**University of Szeged**

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# The safety of cannabinoids and cannabis-based products

**Ph.D. Thesis**

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## *Dedication*

*I wish to dedicate this work to my wife, Zsófia who loved and wholeheartedly supported me throughout the journey, my family whose care, dedication and encouragement helped me through all struggles. I am thankful for the Lord Almighty for giving me the strength to complete this dissertation. Praise be to God!*

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## List of publications related to the thesis

1) Czégény Zs, Nagy G, Babinszki B, **Bajtel Á**, Sebestyén Z, Kiss T, Csupor-Löffler B, Tóth B, Csupor D: CBD, a precursor of THC in e-cigarettes, SCIENTIFIC REPORTS 11 : 1 Paper: 8951 , 6 p. (2021), doi: 10.1038/s41598-021-88389-z
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## Further publications

1) P Orvos, B Pászti, L Topal, P Gazdag, J Prorok, A Polyák, T Kiss, E Tóth-Molnár, B Csupor-Löffler, **Á Bajtel**, A Varró, J Hohmann, L Virág, D Csupor: The electrophysiological effect of cannabidiol on hERG current and in guinea-pig and rabbit cardiac preparations, SCIENTIFIC REPORTS 10 Paper: 16079, 9 p. (2020), doi: 10.1038/s41598-020-73165-2
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# Table of content

# Abbreviations and symbols

5-$HT_3$Rs – serotonin receptors
7-OH-CBD – 7-hydroxy cannabidiol
$A_{1A}R$ – adenosine receptor
AD – Alzheimer's disease
AEs – adverse effects
ALS – amyotrophic lateral sclerosis
ALT – alanine transaminase
ASM – anti-seizure medications
AST aspartate aminotransferase
APD – action potential duration
$Ca_v$ – T-type voltage-gated calcium channels
$CB_1R$ and $CB_2R$ – cannabinoid receptors type 1 and type 2
CBD – cannabidiol
CBDAS - cannabidiolic acid synthase
CBGA - cannabigerolic acid
CBGAS - cannabigerolic acid synthase
CBN – cannabinol
CBNA - cannabinol acid
CBR inotropic cannabinoid receptor
$C_{max}$ – maximum plasma concentration
COVID-19 – corona virus disease of 2019
COX-2 – cyclooxygenase-2
CRM – certified reference material
CTO – column temperature controller
CYP – cytochromes P450
D2 and D3 – dopamine receptors
DGU – vacuum degasser
DRE – drug-resistant epilepsy
DS – Dravet syndrome
EB – epidermolysis bullosa
EEG – electroencephalographic
EFSA – European Food Safety Authority
ELISA – enzyme-linked immunosorbent assay
EU- European Union
FAAH – fatty acid amide hydrolase
FDA – Food and Drug Administration
GABA-$_AR$ – GABA-A receptors
GlyRs – glycine receptors
GPP - geranyl diphosphate
GPR55 – G protein coupled receptor 55
hiPSC-CM – human-induced pluripotent stem cell derived cardiomyocytes
ICD – Internation-al Statistical Classification of Diseases and Related Health Problems
ICNCP - International Code of Nomenclature for Cultivated Plants
IL-6 – interleukin-6
$K_v$ – voltage dependent potassium channel
LGS – Lennox-Gastaut syndrome
LPS – lipopolysaccharide
MEP - methylerythritol phosphate pathway
MS – multiple sclerosis
NAM – allosteric negative modulator
$Na_v1.5$ – cardiac sodium channels
$Na_v$ - voltage-gated sodium channel
NFκB – nuclear factor kappa B
OA - olivetolic acid
OLE – open-label extension
ORs – odds ratios
OR – opioid receptor
OTC – over-the-counter
OUD - opioid use disorder
PAM – allosteric positive modulator
PD – Parkinson's disease
PDQ-39 – Parkinsons's Disease Questionnaire 39
PICO – patients, intervention, comparison, outcome
PICO – Population (P), Intervention (I), Comparison (C), Outcome (O)
PPAR γ – peroxisome proliferator-activated receptor γ
PRISMA – Preferred Reporting Items for Systematic reviews and Meta-Analyses
PROSPERO – International Prospective Register of Systematic Reviews
PTFE – polytetrafluoroethylene
RCTs – randomized, placebo-controlled trials
ROS – reactive oxygen species
RRs –risk ratios
SIL – autosampler
SPD – diode array detector
SPS – simulated public speaking
$t_{1/2}$ – half-life
TBC – two-bottle choice
THC – tetrahydrocannabinol
THCAS - tetrahydrocannabinolic acid synthase
TLR4 – toll-like receptors
$T_{max}$ – time of maximum concentration
TNF-α – tumor necrosis factor α
TREs – treatment-resistant epilepsies
TRPs – transient receptor potential channels
TSC – tuberous sclerosis complex
UC – ulcerative colitis
UHPLC – ultra high-performance liquid chromatography
UN – United Nations
δ,μ-OPR – opioid receptors
$\Delta^9$-THC – $\Delta^9$-tetrahydrocannabinol

# 1. Introduction

The importance of studying *Cannabis sativa* L. has long been a focus of many scientists, and even the history of hemp as a medicine dates back to centuries. The structure elucidation of the major and most abundant cannabinoids, $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) and cannabidiol (CBD) was a huge landmark in the field of cannabinoid research thanks to Raphael Mechoulam, who elucidated the structure of these compounds in 1960's. Today, the significance of cannabis is even greater due to the increasing popularity of CBD and other cannabinoids as food-supplement and medicines [7].

*Cannabis sativa* L. is known for its psychoactive effects, more commonly 'getting a high feeling', but some of its constituents, such as CBD, are not psychoactive. The toxicological and pharmacokinetic studies of cannabinoids have evolved in parallel with by the application of the chemical components, but the results of the safety studies could not keep up with the success of the application of cannabinoids. The administration of CBD-containing or enriched oils and food-supplements has been a risk factor to consumers due to the diversity of CBD concentrations in the products. There are no comprehensive studies that would investigate the various CBD-containing products in Hungary, although the number of products on the market would indicate the performance of such studies.

Currently, several active ingredients of cannabis (CBD and THC) and the semisynthetic derivatives of THC (nabilone) are marketed as medicines, but various other products can also be found on the market such as food-supplements, wellness, and beauty products. The problem with CBD products is not only the chemical profile, but also the presence of impurities and other minor cannabinoids that could come from the manufacturing technologies. In this way, analytical investigation of such products is highly suggested. Additionally, the legal status of cannabis and CBD is questionable in the European Union (EU) and outside of the Community because of its pharmacological effects administered as food-supplements. Nowadays, in addition to its rational use, there is also a significant unprofessional use based on exaggerated expectations. This is in part due to the use of hemp anomalies in the legal regulation of cannabis. Furthermore, the fact that cannabinoids are active substances in antiepileptic medicines, can lead people to the use of cannabis and CBD-containing products with hope for several therapeutic fields without any medical control.

Statistical analysis is necessary for all types of research. Meta-analyses are good tools for evaluating big data connected to a defined topic. The number of clinical trials conducted with CBD is limited and emphasizes different outcomes. Adverse events are crucial parts of any authorization process because without a reliable safety profile, active ingredients cannot be accepted. Applying the proper search terms and searching in the appropriate databases meta-analyses can be easily performed. Focusing on adequate outcomes, meta-analyses can be a solid source of evidence-based medicine. The long-term use of cannabinoids in evidence-based medicine is greatly influenced by research that can provide a more accurate picture of their risk-benefit profile.

Based on the current domestic legislation, including member states of EU and international conventions in force on hemp and its constituents, we receive a mixed opinion of the plant. There is a lack of unified regulation in the EU on the marketing and quality of CBD products. Thus, the popularity of CBD makes its way through advertisements, social media, manufacturers, and other untrusted sources, respectively. All these facts highlight the need for proper quality control of CBD-based products that require swiftly and easily applicable analytical methods to determine their CBD content. In 2022, the European Food Safety Authority (EFSA) published a statement on the safety of CBD as a novel food, and determined various gaps and uncertainties and concluded that the safety of CBD as a novel food cannot currently be established [8].

The work presented in this thesis is part of a research project on cannabinoids at the Institute. The previous results of the research team have shown that even the study of cannabinoids, which have been relatively well researched in terms of bioactivity, can still hold surprises [5,6], and an analysis of the literature shows that there are more questions than answers in the field of cannabinoid research. In my work, I present investigations conducted using different methodologies, with the common feature that they all focus on promoting a more rational and safer use of cannabinoids.

# 2. Aims of the study

The aim of our work was to analyze the safety of two widely used cannabinoids, dronabinol and nabilone, to collect basic research data on the safety of CBD-containing e-cigarettes, and to conduct a chemical analysis of CBD-containing food-supplements. To this end, we aimed to:

- analyze the safety of dronabinol and nabilone in a meta-analysis based on data from clinical trials;
- investigate the effect of temperature on the composition of CBD pyrolysis products under different conditions;
- develop a reliable and robust method for the screening of commercially available CBD-containing products in Hungary;
- qualitatively and quantitatively describe the examined products.

# 3. Literature overview

## 3.1. Botany of *Cannabis*

*Cannabis* belongs to the Cannabaceae family and according to the literature originates in Asia [9]. *C. sativa* is an annual herb and one of the most widely used and oldest horticultural plants [10]. There is a still ongoing debate about the taxonomy of the plant [11,12]. This issue has influenced the nomenclature of the plant, and guidelines suggest following the International Code of Nomenclature for Cultivated Plants (ICNCP) and do not emphasize epithets such as *sativa* or *indica* [9].

Genus *Cannabis* has many species all over the world from north to south. It produces a very specific group of molecules called cannabinoids. According to the studies by ElSohly et al. there are a total number of 565 compounds in the *Cannabis* plant and 120 are phytocannabinoids. Classification can also be carried out based on phenotypes referring to three main groups based on the $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) and cannabidiol (CBD) levels. There are phenotype I or drug-type with higher $\Delta^9$-THC level than 0.5% and less cannabidiol than 0.5%; phenotype II or intermediate type with CBD as the major cannabinoid but THC is also present; phenotype III or fiber-type (hemp) with low $\Delta^9$-THC level. Cannabis is predominantly dioecious (male and female flowers occur in separate plants) and occasionally monoecious (male and female flowers occur in the same plant, hermaphrodites) [10]. The valuable compounds of the plant are produced in the glandular trichomes mainly on the surface of the female flowers. The resin is produced by the secretory disk cells of the trichomes, which is rich in various cannabinoid acids and contains a great number of other compounds, mostly terpenes. Different types of trichomes could be differentiated like capitate-sessile, capitate-stalked, and bulbous glandular trichomes. Several factors could influence the production of cannabinoids in the plant, including environmental and genetic factors. Altitude, light source and intensity, temperature, fertilization, etc. are often mentioned as key control factors in the productions of cannabinoids. The role of cannabinoids and other compounds is still not fully understood, but there is suggestion that they act as defenders of the plant against ultraviolet (UV) radiation, herbivores, and insects, respectively [13].

## 3.2. Biosynthesis of phytocannabinoids

Phytocannabinoids are $C_{21}$ terpenophenolic compounds with a terpen and a phenolic moiety. The biosynthetic pathway can be divided into two different pathways and several different cell types. In the cytosol, there is the cytosolic mevalonate, and in the plastids,

the plastidial methylerythritol phosphate pathway (MEP). Geranyl diphosphate (GPP) and olivetolic acid (OA) are key intermediers of biosynthesis coupled by cannabigerolic acid synthase (CBGAS) that results in cannabigerolic acid (CBGA). Then, biosynthesis continues, and other enzymatic steps are taken with tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), and other synthases. The products of biosynthesis are various types of cannabinoid acids which are not active forms of the compounds, but still possess some biological activities (**Figure 1**). These final acidic cannabinoids still undergo further conversions, mainly decarboxylation by exposure to heat, radiation and sometimes during inappropriate storage [14,15]. Time and ageing can also affect the level of cannabinoids in the plant and investigations showed that the concentration of cannabinol acid (CBNA) and cannabinol (CBN) increased in cannabis samples stored for a long time [16].

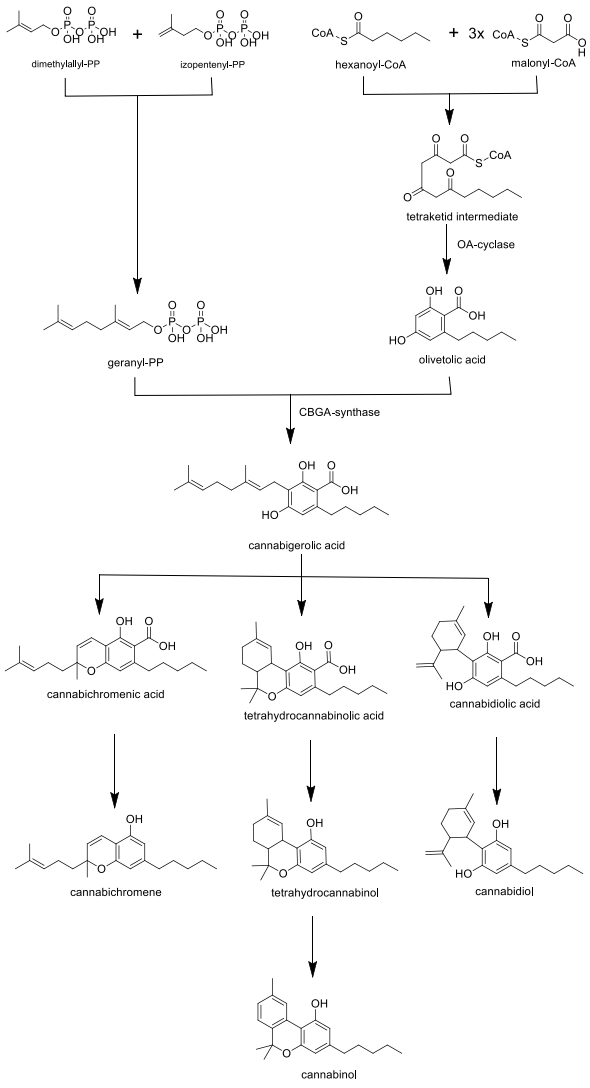


**Figure 1.** Biosynthesis of phytocannabinoids

## 3.3. *Cannabis* constituents

Cannabis has a very complex chemical profile. It contains mainly cannabinoids, but other constituents are present in the plant. The term cannabinoid refers to a group of chemical compounds that have a core structural part but vary in chain substitutions and ring closure positions. The cannabinoids that are isolated from the *Cannabis sativa* plant are called phytocannabinoids. These molecules can be further classified into main categories of cannabinoids that show great similarity. According to Rock and Parker, there are 11 types of cannabinoids including the $\Delta^9$-THC, $\Delta^8$-THC, CBD, CBG, CBC, CBND, CBE, CBL, CBN, CBT, and miscellaneous [17]. The concentration of such cannabinoids depends on numerous cultivation factors that could greatly influence the level of the constituents. Thus, the exact amount of cannabinoid found in the plant can be described by a certain range. The content analysis of the cannabinoids could be carried out by using analytical instruments mostly HPLC-UV, GC-FID, and GC-MC techniques. Gloerfelt-Tarp et al. in their article used near InfraRed-based chemometric application for the quantification of cannabinoids. This method has the advantage of not destructing the sample along with cost and time effectiveness. It also has a good prediction percentage about the position of the searched compound and its content level. According to the results of Gloerfelt-Tarp et al. the content of the major cannabinoids for CBD ranges from 0.01–0.52%, for $\Delta^9$-THC from 0.01–1.85%, for CBG from 0.01–0.08%, and for CBN from 0.01–0.76%, respectively [18].

The main compound of the plant is the $\Delta^9$-THC. It is probably the most studied constituent of all cannabinoids which is responsible for the psychoactive effect. The structural variation of the tetrahydrocannabinol-type compounds is very rich. Multiple isomers and possible artifacts can be present in the plant which can occur after degradation or transformation. The structural similarities among these compounds can influence the pharmacological mechanisms as well. $\Delta^9$-THC has partial agonistic effect at both $CB_1$ and $CB_2$ receptors [19].

CBD was the first genuine phytocannabinoid to be isolated in 1940 but its structure had to be corrected after the development of spectral techniques. It is a non-psychoactive cannabinoid but has multiple other possible medicinal properties. CBD is and allosteric inhibitor of $CB_1$ and further modulates the activity of the physiological mechanism of $\Delta^9$-THC [19].

CBG is the unstable but major precursor molecule for cannabinoids. It was the first natural cannabinoid to be synthesized in 1964. At the beginning, CBG did not have significant attention in the field of cannabinoid research. Besides *Cannabis* sp.*,* other species such as *Helichrysum umbraculigerum* may contains CBG in various concentration. CBG acts as an antagonist on the menthol receptor TRPM8 which is important in the treatment of prostate cancer. It can also active α-2 adrenergic receptors and moderately inhibit the $5HT_{1A}$ serotonin receptors [19].

CBN was the first cannabinoid isolated from *cannabis*. CBN is formed in the plant naturally during the process of aging and due to light exposure and other degradation factors e.g. oxidation. The pharmacological activity of CBN is weaker compared to $\Delta^9$-THC in terms of $CB_1$ and $CB_2$ affinity.

## 3.4. Medical cannabis

China is the birthplace of *Cannabis* cultivation, which later expanded to other continents [10]. The term "medical cannabis" describes the use of cannabis or cannabinoids as a therapeutic agent to treat illness or reduce its symptoms. Cannabis and cannabinoids can be used topically, sublingually, or orally. Additionally, cannabis can be added to meals, smoked, inhaled, or turned into tea. Cannabinoids can be consumed as an herbal supplement, obtained organically from the plant, or produced artificially [20,21]. The ratio of THC to CBD in the plant determines the medicinal efficacy of cannabis. In addition to cannabinoids, the plant can also include other substances such as terpenes, flavonoids, stilbenoids, amino acids, fatty acids, alkaloids, hydrocarbons, carbohydrates, and phenols that may have health benefits [22].

The discussion of medical cannabis and the application of pure cannabinoids as a therapeutic agents should be distinguished. Any part of the plant that is used, as a whole, refers to medical cannabis. This way of administration has a long-time known history originating from the very early phases of human history. Advantages and disadvantages both come with the application of medical cannabis. Legislation, complex biochemical profile, uncontrolled quality of the herb and/or its products can all be noted as disadvantages. On the other hand, better patient compliance, more controlled cannabinoid yields due to modern straining strategies, faster way of action can be considered as advantages. Adverse event profile can be controversial in terms of benefits and drawbacks, but the goal is to minimize such events in any form of application.

MacCallum and Russo in their review give an insight to a practical approach in terms of medical cannabis [23]. The most important question of modern therapies whether there is enough evidence to use a certain active ingredient or not. *Cannabis* due to its chemical variety, adaptability and other factor belongs to the group of unstable and unsure level of cannabinoid, respectively. The most common routes of administration of cannabis are related to smoking, vaporization, oral (e.g. oils, tinctures, capsules etc.) and topical (e.g. ointments, suppositories etc.) routes. Some of them require extensive heating that can lead to the transformation of chemical constituent and/or degradation of the valuable active component. Consequently, controlled patient compliance, blood level concentrations etc. are not able to be performed or achieved. Besides cannabinoids, other compounds can have unique pharmacological effects that may influence the outcome of the therapy e.g. monoterpenoids, flavonoids etc. The pharmacokinetic aspects of the different administration routes cannot be neglected because the bioavailability can significantly differ from oral to inhalation. Although, medical cannabis can be useful in cases when the conventional therapies failed to provide hope and cure. The therapeutic protocol in case of cannabis requires dose titration in patients with a low start that can gradually be increased with care. CBD-predominant chemovars are suitable to provide a good control for the therapeutic titration. In terms of adverse events, cannabis has a good safety profile with a low number of reported cases of serious adverse events. Despite the fact, it should be administered carefully [23].

The pure cannabinoids in many aspects overlap with the medical cannabis. These molecules can be isolated by various chromatographic methods and synthesized chemically. The pharmacological effect of the isolated and synthesized cannabinoids is equal. Pure cannabinoids act on a well specified target in the human body, mainly at the endocannabinoid system. Before the authorization of any cannabinoid-based medicine these molecules go through a very strict control procedure. Consequently, the quality of pure cannabinoid medicines has a higher quality level compared to the medical cannabis. In this way, the therapeutic control of the patients could be more precisely monitored with a well-defined concentration. The entourage effect of cannabinoids in case of the isolated cannabinoid compounds can be more controlled which could provide specific therapeutic possibilities. The dosing strategy and titration could be performed more accurately. In view of the adverse events, pure cannabinoids have a lower number of shown adverse events compared to medical cannabis [24].

## 3.5. Preclinical aspects of CBD

Multiple reasons drive the research behind CBD. It is the second most abundant cannabinoid in *Cannabis sativa* and mainly accumulated in the fiber type cannabis chemovars. CBD also has many promising pharmacological effects and compared to THC it is not psychoactive. There is also a tendency of widespread application of CBD in food-supplements and medicines as well [25]. This phenomenon brings along a multitude of questions to be answered. The preferable safety profile of CBD allows it to be a good remedy for a number of health issues including inflammatory and neurodegenerative diseases, epilepsy etc. CBD has an asset of point of attacks by acting on various ion channels, receptors, and modulating enzymes as well. Thus, it bears a huge potential for targeted drug development and potential solution for unsolved medical questions. The chemical modification of the molecule also holds a cascade of possibilities for overcoming the challenges of CBD due to its nature. Banerjee et al. in their publication discuss the different derivatization techniques of CBD. By creating *O*-acyl, -alkyl, and miscellaneous derivatives Banerjee et al. conclude that the functionalization of CBD broaden the acceptance and application spectrum of the molecule [26]. CBD possesses a list of positive features for being abundantly available both via isolation and synthesis, lacking a psychoactive profile compared to THC which makes it a preferable active ingredient. CBD has a distinctive pharmacological way of action but at the same time able to modulate the effect of THC. It has a popularity worldwide because of its legal but not regulated status After all, there is not just a scientific but market wise space created for the distribution of CBD leading to cases of uncertain quality and unrevealed health concerns to consumer and all end-users [25]. In summary, CBD has all the potential for solving practical and realistic questions in the light of scientific and health care issues.

Countless studies and articles deal with the potential aspects of CBD. On the basis of the various molecular targets of CBD, the examined outcomes have a broad spectrum. CBD represents a potential target molecule for various indications at different molecular levels.

### 3.5.1 Pharmacokinetic properties of CBD

The bioavailability of CBD greatly depends on the method of administration. There is a lack of information on its bioavailability as a single agent because it is usually investigated in combination with THC. The lipophilic character and its extended first-pass metabolism via the liver leads to a poor bioavailability which is estimated to be

around 6%. Oral bioavailability could be improved by different formulation methods and the application of various delivery methods. In this way, the maximum plasma concentration ($c_{max}$) could be increased multiple times [27].

By inhalation, CBD can reach rapid plasma peaks in minutes and in connection with those higher $c_{max}$ levels, if we compare it with oral administration. In this way, bioavailability can also range greatly from 11% to 45%. CBD has other routes of administration such as intravenous and transdermal, but these are not the most significant of all possible ways. The pharmacokinetics of CBD can be affected by many environmental factors that affect the maximum concentration time ($T_{max}$) and the half-life ($t_{1/2}$) of the compound. Food intake, hormonal difference, body fat percentage, and other elements can lead to pharmacokinetic differences of CBD [27].

CBD can be distributed in many parts of the body, but mainly accumulates in adipose tissues due to its high lipophilicity. It also reaches highly vascularized tissues and organs, such as the heart, brain, liver, lungs, and spleen. CBD metabolized in the liver via various cytochrome P450 enzymes and gives an active metabolite of 7-hydroxy cannabidiol (7-OH-CBD) which then undergoes other metabolic hydroxylation and glucuronidation processes and is finally excreted through urine [27].

Due to its broad spectrum of molecular targets, CBD can interact with other chemical compounds and medications, leading to changes in plasma concentrations, altered action mechanisms that affect the pharmacology of drugs, and can show multiple adverse effects, although CBD is considered to be a safe active ingredient.

### 3.5.2. Molecular targets of CBD

Castillo-Arellano et al. in a review identified 56 different molecular targets that include various enzymes, ion channels, and receptors. The research group investigated molecular targets and pharmacological implications in animal models and human diseases at both the *in vitro* and *in vivo* levels and found valuable relationships between the targets and the pharmacological applications. Among the identified targets the most important are the cannabinoid receptors type 1 and type 2 ($CB_1R$ and $CB_2R$), glycine receptors (GlyRs), GABA-A receptors (GABA-$_A$Rs), serotonin receptors (5-$HT_3$Rs), voltage-gated sodium channels ($Na_v$), T-type voltage-gated calcium channels ($Ca_v$), transient receptor potential channels (TRPs), peroxisome proliferator-activated receptor γ (PPAR γ), and other enzymes, e.g. fatty acid amide hydrolase (FAAH), cytochromes P450 (CYP) etc. [28]. The targets that are strongly related to the clinical relevance of CBD are GABA-$_A$R, $Na_v$,

$Ca_v$, $CB_1R$, GPR55 (G protein coupled receptor 55), CYPs in terms of epilepsy. Other ones that are responsible for the antinociceptive effect are GABA-$_AR$, TRPs, adenosine receptor ($A_{1A}R$), GlyRs, PPAR γ, 5-$HT_{1A}R$, $CB_1R$, TRPs, $Ca_v$, opioid receptors (δ,μ-OPR), GPR55, and FAAH.

The key to CBD as an active compound lies in its complex mechanism of action as an agonist, inverse agonist, or even antagonist and can even behave as an allosteric negative (NAM) or positive (PAM) modulator.

Different types of ions such as $Na^+$, $K^+$, $Ca^{2+}$, and $Cl^-$ play a dominant role in many pathological conditions. CBD can modify and inhibit $Na_v$ channels in both the rest and inactive states. It has a special affinity for all $Na_v$ channels. CBD can also affect the $Ca_v$ in this way and contribute to the therapy of several diseases such as epilepsy, sleep difficulties, and pain management, but the exact mechanism is still not clear. Inhibition by CBD in $Ca_v$ has been determined by using the patch clamp methodology.

TRP receptors also navigate ions throughout the body like $K^+$, $Na^+$, $Mg^{2+}$ or $Ca^{2+}$ which also affect many physiological processes in the body, and CBD can interact with these receptors. Among the many TRPs subclasses, six are inhibited or activated by cannabinoids, and these are called inotropic cannabinoid receptor (CBR). These are TRPV1, TRPV2, TRPV3, TRPV4, TRPA1, and TRPM8. TRPV1 is especially connected to CBD because it is a full, but not potent, agonist of this type of channel, and this mechanism is often associated with the anxiolytic, anti-hyperalgesic, and anti-inflammatory effects of CBD in animal models. The pain-relieving character of CBD may also be related to its activity on opioid receptors. On the δ-OPR CBD acts as a PAM and can reduce the activity of μ-OPR. These OPRs are present in the cardiovascular and immune systems.

The non-psychoactive effect of CBD is related to the differences between the two cannabinoid receptors both structurally and on the location of the receptors. $CB_1Rs$ are mostly expressed in the brain and central nervous system, and $CB_2Rs$ are mainly found in immune cells. CBD is found to be a non-competitive NAM or antagonist of $CB_1R$ and CB2R, compared to THC, which activates the aforementioned receptors [28].

CBD can activate PPARγ receptor which is involved in the anti-inflammatory mechanism. PPARγ expression is enhanced in the pathophysiological condition of multiple sclerosis (MS), and CBD was found to activate PPARγ in MS. In a rat model of

Alzheimer's disease (AD), CBD prevented neurodegeneration in rats by reducing pro-inflammatory molecules and stimulating hippocampal neurogenesis [28].

The mechanism behind the antiepileptic effect of CBD is related to the modulation of GABA-$_A$R, $Na_v$, and $Ca_v$. CBD inhibits ion channels and increases the inhibition of GABA-$_A$R and $Na_v$ via metabolic pathways through CYPs. CBD also has a strong protein binding character, which increases neurotransmitter release [28].

In various rodent models, the administration of CBD led to an analgesic effect by antagonism of $A_1$$_A$R, $CB_1$R, and TRPA1, suggesting the analgesic effect in the supraspinal region. Another study also suggested that CBD has an analgesic effect through TRPV1 antagonism. Acting on the $CB_1$R and PPARγ CBD reduces the expression of the inflammatory marker of cyclooxygenase-2 (COX-2) and the nuclear factor kappa B (NFκB). In different pain models, the results suggested that the modulation of GlyR and 5-$HT_1$$_A$R has analgesic effects in allodynic type of pain [28].

Ghovanloo et al. in a mini-review discussed the interactions of CBD with voltage-gated sodium channels ($Na_v$). The non-psychoactive feature is not related to endocannabinoid receptors (CBRs), although it has other possible pathways and molecular targets in which sodium ion channels are particularly important. $Na_v$ channels play an important role in the electrical signaling of neurons [29]. CBD has a low affinity for CBRs, but on the other hand it has a mild antagonistic effect, which suggests that molecular mechanisms could be responsible for the non-psychotropic effects. The authors of the review noted that CBD directly modulates the biophysical properties of the biomembrane, thus, it may facilitate an allosteric modulation of membrane proteins. $Na_v$ are peculiarly interesting in terms of CBD efficacy because the medical conditions which CBD is approved for are related to some kind of $Na_v$ channel deficiency. Dravet syndrome is commonly linked to the genetic mutation in $Na_v$1.1.

Excitability-related disorders such as pain, seizures, muscular problems, arrhythmias, and other disorders are also related to $Na_v$ deficiencies. CBD can modulate membrane elasticity, which has been shown to allosterically stabilize $Na_v$ channel inactivation. Consequently, CBD has been established to have an inhibitory effect on the $Na_v$ channel [29].

The investigations of CBD included voltage-clamp experiments, and the results showed that CBD inhibits all human $Na_v$1.1-7 from the inactivated states. The experiments also showed that CBD imparts similar effects on $Na_v$ gating by inhibiting

$G_{max}$ without changing the voltage dependence of activation, but hyperpolarizing steady-state inactivation and slowing recovery form inactivation. Ghovanloo et al. found that $Na_v$ enter deeper inactivated states because CBD slows the recovery kinetics even further.

CBD interacts with the interface of the channel pore and fenestrations in which CBD directly blocks the pore and alters the membrane elasticity which indirectly stabilizes $Na_v$ channel inactivation. The authors in their study noted that CBD-dominant nutraceutical products can inhibit $Na_v$ even more potently than pure CBD which is believed to be related to other components, e.g., phytocannabinoids or terpenes, which can further modulate or inhibit $Na_v$ [29].

Depending on the concentrations, CBD can modulate, hyperpolarize the voltage-dependent potassium channel $K_v7.2/3$ and can alter the excitability processes of the neurons. This finding could support the role of CBD as an anticonvulsive and analgesic agent, independently of other modulatory effects of the ion channel [29].

Fouda et al. investigated the effects of CBD in lipopolysaccharide (LPS)-induced cardiotoxicity through Toll-like receptors (TLR4) and cardiac sodium channels ($Na_v1.5$). The researchers incubated human immune cells (THP-1 macrophages) with LPS and MPLA (TLR4 agonist) separately and in combination with CBD and C34 (TLR4 antagonist) and found that the two compounds mentioned above inhibited the release of the compound of interleukin-6 inflammatory markers (IL-6) and tumor necrosis factor α (TNF-α) [30].

Enzyme-linked immunosorbent assay (ELISA) assay was performed to detect the levels of pro-inflammatory cytokines and found that CBD or C34 attenuated the LPS or MPLA-induced reduction in cell viability. Researchers also investigated the relationship between apoptosis and human-induced pluripotent stem cell derived cardiomyocyte (hiPSC-CM) cytotoxicity using caspase 3 activity for apoptosis studies. The results showed that CBD or C34 significantly attenuated the increase induced by LPS or MPLA in the caspase 3 activity of hiPSC-CM. The formation of reactive oxygen species (ROS) was also tested with CBD or C34 and found that the compound attenuated LPS or MPLA-induced elevations in ROS levels. Although alteration of cell viability, apoptosis, or ROS levels was not experienced under control conditions [30].

The effect of CBD on the $Na_v1.5$ channel was also studied by applying voltage clamp measurements. LPS significantly shifted $Na_v1.5$ activation midpoint of $Na_v1.5$ (V1 / 2) in the positive direction, and CBD reversed the effect of LPS.

The authors also investigated the cardiac action potential using the O'Hara-Rudy model. LPS and MPLA prolonged the duration of the simulated action potential (APD). The results showed that CBD or C34 rescued LPS- or MPLA-induced prolongation of APD [30].

De Almeida and Devi also investigated the molecular and signaling pathways for CBD in their review. They reviewed seven molecular targets in depth. GPR55 is said to be a 'third' cannabinoid receptor due to some effects of CBD that could not be related to CB. By applying FLAG-tagged human GPR55, they demonstrated that CBD showed antagonistic effect in GPR55. They also discussed the effect of CBD on 5-$HT_{1A}$ receptors by using the selective antagonist NAN-190 on 5-$HT_{1A}$ and found that CBD competes for the orthosteric binding site of 5-$HT_{1A}$ receptors. They revealed that behavioral studies examining the effect of CBD showed that it has antidepressant effects via 5-$HT_{1A}$ receptor modulation. The authors also collected data on the anti-allodynic effect of CBD acted throughout 5-$HT_{1A}$ activation in the central and peripheral nervous systems, regulating neuronal excitability and neurotransmitter release [31].

Other targets were also discussed in the review of De Almeida and Devi, including dopamine receptors (D2 and D3) because CBD is said to have a partial agonist effect on the D2 receptor. Other researchers found that CBD might bind more favorably to D3 dopamine receptors compared to D2 and probably has a partial agonist effect of D3 [31]. De Almeida and Devi also examined CBD-related adenosine receptors and found that CBD mediated anti-inflammatory markers in animal models that act on the $A_{2A}$ adenosine receptor. Going after the idea of Vaysse et al. [32] the authors collected information on the opioid receptor (OR) modulating effects of CBD. The authors found that CBD at a concentration of 30 μmol/L concentration behaved as a negative allosteric modulator on the μ and δ ORs. In an animal study investigating the reinforcing properties, motivation, and relapse for ethanol consumption in the two-bottle choice (TBC) paradigm in mice, CBD decreased ethanol intake and the number of effective responses in oral ethanol self-administration. Hurd in her work [33] noted that CBD has a potential therapeutic potential in the treatment of opioid-use disorders (OUD) due to the positive effects in other medical conditions such as anxiety, sleep disorders, behavioral characteristics of drug addiction and neuroprotective effect. This relationship could be based on the strong connection between the endocannabinoid system and the opioid system [31].

Like many other publications, De Almeida and Devi discussed the role of CBD in ion channel activities and suggested that the TRPV1 receptor is a key molecular target. Electroencephalographic studies (EEG) showed that CBD had anticonvulsant effects in mice model of seizure induction. CBD increased seizure latency and reduced seizure duration when injected intraperitoneally and these effects were tested by selective antagonists on the TRPV1, $CB_1$, and $CB_2$ receptors. They also found that CBD also engages the sodium ($Na^+$) and calcium ($Ca^{2+}$) channels. Consequently, CBD could have a great impact on neuronal excitability through the modulation of $Na^+$ and $Ca^{2+}$ [31].

PPARγ receptors could also be potential targets for CBD because it improves lipid and glycemic parameters in type 2 diabetes (DM2). The application of a selective antagonist that blocks PPARγ CBD significantly reduced the effects on reactive gliosis in primary astroglial cultures of rats. Acting in this way, CBD could be a therapeutic option for Alzheimer's disease (AD) [31].

## 3.6. Clinical background of CBD

Potential therapeutic uses for both synthetic and phytocannabinoids have been increasing in the medical community. Based on preclinical data and the number of studies investigating the molecular targets and signaling ways behind the mechanism of action of CBD there are generally targeted medical conditions, e.g., pain, seizure disorders, appetite stimulation, muscle spasticity, nausea and vomiting, but it might be useful for the treatment of central nervous system disorders and also for cancer [34].

There are several approved medicines containing pure CBD (Epidyolex) or CBD:THC extract (Sativex) with various indications. Sativex was approved for the treatment of spasticity, while Epidyolex was approved for the treatment of seizures associated with Lennox-Gastaut syndrome (LGS) or Dravet syndrome (DS) in pediatric patients.

Legare et al. collected data from clinical trials conducted with CBD and THC as well and summarized the conclusions. In their publication, they mention about therapeutic potential of cannabinoids in the treatment of chronic pain. The reviewed meta-analysis concludes that in the investigated RCTs dronabinol, nabilone, and nabiximols showed efficacy in the reduction of neuropathic pain. In chronic or intractable pain of non-neuropathic origin, nabilone was found to be ineffective in alleviating radiation therapy-induced pain. Data from clinical trials using Sativex or high CBD nabiximols for chemotherapy-induced pain produced non-significant results with high variability [34].

According to the review by Sholler et al., there are few clinical studies that have evaluated the efficacy of CBD alone for the indication to treat pain or reduce inflammation [35]. The authors report that in such studies CBD was co-administered with THC. In a randomized, placebo-controlled trial, a transdermal CBD gel was tested to treat knee pain associated with osteoarthritis. The pain scores analyzed showed controversy in the results. There are several clinical trials with a small number of patients investigating chronic pain treatment in patients with a history of kidney transplantation. The results of these studies still require further well-designed clinical trials [35]. Fiani et al. in their review gave more detail on the current application of CBD in various neurological disorders. They found that non-cancer-related pain has been mainly related to nabiximols (THC: CBD 1:1), so further clinical trials should be carried out [36].

Epilepsy is a condition that affects both children and adults and arises from an abnormal excitation-inhibition balance of neurons in the brain. CBD has greater efficacy in the clinical setting compared to other preparations made from *Cannabis*. According to some open-label studies, the efficacy of CBD was around 50% and showed variability depending on individual factors between patients. Studies also show that CBD has a synergistic effect applied with clobazam, another antiepileptic agent. Pauli et al. in their mini-review presented data from clinical trials related to CBD. Clinical trials have been conducted for Epidyolex, which still counts as the gold standard for scientific evidence. Epidyolex was approved for the treatment of LGS and DS [37]. Kühne et al. in their publication provide real-world data on the effect of CBD in the treatment of various epilepsy subtypes. In their retrospective multicenter study, they investigated the efficacy and tolerability of CBD in patients with epilepsy in 16 epilepsy centers in Germany. CBD therapy was off-label in the severity of the cases. The starting and titration doses were lower than the recommended doses. The results showed that 36.9% of all patients experienced a reduction in the frequency of seizures of more than 50%, which was independent of their epilepsy types and the co-medication of clobazam. Adverse effects were frequently reported but remained mild. The conclusion of the work of Kühne et al. demonstrated that the overall seizure freedom rate of CBD was comparable to many other antiseizure medications (ASM) in the treatment of drug-resistant epilepsy (DRE) with a positive safety profile. However, they call for further research to investigate the extended application and approval of CBD for other epilepsy subtypes and for children under the age of two [38].

Amyotrophic lateral sclerosis (ALS) is another condition linked to neuronal malfunction. Both THC and CBD were tested for this condition and studies found that THC and CBD reduced pruritus in patients with ALS, but the clinical data supporting this observation are limited [34].

CBD was also investigated in the treatment of Parkinson's disease (PD). The effects of cannabinoids on the $CB_1$ receptor may ensure therapeutic benefit for patients with PD. A double-blind study investigating the effects of CBD showed a significant improvement in the intervention group compared to the placebo groups according to the Parkinson's Disease Questionnaire 39 (PDQ-39) [34]. Pagano et al. in a review mention that CBD treatment of PD patients over a 4-week period in an open-label study significantly reduced psychotic symptoms, i.e. illusions and hallucinations, and also minor symptoms like withdrawal and depression [39]. On the contrary, Sholler et al. note that in their review, two published clinical trial data were included stating that 6 weeks of oral CBD improved self-reported well-being, but did not alter clinically observed disease symptoms. The other clinical trial included in their review did not confirm the positive effects of CBD in PD and concluded that it is difficult to maintain a firm conclusion on the efficacy of CBD in the treatment of PD [35]. Fiani et al. found that research on CBD in the field of PD suggests that CBD has a mild beneficial effect on PD-related tremor [36]. This statement is mainly the consequence of the small number of clinical trials that cover the effects of CBD in PD.

CBD is commonly used to treat anxiety problems. The endocannabinoid system is linked to have a therapeutic benefit to reduce anxiety by modulating mood. A double-blind study focusing on patients with anxiety disorder found that 600 mg of CBD administered 1.5 h before public speaking was able to reduce anxiety compared to healthy volunteers. Clinical trial investigating this indication of CBD are currently ongoing [34]. Sholler et al. in their work reviewed the evidence derived from clinical trials. They noted that the results of a survey of young adults showed that the main reason for the use of CBD is to alleviate anxiety. In several studies, the score of study subjects was measured by different public speaking tests, e.g., simulated public speaking (SPS) which reliably describes self-reported anxiety levels in humans. CBD produced a U-shaped dose-response curve in the decrease in anxiety. 300 mg of CBD was the most effective of all doses [35]. Larsen and Shahinas in their review collected data on the dosage, efficacy, and safety of CBD in human trials. In their publication, the CBD dose ranged from 150–

900 mg in most of the investigated studies. The results were mixed, either supporting the effect of CBD or opposing such findings. Some studies using a small dose of CBD (16 mg) by inhalation were effective in extinction and consolidation of fear [40].

Treatment of skin disorders is a relatively new application of CBD. However, topically applied CBD may be a possible way of treating various skin disorders. An observational study reporting cases of self-initiated topical use of CBD in patients with epidermolysis bullosa (EB) suggests that CBD may improve quality of life in such patients [39].

The number of gastrointestinal diseases, including ulcerative colitis (UC), has increased. Based on anecdotal evidence of the benefits of cannabinoids under such conditions, CBD was tested in such cases. A double-blind, placebo-controlled study over 10 weeks investigated patients with UC taking Epidyolex 50–250 mg. Their goal was to measure changes in Mayo scores of patients that are used to index the severity of UC. The secondary outcome was the measurements of UC symptoms and calprotectin levels. The results showed that there was a decrease in all the measured parameters [37].

Sholler et al. in their review discussed the effects of CBD on sleep quality. In the mentioned study healthy volunteers with sleep difficulties such as falling or staying asleep were included and CBD was administered at different doses. Those who received 160 mg of CBD reported having a longer duration of sleep. Another randomized double-blind trial investigated sleep as the primary outcome in healthy volunteers and found that CBD did not alter sleep measures. Thus, the confidence of CBD in the treatment of sleep difficulties requires further research [35].

The evaluation of adverse effects is strongly related to the clinical application of CBD and is an important factor for all kinds of drug approval and administration. Chesney et al. investigated the adverse effects of CBD in a systematic review and meta-analysis that included data from randomized clinical trials. The focus of the studies involved in the systematic review was on clinical data on schizophrenia, problematic cannabis use, Huntington’s disease, DM2, non-alcoholic fatty liver disease, Crohn's disease, and healthy volunteers. The authors also investigated the withdrawal rate of the pooled studies and found that there was a higher withdrawal rate in the CBD group due to high doses and adverse effects. Adverse effects were classified by severity. Serious adverse effects appeared in smaller numbers and were related to pneumonia and abnormal liver function test. Adverse effects appeared in a higher proportion in CBD-treated patients, but

differences were also observable whether the treated clinical group included children with epilepsy or not. Decreased appetite, diarrhea, sedation, and somnolence was the most frequently reported AE while in epilepsy studies decreased appetite, diarrhea, and somnolence were the most frequent without sedation. The conclusion of the publication is that CBD is well tolerated and has few adverse effects. The risk lies behind the ability of CBD to inhibit the liver metabolism of other medications, thus increasing the risk of potential interaction or altered effect of drugs [41]. Fazlollahi et al. in their publication investigated the frequency and risk of developing AE in patients with epilepsy who used CBD. The authors determined the overall incidences in the CBD treated groups and in the control groups. Calculated the overall risk ratios (RRs) for any grade and severe adverse effects (AEs) respectively for the CBD and control group as well. AEs were also classified into any grade and mild, moderate, and severe AEs. The frequency of AEs was higher in the CBD treated group than in the control group. The category of any grade of AE included diarrhea, somnolence, decreased appetite, and elevation of alanine transaminase (ALT) or aspartate aminotransferase (AST). In the mild category of AEs the incidence of diarrhea was higher while decreased appetite, somnolence belonged to the moderate category of AEs. The severity of serious adverse effects was calculated without further discussion. The findings of Fazlollahi et al. showed that the frequency of any grade AE in patients with epilepsy was more than two times higher for those who received CBD than for controls. However, the authors state that further research is required to investigate the therapeutic effects of CBD and related adverse effects [42]. Madeo et al. in a systematic review updated on clinical toxicity and adverse effects of CBD [43]. Their focus was on neurological studies including CBD as a treatment drug. The settings of the trials varied, but patients administered highly purified CBD. In observational cohort studies 'open label extension' (OLE) in patients with LGS, DS, and tuberous sclerosis complex (TSC), pyrexia and disorders of the gastrointestinal tract, including diarrhea, vomiting, and decreased appetite, were the most common. The most frequent neurological AEs were somnolence, sedation, drowsiness, and convulsion. Those studies that investigated the antipsychotic, antidepressant, anxiolytic, anticraving, and precognitive effects of CBD reported dizziness, drowsiness, recurrent fatigue, recurrent feeling of strong blood flow, and recurrent headache, fatigue, low mood, hot flashes and cold chills, somnolence, increased appetite, diarrhea, weight gain, lethargy, nausea, and tiredness which were mild to moderate in terms of severity, respectively.

CBD possesses analgesic and anti-inflammatory properties, thus the AEs connected with such medical conditions were evaluated. In the case of patients, who underwent arthroscopic rotator cuff repair and received CBD, they reported an increase in transaminase level. Another study, including healthy volunteers, found that common AEs such as lethargy and upset stomach were reported in both the treated and control arms. In this case, subtle mood changes, frequent urination, and wooziness were also reported. CBD was also tested in patients with osteoarthritis and psoriatic arthritis. The patients co-administered other medications, so the reported AEs were not related to CBD. Due to its anti-inflammatory properties, CBD has been tested in patients with mild to moderate symptoms of COVID-19. The patients had concomitant medications in addition to CBD and the reported AEs were somnolence, fatigue, decreased appetite, lethargy, weight loss, and diarrhea. The authors compared the updated review with their previous work and concluded that scientific data has been expanded by data on the long-term efficacy and tolerability of CBD for the treatment of treatment-resistant epilepsies (TRE), suggesting that CBD has a good safety profile in both children and adults. Although the authors highlight a concerning issue regarding the safety evaluation of over-the-counter CBD products and preparations (OTCs) since these products are poorly regulated and could contain other cannabinoid compounds or possible contaminants [43].

## 3.7. Analysis of *Cannabis* from a legal point of view

Besides the medicinal character of *Cannabis*, other features of the plant have deep roots in human society. Taking this into account, there is no doubt that the control of the plant is as old as law. The legality and jurisdiction of *Cannabis* and CBD have been a confusing topic for a long time. *Cannabis indica* was first mentioned in the 1925 International Opium Convention held in Geneva, which contained a trade limitation of the plant [44]. The United Nations (UN) has several drug-related treaties, but the most influential are the Single Convention on Narcotic Drugs of 1962 as amended by the 1972 Protocol and the Convention of Psychotropic Substances of 1971 [45]. We have reviewed the content of the treaties and conventions and made a general summary for better understanding of the topic in a previous paper. In recent years the acceptable limit for THC in the plant varied, but it was stable in these studied documents. The view of cannabis has been shaped by the aforementioned reports that not only affected the attitude toward the plant, but also the opportunities for clinical and industrial use have been reduced due to strict policies [2]. Fiani et al. investigated the situation of CBD in the United States regarding its legality

and ethics [36]. The regulation of cannabis is difficult due to its rich chemical profile and many constituents. This complexity also results in inconsistencies in the chemical profile that can misinform patients, clinicians, and scientists about the use of cannabis. Cannabis use has a long history in the US and has been regulated but has recently been removed from the class of Schedule 1 substances due to the Agricultural Improvement Act of 2018. As a result of the current jurisdiction, CBD will be more easily accessible to scientists to prove its supposed and real pharmacological effects [36].

# 4. Materials and methods

## 4.1. Meta-analysis of clinical data on the safety of dronabinol and nabilone

The following PICO (patients, intervention, comparison, outcome) format was applied: P: adult patients; I: dronabinol or nabilone; C: placebo; and O: frequency of adverse effects. The meta-analysis was reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement. The meta-analysis protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) a priori (registration number CRD42021240190).

### 4.1.1. Search strategy

The literature search was conducted until 21 February 2020, using the following search strategy: [dronabinol OR nabilone] for EMBASE; [("dronabinol"[MeSH Terms] [All Fields]) OR ("nabilone"[MeSH Terms] [All Fields])] for PubMed; [dronabinol OR nabilone] for Cochrane Central Register of Controlled Trials; and [TOPIC: (dronabinol OR nabilone) Timespan: All years. Indexes: SCI-EXPANDED, SSCI, A&HCI, ESCI.] for Web of Science. No publication date or publication status or language restrictions were applied. For transparency, the meta-analysis was based on publicly available data; neither the authors of the articles nor the manufacturers of the studied products were contacted for additional information.

### 4.1.2. Eligibility criteria

All randomized, placebo-controlled trials (RCTs) evaluating the clinical effects of dronabinol or nabilone and reporting adverse effects were included. For each outcome, at least three clinical trials involving different patient populations were required to perform a statistical analysis

#### 4.1.3. Study selection

Record management was performed using the Mendeley 1.17.9 software. After removing duplicates and records without an abstract, the remaining records were screened for eligibility on the basis of article titles and abstracts. The eligibility of the full texts of the remaining records was independently assessed by two reviewers. Disagreement between reviewers was resolved by discussion or, if necessary, by consulting a third reviewer.

#### 4.1.4. Data extraction and synthesis of the results

Data collection was carried out according to the PRISMA guidelines. Study characteristics and results were extracted independently by two reviewers. Discrepancies in the extracted data were resolved by discussion. The following data items were extracted from the included papers: study design, sample size and characteristics of the patient population, duration, intervention details, and numbers of different AEs.

#### 4.1.5. Risk of bias

The risk of bias was assessed using the Cochrane Collaboration tool, which includes seven specific domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other scores of biases. For each domain, studies were judged to have a high (red), unclear (yellow), or low (green) risk of bias. Disagreements in the quality of the studies were resolved by discussion. The summary table and figure of the risk of bias were generated by RevMan 5 software [46].

#### 4.1.6. Statistical analysis

Pooled odds ratios (ORs) were calculated for dichotomous outcomes. A random-effect model was applied in all analyses with the DerSimonian–Laird estimation. Statistical heterogeneity was analyzed using the $I^2$ and $\chi^2$ tests to obtain probability values; $P < 0.10$ was defined to indicate significant heterogeneity. The $I^2$ test represents the percentage of total variability across studies because of heterogeneity. The $I^2$ values of 30%–60%, 50%–90%, and 75%–100% corresponded to moderate, substantial, and considerable heterogeneity, respectively, according to the Cochrane Handbook [46]. Forest plots displayed the results of the meta-analysis. Sensitivity analyses were also carried out omitting one study and calculating the summary OR, weighted mean difference with the 95% CI to investigate the influence of a single study on the final estimation. Publication bias was assessed by performing Egger's test and a funnel plot was utilized for visual evaluation [47]. A leave-one-out sensitivity analysis was performed by iteratively removing one

study at a time to confirm that our findings were not driven by any single study. Statistical analyses were performed with Stata 16 SE (Stata Corp).

## 4.2. Pyrolysis studies of cannabidiol (CBD) with Py-GC/MS

A 1 mg/mL CBD in methanol solution was purchased from Supelco (certified reference material, Cerilliant), and kept at –20 °C until analysis. The experiments were carried out in a gas mixture of 9.34% (n/n) oxygen and 90.66% (n/n) nitrogen or in a helium atmosphere.

The experimental method to simulate low-temperature tobacco heating conditions was developed in our earlier study [48]. This method was adopted and modified using various temperatures [49] to study the breakdown pattern of CBD; therefore, the experimental conditions are briefly described here.

Py-GC/MS analyses were performed using a Pyroprobe 2000 (CDS Analytical, Oxford, PA, USA) pyrolizer equipped with a platinum heating coil and a quartz sample tube. A total 15 µL aliquot of solution was dispensed in 5 µL portions onto a piece of quartz wool placed in the quartz tube and it was rested for 3 minutes at room temperature to allow evaporating the majority of the solvent after each portion. The quartz tube was placed in the Pyroprobe, at room temperature, which was then inserted into the preheated pyrolysis chamber. The temperature of the pyrolysis chamber was 280 °C, except for the pyrolysis experiments carried out at 250 °C, when the chamber temperature was also set at 250 °C. The pyrolysis chamber was flushed at a flow rate of 276 mL/min using the applied gas mixture. The sample was then heated at a maximum heating rate (set at 999 °C/s) to the final pyrolysis temperature. The experiments were performed at five different pyrolysis temperatures in a temperature range of 250–500 °C, using a 5 min isotherm period. Oxidative experiments were performed in a gas mixture of 9% oxygen and 91% nitrogen. To reveal the role of oxygen in the thermal degradation reactions, additional experiments were performed in a helium atmosphere, applying the same pyrolysis temperatures. The volatile products were purged on-line to a DB-1701 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) of the GC/MS (Agilent 6890 GC/5973 MSD) system. At the end of the pyrolysis, the pyrolysis gas flow was closed, and the helium carrier gas was supplied to the GC/MS. A solvent delay of 7 min was applied to protect the MS. The GC oven was programmed to an isotherm period of 7 min at 40 °C before increasing to 280 °C at a rate of 10 °C/min. The range of m/z 29–400 was scanned by the mass spectrometer in EI mode (70 eV). At least three parallel experiments were

performed at each temperature. Identification of the pyrolysis products was based on the combined Wiley Registry 9th edition/NIST 2011 mass spectral library and data from the literature [36-40]. The percentages of the compounds were estimated using the peak areas of the total ion current chromatograms.

## 4.3. Quantification of CBD content in food-supplements and hemp seed oils by UHPLC-UV methods

### 4.3.1. Chemicals, reagents, and analyzed products

Food-supplement samples were purchased from Hungarian online sources. The products included CBD-enriched oils, gelatine capsules containing CBD oil, e-cigarette liquid, and hemp seed oils. The total number of products analyzed was 27. The label of the analyzed samples was also evaluated in addition to cannabinoids and contained different ingredients and excipients. The characteristics of the samples are summarized in **Table S7**. The identification of the source or the products i.e. full-spectrum, broad-spectrum, distillate, were determined by the labeling and in some based on the attached handouts. All products were stored at +4 °C until sample preparation.

The solvents (i.e. methanol) used for sample preparation were analytical grade purchased from Sigma-Aldrich, St. Louis, Missouri, USA. HPLC grade water was in-house ultrapure by applying Direct-Q® 3 UV Water Purification System. The ammonium formate was of 97% purity and was purchased from ThermoFischer (Kandel, Germany). The analytical grade reference standard CBD was purchased from Cayman Chemical, Michigan, USA (item number 90080, batch number 0592969-115).

### 4.3.2. Sample preparation

For sample preparation 300 µL of the product was diluted to 10 mL with methanol. The gelatine capsules were cut open and an appropriate amount of liquid content was collected. The diluted samples were ultrasonicated for ten minutes at room temperature and subsequently diluted tenfold with methanol. Finally, the diluted products were filtered through a syringe filter with a polytetrafluoroethylene membrane (PTFE) (d = 13 mm, porosity: 0.45 µm, Natong FilterBio Membrane Co., Ltd. Nantong City, China) and immediately injected. The volume of the injected sample was 3 µL. All measurements were performed in triplicate.

### 4.3.3. UHPLC conditions

The experiments were carried out on a Shimadzu Nexera X2 UHPLC liquid chromatography system equipped with a vacuum degasser (DGU-20A5R), two binary

pumps (LC-30AD), a mixer assembly, an auto sampler (SIL-30AC), a column temperature controller (CTO-20AC), a diode array detector (SPD-M20A) and communication bus module (Shimadzu Corp., Kyoto, Japan). For separation, a Kinetex Polar C18 column (100 x 3 mm, 2.6 µm, Phenomenex, Torrance, CA, USA) reverse phase column was applied equipped with a guard column with the same packing material. The elution was carried out using 50 mM ammonium formate dissolved in water-methanol 9:1 (v/v) as mobile phase A and 50 mM ammonium formate dissolved in water-methanol 1:9 (v/v) as mobile phase B. The elution started with 19 min long isocratic step with using 75% mobile phase B, then the gradient was changed in 0.5 min to 100% of mobile phase B which was upheld for 1 min, finally the gradient was set up to initial 75% B in 0.5 min and for another 3 min to afford column equilibration for next injection. The total runtime was 24 min. The flow rate was set at 0.5 ml/min and the monitoring wavelength was set at 210 ± 10 nm. An injection volume of 3 µL was used.

#### 4.3.4. Validation of the UHPLC-PDA method

In this study, validation was applied to the quantitative analysis of CBD in food products and food-supplements. The analytical method was carried out following the Harmonized ICH Guideline [50] with further experiments. The analytical method validation included establishing the calibration curve for the CBD reference compound, assessing system suitability, linearity, determining the limit of detection (LOD) and limit of quantification (LOQ), recovery, precision, repeatability, intermediate precision, stability, and filter compatibility.

# 5. Results

## 5.1. Safety of dronabinol and nabilone

### 5.1.1. Literature search and study selection

Using the search terms dronabinol and nabilone for the literature search of EMBASE, PubMed, Web of Science databases and the Cochrane Central Register of Controlled Trials, and removing duplicates, the search yielded a total of 7859 potentially relevant reports. The included RCTs were selected according to the flow chart presented in **Figure S1**. After screening the titles, 192 publications remained and, by further screening the abstracts, 101 hits were retrieved for full-text screening, of which 82 RCTs were also excluded. The reasons for excluding articles were not clinical studies, not a placebo-controlled setting, missing or inappropriate data, and other study drugs than nabilone or dronabinol. Briefly, 26 papers were excluded since these did not report clinical trials, 22 trials were not placebo-controlled, 15 were excluded due to missing or inappropriate data, whereas in 22 trials other study drugs were used than nabilone or dronabinol. 19 RCTs were considered to be appropriate for quantitative analysis, [51–69] and 16 of these were included in the meta-analysis. Although three studies were considered for inclusion, the criterion of the minimal number of studies with the same outcome was not met in any of the outcomes reported; therefore, they were excluded from the meta-analysis. In 6 studies, nabilone was the study drug (**Table S2**), [55,57,59,62,66,67], while in 10 studies (**Table S1**), [51–54,56,60,61,63,65,69] dronabinol was used.

### 5.1.2. Risk of bias assessment

In general, the methodical quality of the trials included in our final quantitative analysis was considered good, mainly with a low or unclear risk of bias (**Figure 2**). None of the studies showed a high risk of selection bias. In nine studies, random sequences or codes were generated by computer programs [54,56,60,61,63,65–67,69]. Therefore, these studies were judged to have a low risk of selection bias. However, the remaining seven studies had unclear risk of selection bias, [51–53,55,57,59,62] because the authors failed to describe the methods used for randomization in detail. Based on the blinding of the personnel and participants, and making the interventions as identical as possible, nine studies were reckoned to have low risk of performance bias [51,52,54,60–63,65,66]. In the remaining studies, [53,55–57,59,67,69] it was not mentioned whether the intervention and the comparator were identical in size, shape, color, and odor. Moreover, the authors of four of these studies failed to describe precisely who exactly was blinded [55,57,59,69].

Ten trials had a low risk of detection bias [51,53,54,56,60,61,63,65–67]. In these studies, the evaluation of the outcomes was done in a properly blinded manner. However, six trials were judged to have an unclear risk of detection bias [52,55,57,59,62,69] because blinding of the outcome assessment was not described in detail, and it was unclear whether the person responsible for the assessment was blinded or not. Almost all studies showed a low risk of attrition bias. However, in one trial more than half of the enrolled patients did not complete the study;[69] therefore, this study was judged to have a high risk of attrition bias. In the study reported by Esfandyari et al., it is unclear whether there were patients lost during the course of the trial; therefore, the attrition bias of this study is unclear [53]. Furthermore, a relatively high proportion of enrolled patients did not finish the study of Malik et al., and the underlying reasons were not fully described, so this study also shows an unclear risk of attrition bias [52]. Six studies showed a low risk of reporting bias [53,61,63,65,67,69]. In four studies, not all the results were clearly indicated numerically; [56,60,62,66]these studies were considered to have an unclear risk of reporting bias. We identified several flaws, for example, inconsistency between the methods and the results section, missing results or p values, in six studies; therefore, these studies were considered to have a high risk of reporting bias [51,52,54,55,57,59]. Overall, all studies showed a low risk of other types of bias. Publication bias was assessed using Egger's test, and funnel plot was utilized for visual assessment. The number of studies allowed to perform this test only in case of headache in dronabinol studies. Inspection of the funnel plot and significance of Egger's test (p=0.015) revealed a small study effect in the case of this AE (**Figure 3**).

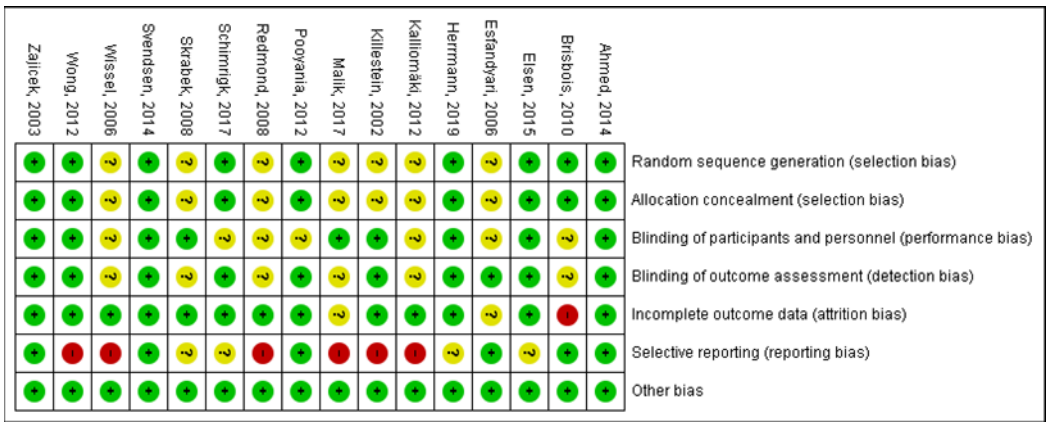


**Figure 2.** Table of biases

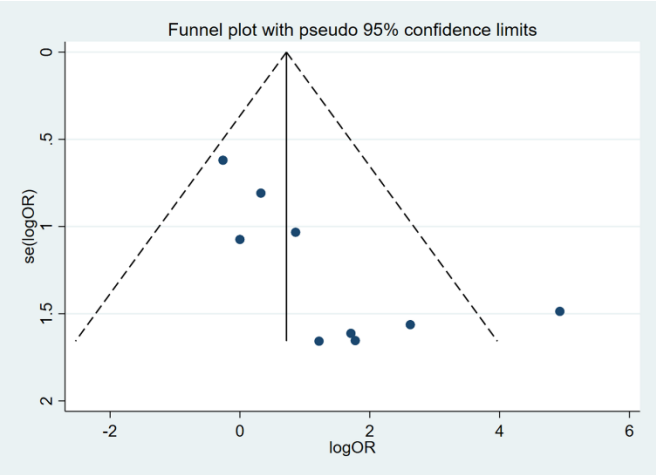


**Figure 3.** Funnel plot of the 95% confidence limit

### 5.1.3. Study characteristics

**Nabilone**

In the case of nabilone, 5 of the 6 included trials used a crossover design [55,57,59,66,67]. Clinical trials were carried out in Canada (n=4), [55,62,66,67] , the UK (n = 1) [59], and Austria/Germany/Switzerland (n=1) [57]. Nabilone was used to alleviate agitation in patients with moderate to severe Alzheimer's disease [66], spasticity in people with spinal cord injury [67], spasticity-related pain [57], fibromyalgia [62]. In two trials, the effects of nabilone on capsaicin-induced pain and hyperalgesia [59], and the analgesic and antihypertensive properties of nabilone on experimental heat pain were studied [55]. The duration of these studies was 1 to 9 weeks. The patients were 18–70 years old (mean age 22.5–50.1 years), except in a trial in which patients with Alzheimer's disease were included and the mean age of the patients was 87 years [66]. The applied dose ranged between 0.5–3 mg daily, in three trials 0.5–1 mg titration doses were used [59,66,67]. Altogether, 154 patients were enrolled and 129 completed the studies.

**Dronabinol**

Dronabinol was studied in 10 randomized, placebo-controlled trials, conducted in Canada (n=1) [69], Denmark (n=1) [63], Germany (n=1) [56], the Netherlands (n=3) [51,60,65], the USA (n=3) [52–54] and the United Kingdom (n=1) [61], and two of these trials were crossover [51–65]. The duration of the study ranged from 2 days to 16 weeks. In the case of one study, dronabinol was administered 4 times, with wash-out periods of 2 weeks [53]. 911 patients enrolled were 18–70 years old (mean/median age 26.0–72.1), and in some studies, only the mean age (46–79 years) was disclosed [51,60]. Data from 774 patients were evaluated. Daily doses of dronabinol ranged between 2.5–15 mg. In two

trials, the efficacy of dronabinol in the alleviation of neuropathic pain in patients with multiple sclerosis [56,63], and another trial focused on the efficacy and safety of the drug in patients with multiple sclerosis (MS) [51]. In one trial, the effect on gastrointestinal transit and postprandial satiation was studied in healthy human subjects [53], while in another trial the effect on gut transit was studied in patients with irritable bowel syndrome [54]. Malik et al. studied the efficacy in functional chest pain [52], van den Elsen evaluated the clinical effect of dronabinol on dementia-related neuropsychiatric symptoms [60], while the safety and tolerability of dronabinol were evaluated in older people [65]. One study aimed to determine if THC can improve taste and smell perception, appetite, caloric intake, and quality of life in cancer patients [69].

### 5.1.4. Outcomes

#### 5.1.4.1. Quantitative analysis –nabilone

In studies evaluating the effects of nabilone, 39 different adverse effects were reported (**Table S3**). These adverse effects were classified according to the International Statistical Classification of Diseases and Related Health Problems (ICD-10) and divided into three main categories [70]: AEs related to the central nervous system, cardiovascular system, and miscellaneous. 15 AEs were related to the central nervous system, while 5 affected the cardiovascular system. AEs were more frequent in the treated group than in the placebo group in both major types (68 vs 24 and 25 vs 6, respectively), and the same applies to the total number of AEs (228 vs. 61). Only 4 AEs (drowsiness, dizziness, headache, dry mouth) were reported in at least three studies and could be meta-analysed. Drowsiness was more than seven times more frequent in patients treated with nabilone than in the placebo group (OR: 7.25; 95% CI: 1.64–31.95, **Figure 4A**), while the risk of dizziness (OR: 21.14; 95% CI: 2.92–152.75, **Figure 4B**) and dry mouth was also higher (OR: 0.94; 95% CI: 0.19–4.72, **Figure 4C**) in the nabilone group. However, the frequency of headache was not different in the two groups (OR: 17.23; 95% CI: 4.33–68.55, **Figure 4D**).

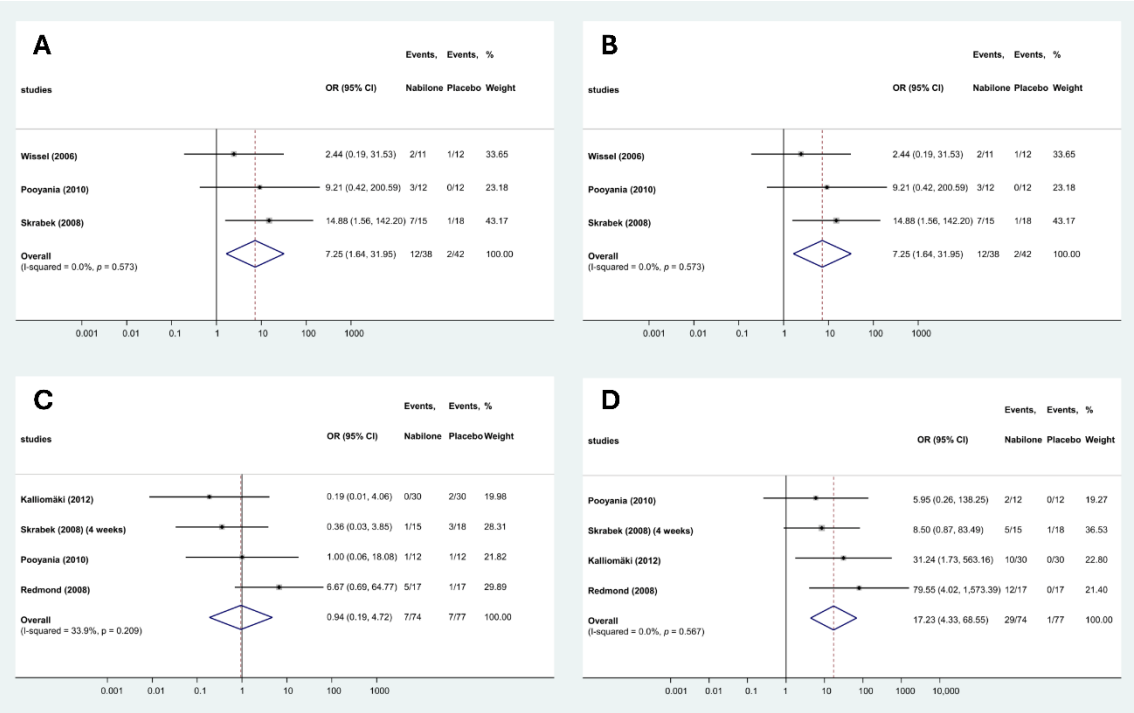


**Figure 4.** Forest plots of different AEs – nabilone

To evaluate the robustness of the results, we performed a leave-one-out sensitivity analysis for each AE by iteratively removing one study at a time and recalculating the summary OR. The summary ORs remained stable in the case of dry mouth and headache, indicating that our results were not driven by any single study. However, in the case of dizziness and drowsiness, no significant differences can be observed for frequency AEs when we leave out the results of Redmond et al. [55] or Skrabek et al. [62].

### 5.1.4.2. Quantitative analysis – dronabinol

In the analyzed clinical trials, 97 different AEs were reported (**Table S4**). These were classified according to ICD-10 and grouped as AEs that affect the central nervous system, respiratory system, musculoskeletal system, gastrointestinal system, urogenital system, and miscellaneous. The frequency of AEs was higher in these domains in the dronabinol-treated groups (46 vs 11, 5 vs 2 and 17 vs 6, respectively) except for AEs related to the gastrointestinal and urogenital systems. The overall risk of adverse events was higher based on the total number of recorded events (325 vs 142). Altogether, 6 individual AEs (nausea, drowsiness, dizziness, headache, fatigue, dry mouth) met the criteria for the meta-analysis. The frequency of dry mouth (OR: 5.58; 95% CI: 3.19–9.78, **Figure 5A**), dizziness (OR: 4.60 95% CI: 2.39–8.83, **Figure 5B**) and headache (OR: 2.90; 95% CI:

1.07–7.85, **Figure 5C**) was significantly higher in the dronabinol groups, while in the case of nausea, drowsiness and fatigue there was no such difference [(OR: 1.45; 95% CI: 0.38–5.43, **Figure 5D**), (OR: 3.77; 95% CI: 0.43–33.25, **Figure 5E**), and (OR: 2.00; 95% CI: 0.82–4.88, **Figure 5F**), respectively].

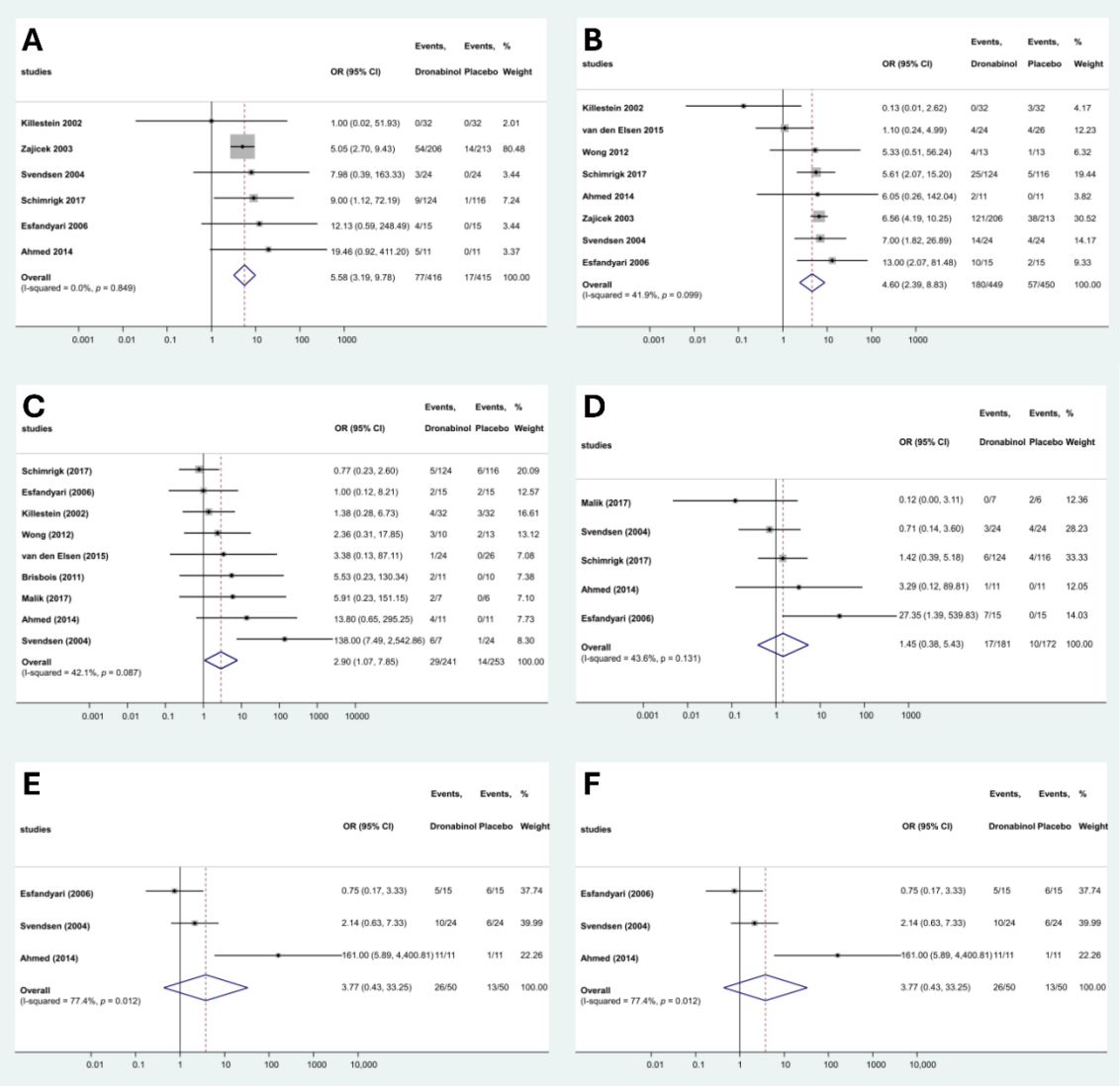


**Figure 5.** Forest plots of different AEs – dronabinol

Furthermore, sensitivity analyses by iteratively removing one study at a time showed similar and consistent results, indicating the robustness of our findings, except for headache, where in the case of removal of the results of Brisbois et al. [66] or Svendsen et al. [63] or Malik et al. [52] or Ahmed et al. [65], the risk of AEs in groups treated with dronabinol or placebo was not significantly different.

#### 5.1.4.3. Qualitative analysis of excluded studies

Although three randomized controlled studies were excluded from the meta-analysis, the results of these may also contribute to the overall picture of the AE profile of nabilone and dronabinol. One trial was left out because the number of studies reporting specific AEs was not sufficient to prepare a meta-analysis [58], while one clinical trial was excluded due to inadequate reporting of AEs (using general terms instead of specifying AEs) [64], and in one study, the numbers of different AEs were combined and could not be evaluated separately [68]. The study of Beaulieu reported the use of nabilone (1 and 2 mg) in patients with postoperative pain compared to placebo and ketoprofen (n=41). The incidence of nausea and vomiting, sleep quality, euphoria, sedation, pruritus, and mood were not different between the study groups. Sedation scores were higher in the 2 mg nabilone group compared to the ketoprofen group, and although euphoria was not significantly different between the four groups, it was more frequent in the nabilone groups [58]. In the case of dronabinol, two studies were left out. Van den Elsen et al. evaluated the efficacy and safety of 1.5 or 3 mg of dronabinol compared to placebo in patients with dementia suffering from neuropsychiatric symptoms in a crossover trial. 184 mild to moderate AEs were recorded, which were distributed similarly in the THC (91 AEs) and placebo (93 AEs) groups. There was no increase in the incidence of AE after administering higher doses of dronabinol [64]. Zajicek et al. conducted a study with patients with progressive primary or secondary multiple sclerosis (n=498). Patients received dronabinol (titrated against body weight and AEs, maximum dose 28 mg daily) or placebo for 36 months. 35% of patients who received dronabinol had at least one serious AE compared to 28% of patients who received placebo. The number and nature of serious AEs did not differ significantly between these 2 groups [68].

## 5.2. Transformation of CBD during pyrolysis

Depending on the level of filling of the e-liquid, the resistance of the coil, and the voltage settings, the coil temperatures of e-cigarettes range from 110 to 1008 °C [71,72]. Typical wetted coil temperature is 200–400 °C, extremely high temperatures (around 1000 °C) have been measured for dry coils without any e-liquid [73]. In our experiments, we studied pyrolysis in the typical operating temperature range of e-cigarettes (250–500 °C) under both inert and oxidative conditions.

The composition of CBD pyrolyzates obtained in inert and oxidative atmospheres are summarized in **Table S5** and **Table S6**, respectively. The data obtained clearly present

the thermal instability of CBD. Depending on temperature and atmosphere, 25% to 52% of CBD transformed into other chemical substances during the experiments. In the absence of oxygen, 23 pyrolysis products were observed, 15 of which were identified (**Table S5**). These compounds represented 81–27% of the pyrolysis products (at lower temperatures, between 250–400 °C, 93–97%). In the presence of oxygen, 22 pyrolysis products were detected (**Table S6**), the 15 identified components represent the 88–96% of the degradation products in the studied temperature range. Most of the degradation products (i.e. 18 compounds) appeared in both inert and oxidative conditions. Additional four products were observed exclusively in the oxidative atmosphere, while five degradation products were observed exclusively in the inert atmosphere.

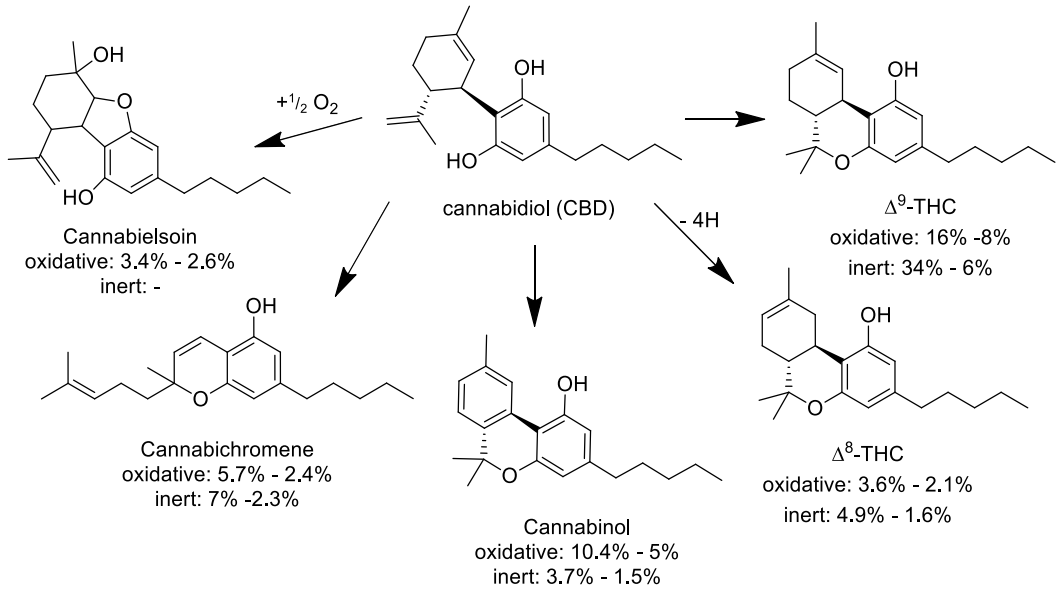


**Figure 6.** The major thermal decomposition routes of CBD

The four most intense products, namely $\Delta^9$-THC, $\Delta^8$-THC, cannabichromene and cannabinol, represent more than 95% of the decomposition products at pyrolysis temperatures of 250 and 300 °C in an inert atmosphere. Under oxidative conditions, an additional product, cannabielsoin, appeared. The ratio of the aforementioned five decomposition products is more than 80% under oxidative conditions up to 300 °C. All of these compounds formed by the cyclization reaction (**Figure 6**). The cyclization of phenolic flavors to bicyclic compounds under simulated tobacco heating conditions at 300 °C was previously reported [74]. The phenolic O of thymol or ethylvanillin is linked to a geometrically favorable position of a sterically adjacent side group of the molecule,

forming a bicyclic compound. Analogously, in the present case, one of the phenolic O of CBD was linked to the tertiary carbon of the isopropenyl group, thus forming a sterically favored six-membered ring and the resulting $\Delta^9$-THC molecule. However, $\Delta^9$-THC formed also under inert atmosphere. The related cyclization can be formally described as an intramolecular Markovnikov addition of phenolic OH onto the double bond of the isopropenyl group. Therefore, no oxidation step is needed for the formation of $\Delta^9$-THC. The psychoactive $\Delta^9$-THC was the main compound detected, accounting for up to 42% and 70% of the decomposition products under oxidative and inert conditions, respectively, at all temperatures applied. One possible reason for the lower $\Delta^9$-THC amount in the oxidative atmosphere measured in our study could be the higher decomposition rate of the formed $\Delta^9$-THC in the oxidative ambient. An increased rate of $\Delta^9$-THC was published in a cannabis resin sample exposed to air compared to that stored in a sealed plastic bag at ambient temperature [72], indicating the role of oxygen in $\Delta^9$-THC decomposition.

Among THC isomers, $\Delta^8$-THC has also psychotropic effects according to the recent review of the Expert Committee on Drug Dependence of the World Health Organization (WHO) [76]. The $\Delta^8$-THC molecule formed by an additional isomerization of cyclization during thermal treatment. Both $\Delta^9$-THC and $\Delta^8$-THC were formed at a higher rate in an inert atmosphere and at relative lower temperatures (250–300 °C). By increasing the temperature, the relative yield of THC decreased, while other decomposition reactions became more pronounced.

According to previous studies, cannabinol is derived by the cyclization, dehydration, and aromatization of CBD, probably through a THC intermediate during long-term storage [77]. In the present study, a notable amount of cannabinol was detected as a thermal degradation product of CBD. The relative amount of cannabinol was significantly higher under oxidative conditions at each temperature studied, indicating the effect of oxygen in the reaction mechanism. In addition to the driving force of aromatization in both atmospheres, dehydrogenation by oxygen with the elimination of water may play an additional role in the oxidative atmosphere. The most intense formation of cannabinol (10.4%) was observed at 400 °C in an oxidative atmosphere.

Cannabichromene also formed through cyclization reaction. However, one of the phenolic O of the CBD molecule attacked the double bond of the cyclohexene ring at C substituted with methyl group in this case, while the cyclohexene ring opened up by C–

C scission to form the chromene frame. Cannabichromene formation was more pronounced in an inert atmosphere compared to the oxidative condition at 250 °C. At higher temperatures, there were no significant differences, and the relative yield of cannabichromene was decreased.

Cannabielsoin was only detected under oxidative conditions. In this transformation, one of the phenolic O of CBD was similarly linked to the double bond of its cyclohexene ring, but in the secondary carbon, while a hydroxyl group formed in the adjacent C group that was substituted with the methyl group via the oxidative medium. The relative intensity of cannabielsoin was around 3% and its quantity was not much affected in the temperature range of 250–500 °C.

At higher temperatures, the share of the decomposition products formed through the cyclization reaction decreased, while the relative intensity of smaller molecules formed by C–C bond scission increased in the pyrolyzate. These identified products were formed by scission of the bond that connects the pentylbenzenediol and the p-mentha-1,8-dienyl moieties of the CBD molecule. Menthatriene isomers appeared at 250 °C in an oxidative atmosphere, while in an inert atmosphere they appeared only above 400 °C, indicating that the presence of oxygen promoted the cleavage of the molecule. The relative amount of menthatrienes was the highest at 500 °C in both inert and oxidative atmospheres.

## 5.3. Quantification of CBD content in food-supplements and hemp seed oils by UHPLC-UV method

### 5.3.1. Label analysis of food products and food-supplements

Twenty-seven products were included in the analysis. The characteristics of the products are summarized in **Table S7**. Eighteen products (CB1–CB7, CB8, CB9, CB16–CB27) were oils enriched with CBD, one product (CB15) was a gelatine capsule, one product (CB6) was an e-cigarette liquid, and five products (CB10–CB14) were hemp seed oils. Because of the poor description of the ingredients the chemical characterization of CBD or hemp extract could not be identified from labels. Sixteen products contained some kind of hemp extract, namely hemp flower extract (CB2), phytocannabinoid extract (CB1) various supercritical extracts (CB3, CB5, CB16, CB18, CB22), hemp seed extract (CB5), alcoholic extract (CB6), hemp extracts (CB8, CB15, CB19, CB25, CB26), CBD hemp extract (CB24), and full-spectrum plant extract (CB21), cannabidiol hemp extract (CB23). The less defined extracts were in products CB23and CB24 (cannabidiol and

CBD extract), phytocannabinoid extract (CB1), hemp seed extract (CB5), alcoholic extract (CB6), hemp extracts (CB8, CB14, CB19, CB25, CB26), and full-spectrum plant extract (CB21). Oils were added to the products i.e. MCT oil, hemp seed oil, hemp oil, walnut oil, linseed oil, poppyseed oil, olive oil, grape seed oil, orange oil, and coconut oil (products CB1–CB5, CB8, CB9, CB14–CB17, CB19–CB27), or hemp seed oil was the only declared ingredient in five products (CB7 and CB10–CB14). The e-cigarette liquid contained glycerol, propylene glycol, terpenes beside CBD. The claimed CBD content was in the range of 10–50 mg in mL of oil or in one piece of capsule, and no CBD content was highlighted on the label of six products (CB7, CB10–CB15).

### 5.3.2. Validation

The goal of our work was to set up a properly validated method in order to reliably measure the cannabinoid content of the investigated food-supplements. A crucial part of our work was the development of an analytical method supported by proper validation and analytical investigation. The investigation included the analysis of CBD-enriched food-supplements from various sources along with preferable sample preparation.

Calibration curve and linearity

One of the major CBD cannabinoid markers was chosen to be analyzed and quantified. The calibration curve was adjusted to the 6 calibration points (**Figure 7**). The regression equation was y = 17205893410.02x – 335045.50 with correlation coefficient 0.9997. The calibration curve covered the range of 0.03–1 µg/injection. The values of LOD (0.02935 µg/injection) and LOQ (0.08895 µg/injection) were determined. The calibration curve covered two orders of magnitude of analyte concentration.

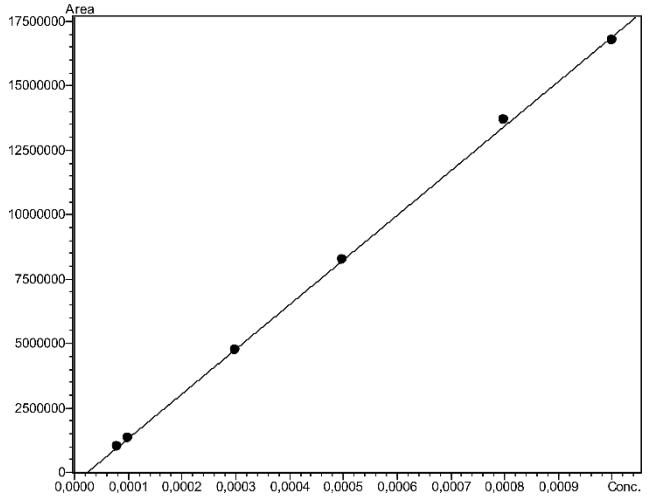


**Figure 7.** Calibration curve for CBD. In the graph the injected amount of CBD (µg/injection) vs AUC is presented.

During the validation process the filter compatibility was determined using PTFE filter of 0.45 μm and 1.74% decrease of the investigated analyte was observed. The analyte seemed to be stability based on *stability* test: the sample stored at –20 °C and injected on days 0, 1, 5, and 7 resulted in on the first day 98.24%, on the fifth day 102.93, and on the seventh day 99.74% of CBD analyte compared to 0-day measurement. Based on these results, the maximum analyte reduction was 1.76%, thus the storage time did not affect the CBD content. The *system suitability* proved this method to be suitable for measurement (low RSD values of the area under the curve (AUC RSD% = 0.27%), retention time (Rt RSD% = 0.17%) and the tailing factor range (1.066–1.089) calculated from five injections). Accuracy was evaluated based on recovery of CBD analyte using product sample CB24. The recovery values for the concentration levels of 50, 100, and 150% were 95.2–99.0% (RSD% = 3.24%), 99.78–100.42% (RSD% = 1.13%), and 96.64–99.32% (RSD% = 0.48%), respectively. Injection of CB24 for ten times afforded the precision assessment of the system: the RSD% of AUCs was 3.89%. The repeatability based on CB24 analysis for six times, the RSD% for CBD value was 1.73%. The intermediate precision was determined by performing sample preparation by two chemists and evaluating the RSD% of obtained results, which was 4.01%.

### 5.3.3. Quantitative analysis of the CBD content

CBD was detected in products according to retention time and UV absorption (**Figure 8**). The quantification of CBD was performed using an external calibration curve. The CBD content of the products was summarized in **Table S7** and presented graphically in **Figure 9a**. The UHPLC chromatograms of the analyzed products are presented in **Supplementary Figures S2–S28.**

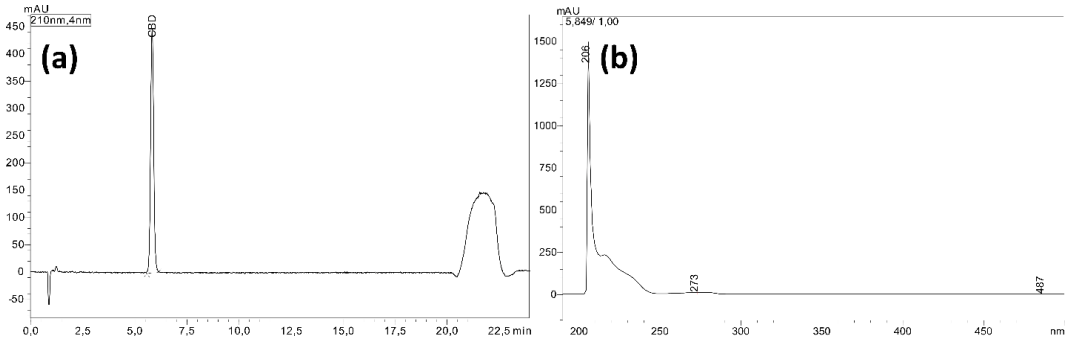


**Figure 8.** UHPLC chromatogram recorded at 210 nm (a) and UV absorption (b) of CBD.

In five products (CB7, CB10–CB14) CBD could not be detected. Although e-cigarette liquid (CB6) and in gelatine capsule (CB15) CBD was present; however, the content of

analyte was below the limit of quantification. In products CB1–CB5, CB8, CB9, CB16–CB27 the CBD content ranged from 12.87 to 54.09 mg/mL. Based on label analysis, the label accuracy was evaluated and classified as under-, accurately-, and over-labeled with detected CBD concentrations <90%, 90%–110%, and >110% of the labelled value, respectively. Information about the CBD content could not be found on the label of products CB7, CB10–CB15, therefore, the accuracy of the labeling could not be assessed. Two products were under-labeled (CB3 and CB24), nine products were over-labeled (CB1, CB4–6, CB8, CB17, CB21–CB23), while eight products were accurately labeled (products CB2, CB9, CB16, CB19, CB20, CB25–CB27) (**Figure 9a**).

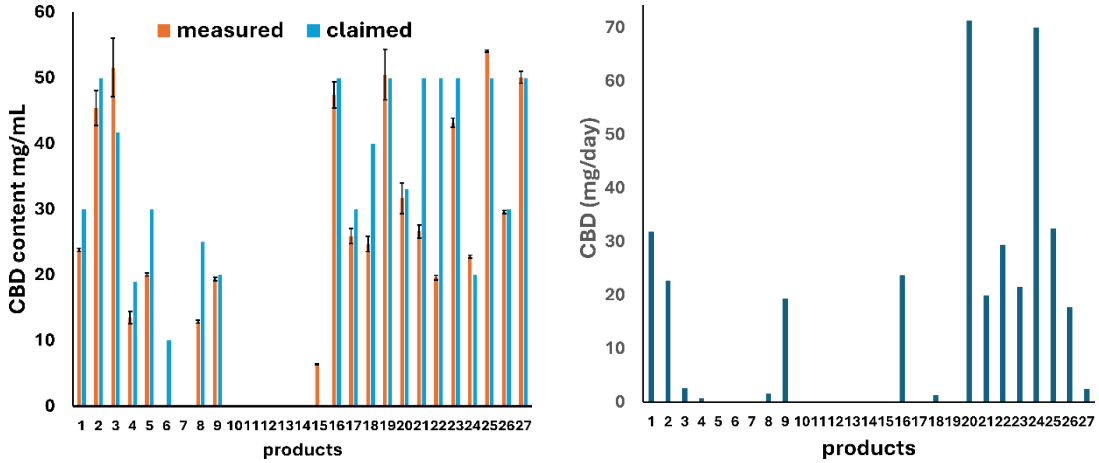


**Figure 9.** Declared and quantified CBD content of the product (a) and the daily CBD intake recommended by the manufacturers of the products (b). Values are presented as mean ± SD (n=3).

The daily intake of CBD was calculated according to the recommended daily consumption of the product (considering 1 drop equal to 0.05 mL) and the measured CBD concentration. The daily CBD intake was 1.24–71.26 mg (**Figure 9b**).

# 6. Discussion

*Cannabis sativa* L. has long been known to people of all kinds. The application of the plant or its extracts vary in terms of safety and tolerability. The pure compounds of *Cannabis* have different effects depending on the molecular mechanism and individual sensitivity.

In the 1960s, cannabis was widely used as a cigarette, which made it relevant to study the pyrolysis of cannabinoids. Based on their results Mikes and Waser hypothesized the cyclization of CBD into THC [78]. Due to the complexity of the plant, performing experiments with the pure compound seemed like a suitable way to test the hypothesis. According to the results of Küppers et al. the pyrolysis of CBD at 700 °C resulted in many pyrolysis products including THC [79,80]. Spronck et al. further investigated the pyrolysis of CBD in several articles [81–84]. The focus of their work was on the isolation of potentially bioactive olivetol derivatives formed during the pyrolysis of CBD. Nowadays, the investigation of the pyrolysis of cannabinoids is relevant because CBD is widely used in e-cigarettes, and previous studies have not analyzed the rate at which this compound is converted to other cannabinoids, such as the psychotropic THC. In our experiments, the mimicked environment was comparable with the one used in electronic cigarettes but did not exactly model the accurate chemical reaction mechanism. The most significant result was the demonstration that CBD is converted to THC in significant proportions by heat treatment, which sheds a special light on the use of CBD in e-cigarettes. THC is a known illegal substance that can lead to abuse and addiction. It has many well documented adverse effects ranging from dysphoria, hallucinations, and paranoia to milder form such as confusion, headache, euphoria etc. [85]. It especially needs to be addressed for people who are unaware of the possible consumption of THC while they are driving or working with heavy vehicles [86]. On top of that, the amount of CBD that is not transformed during pyrolysis creates another type of threat. In their article, Orvos et al. investigated the electrophysiological aspects of CBD both *in vitro* and *in vivo*. In the potential cardiovascular risks connected to CBD, hERG, and $I_{Kr}$ channels play an important role. The application of CBD-containing e-cigarettes without control may lead to an increased blood plasma level of CBD, thus it can trigger possible proarrhythmic adverse events. The occurrence of possible adverse events, based on the scientific results, may be especially high in people with impaired CBD metabolism and/or if the repolarization reserve is weakened [5].

In addition to its use as a recreational drug, *Cannabis sativa* has become increasingly important as a medicinal plant in recent decades, and its compounds and their derivatives are also marketed as medicines. The experience gained from the illegal use of the plant and the wide range of pharmacological effects of cannabinoids justify the question of whether the safety of cannabinoid-based medicines can be considered acceptable. The efficacy of nabilone and dronabinol has been confirmed in several clinical trials and meta-analyses [87,88]. However, data on safety and AEs are also necessary for the assessment of risk-benefit ratios. We presented the results of the first systematic review and meta-analysis on the AE profiles of nabilone and dronabinol based on the results of randomized, double blind, placebo-controlled trials. In case of nabilone, four AEs were meta-analyzed. Drowsiness, dizziness, and dry mouth were more frequent in the patients treated with nabilone than in the placebo group, whereas the frequency of headache was not different in the two groups. In patients treated with dronabinol, more adverse effects could be meta-analyzed. The frequency of dizziness, dry mouth and headache was significantly higher in the dronabinol groups, whereas in case of nausea, drowsiness, and fatigue no significant difference could be observed. Dizziness and dry mouth are common in case of the application of both pharmacons. The adverse effects discussed are diverse, but not severe. In the analyzed clinical trials, 40 different adverse effects were reported for nabilone and 111 for dronabinol; however, the majority of these was not recorded in at least 3 trials that would be sufficient for meta-analysis. In case of radiotherapy-induced nausea and vomiting, international guidelines recommend the use of serotonin receptor antagonists (e.g. granisetron, ondansetron, tropisetron) and dexamethasone as prophylaxis [89]. In case of chemotherapy-induced nausea and vomiting, the recommendations are more diverse; however, serotonin receptor antagonists and dexamethasone are the most commonly used medications [90]. The long-term use of dexamethasone is related to several adverse events, whereas in case of serotonin receptor antagonists the most frequent adverse effects are headache, constipation, weakness and somnolence [91]. Although the side effect profiles of cannabinoids have not been clinically compared with the therapies recommended by guidelines, based on the available evidence, the risk-benefit ratio of cannabinoids does not seem to be inferior. Further, high-quality trials of appropriate patient size, examining the adverse effects of dronabinol or nabilone with comparable and more uniform endpoints would allow to assess the safety profile of these compounds with a lower risk

of bias. Moreover, a considerable number of trials reporting the same or similar adverse effects that can easily be grouped and that are related to different doses of these drugs would enable the assessment of the dose dependency of the adverse effects.

Cannabinoids are used not only as medicines but also as food-supplements. The most popular is CBD which is promoted for its many medicinal properties, however, only a few applications are evidence-based. The vast majority of products with CBD content designed for oral administration belong to unregulated products. Due to a lack of information on the long-term effects of CBD consumption and reliable data on CBD toxicity, authorities chose a restrictive position by not recommending these products for consumption. Uncertainties regarding toxicity and CBD-related adverse effects are further supported by controversial data reported by recent clinical trials. 62% (n=26/42) of clinical studies, that applied 50–4,500 mg/day CBD, reported mild to moderate events; however [92–117]. At higher applied dose (≥750 mg CBD/day), elevated liver enzyme levels, respiratory and cardiovascular conditions prone to infections, have been reported more frequently than at lower doses [94,95,102,106,107]; however, other clinical studies with the same doses have not observed these unwanted events [100,104,105,111,115,118–124]. The reported data support worries of the FDA warning letters claiming that a safe dose of CBD has not been established yet [125].

Another possible risk source for CBD-containing food-supplements or foodstuffs could be the uncertainty about the composition of products. Generally, there are three major types of CBD-preparations: full-spectrum CBD, broad-spectrum CBD, and CBD isolate products. Full-spectrum CBD is a raw extract obtained from *Cannabis sativa,* which in addition to CBD contains other cannabinoids, including THC and other plant metabolites. Broad-spectrum CBD is THC-free, whereas CBD isolate contains only cannabidiol. There is no clear chemical characterization of full-spectrum and broad-spectrum CBD; therefore, the concentration of other phytocannabinoids remains unclear in the products [126]. The risk of non-psychoactive phytocannabinoids in these products, besides CBD, is also unknown, since there are no data on their toxicity or safety in long-term consumption. A study that examined THC contamination of unregulated CBD-containing products revealed that THC concentration levels were in the range of 5–500 µg/mL in 24 products, 500–1,000 µg/mL in 17 products, and more than 1 mg/mL for 11 products [127]. In Korea, hemp seed oils have been investigated and 0.3–19.73 µg/mL of THC and 6.66–63.40 µg/mL of CBD content have been reported [128]. Consumption of

THC-free labeled products might lead to unintentional exposure to THC and related side effects.

The CBD content of various products has been analyzed by several research groups to determine the accuracy of the labeling. An analysis carried out in South Africa revealed that only 7.5% of the investigated products (n=3/40) had a CBD content in the range of 90% to 110% of their claim on the label, while two oils were under-labeled with +27.48% and +49.42% CBD content, respectively [129]. In the United States, 84 products have been purchased online. Quantification of the CBD content of these products revealed that 30.95% of the analyzed products were accurately labeled (n=26/84) and 42.85% contained more CBD than claimed on the label (n=36/84) [130]. Another study conducted in the United States found that 89 of 105 products had information on CBD content. An analysis of these 89 products found that only 24% have been accurately labeled (n=21/89) and 58% (52/89) of the products have been under-labeled [131].

The CBD content of the products analyzed by our research group ranged from 6.40 to 54.09 mg/mL. The labels of nineteen products highlighted the amount of CBD in the product. Eight of nineteen products (42%) were accurately labeled, whereas two were under-labeled, and nine were over-labeled (58%). The accuracy of the labeling is in accordance with data reported by international screenings. Considering the recommended daily doses listed on the product labels, the minimum daily dose of CBD was 0.68 mg and maximum was 71.26 mg. Based solely on these data, severe adverse effects should not be expected when using these products. However, the labeling of the products did not clearly define the type of active substance (i.e., full-spectrum, broad-spectrum, CBD isolate), so the presence and risks related to other cannabinoids could not be excluded. The hemp seed oils analyzed, which were clearly and undoubtedly marketed as vegetable oils, without any indications, appeared to be cannabinoid-free. This fact might suggest that the raw material used for oil production was not contaminated with other hemp organs.

There are identified problems in the regulation of dietary supplements that go beyond the shortcomings in the regulation of e-cigarettes, and our research has confirmed this. The loose control over food-supplements in the EU and in Hungary brings along the appearance of poor quality or not adequately inspected products. The safety of consumers is an important goal in the research of product analysis. Performing a quantitative analysis of a bunch of CBD-containing products in Hungary suggests that the quality of the

products vary. Labeling accuracy tends to have a large deviation. The analysis of the purchased products shows a surprising picture that relates the prices of these products and the illustrated beneficial claims on the websites of the distributors. The developed analytical method combines the features found on various websites of column manufacturers and data found in the analytical literature of the field. Therefore, it has the potential for easy applicability, robustness, and reliability. UV detection limits the quantification of minor compounds in the analyzed products. From this point of view, for the analysis of minor compounds, a coupled instrument might count as a better choice.

Our applied UHLPC-UV analytical method was suitable for our investigations of CBD-containing food-supplements and hemp oils. Based on the review of Nahar et al. [132] in which they conclude that LC-based methods have dominated the field of cannabinoids analysis. Our findings also resonate with the data found in the publication of Siddiqui et al. [133], thus we can agree that the study of CBD regarding consumer product marketing, medical use, side effect profile etc. is extremely important. The conclusion is that further analytical research and a better understanding of consumer attitudes can lead to safer applications and improved analytical methods towards quality control. Our findings suggest that analyzed hemp seed oils are free of phytocannabinoids. The analyzed food-supplements were mostly not accurately labeled and without a clear description of the origin of CBD and possible phytocannabinoid content. Our study provides further evidence that the safety issues of CBD-containing food-supplements must be addressed by authorities, and restrictive regulations are justified.

# 7. Conclusions

Based on the performed studies and experiments, several new scientific results could be concluded. One benefit of the dissertation is a new addition to the therapeutic use and validity of cannabinoid-derived medicines. Such statistical evaluation makes a proper ground for health care providers to make evidence-based decisions during therapy planning in terms of adverse effect profile, which contributes to the better compliance between patients and health care professionals. Another added value of the dissertation is the better understanding of the pyrolysis chemistry of cannabidiol (CBD) used in e-cigarettes. There is limited number of studies investigating the safety and toxicology of pyrolysis products, especially, regarding CBD and other cannabinoids. Our findings can be a starting point for further pharmacological studies on minor cannabinoids and pyrolysis compounds. Finally, our UHPLC-UV validated method could easily be applied by laboratories interested in cannabinoid containing food-supplement analysis. Concerning the results of the tested food-supplements, we can get a genuine picture of the quality of online available products. Thus, legitimate information could be provided to a broader audience. The attention of authorities, consumers and scientists could be attracted to an already popular topic of CBD, supported with the highlight of proper quality control issues, safety risks and potential health hazards.

The major new scientific results presented in this thesis are the following:

- statistical evaluation of safety data from randomized clinical trials with cannabinoid-derived medicines i.e. dronabinol and nabilone, which gives a realistic picture of the safety of the two compounds based on clinical data;
- successful identification of pyrolysis compounds formed during the pyrolysis of CBD;
- validation of UHPLC-UV method for the testing of food-supplements containing CBD;
- content testing and quantification of CBD in food-supplements.

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# SUPPLEMENTARY MATERIAL

# SUPPLEMENTARY MATERIALS – TABLES

**Table S1.** Summary of dronabinol studies

| First author, year | Country | Posology | Duration | Enrolled patients | Patients who have completed the trial | Mean age [yrs (SD)] | Sex [M/F (N)] | Outcomes | Reported adverse events |
|---|---|---|---|---|---|---|---|---|---|
| Malik, 2007 | USA | 5 mg bid for 4 weeks | 4 weeks | 19 | 13 | placebo: 42 (ND)<br>active: 44 (ND) | 2/11 | Effect of dronabinol on pain threshold, frequency, and intensity in functional chest pain (FCP) | Loose stools, nausea, headache, fatigue |
| Schimrigk, 2017 | Germany | titration to daily doses 7.5–15.0 mg | 16 weeks | 240 | 169 | placebo: 47 (9.7)<br>active: 48.4 (9.6) | 65/175 | Positive risk-benefit ratio of dronabinol<br>in the treatment of neuropathic pain in MS patients | Insomnia, Nausea, Dizziness, Vertigo, Headache, Fatigue, Dry mouth |
| van den Elsen, 2015 | The Netherlands | 1.5 mg tid for 3 weeks | 3 weeks | 50 | 50 | placebo: 78 (7)<br>active: 79 (8) | 25/25 | Efficacy and safety of THC<br>in the treatment of dementia-related neuropsychiatric symptoms (NPS) | Delirium, Cognitive disorder, Euphoric mood, Bradykinesia, Somnolence, Agitation, Nasopharyngitis, Pneumonia, COPD, Back pain, Muscle weakness, Muscle spasms, Pain in extremity, Renal impairment, Urge incontinence, Dry eye, Eye haemorrhage, Miosis, Balance disorder, Chest pain, Skin disorder, not otherwise specified, Dizziness, Sensory loss, Restlessness, Aphasia, Apraxia, Headache, Fatigue, Malaise, Presyncope, Syncope, Decreased appetite, Increased gamma-glutamyl transferase, |
| Ahmed, 2014 | The Netherlands | 3–6.5 mg | 6 weeks | 12 | 11 | placebo & active: 72.1 (5) | 6/6 | Safety and tolerability effects of THC in elderly | Euphoria, Concentration problem, Visual hallucination, Relaxation, Dry eye, Blurred vision, Nausea, Coordination disturbance, Drowsiness, Dizziness, Headache, Malaise, Dry mouth |

| First author, year | Country | Posology | Duration | Enrolled patients | Patients who have completed the trial | Mean age [yrs (SD)] | Sex [M/F (N)] | Outcomes | Reported adverse events |
|---|---|---|---|---|---|---|---|---|---|
| Wong, 2012 | USA | 2.5 or 5 mg bid | 2 days | 36 | 36 | placebo: 36.7 (3.1)<br>active (2.5 mg): 47.7 (7.9)<br>active (5 mg): 42.3 (4.5) | 2/34 | Gut transit in IBS-D and dronabinol' transit effect | "Loopy", foggy thinking, hot flushes, drowsiness / discomfort, dizziness / lightheadedness, headache |
| Brisbois, 2011 | Canada | 2.5 mg bid (patients had the option to increase their drug dose to a maximum of 20 mg/day) | 3 weeks & 1 day | 46 | 21 | placebo: 65.5 (8)<br>active: 67 (10.9) | 12/9 | Effects of THC on Chemosensory Perception | Confusion, Seizure, Troubles sleeping, Pneumonia, Thrush, Stomach cramps, Bowel obstruction/constipation, Diarrhea, Vaginal discharge, Unsteady feet, Shortness of breath/ fluid on lungs, Nausea/Vomiting, Hives/Rash, Fever, Headache, Pain, Tired/Drowsy, Oedema, Low blood count |
| Esfandyari, 2006 | USA | 5–7.5 mg bid | 2 days | 30 | 30* (27)<br>* 3 patients did not complete the study; however, their missing data is included in the ITT analysis | placebo: 29 (1)<br>active: 26 (2) | 14/16 | Effect on dronabinol of gastrointestinal transit and postprandial satiation | Excitement, Euphoria/Relaxed, Disturbed mental concentration, Nausea, Numbness, Flushing, Drowsiness, Dizziness / Light-headedness, Headache, Vasovagal, Dry mouth |
| Svendsen, 2004 | Denmark | titration to 5 mg bid | 3 weeks | 24 | 24 | placebo & active: 50 (median) | 10/14 | Effect of dronabinol on central neuropathic pain in MS patients | Euphoria, Feeling of drunkenness, Speech disorders, Hyperactivity, Nervousness, Aggravated MS, Migraine, Sleep difficulty, Upper airway infection, Muscle weakness, Myalgia, Hot flushes, Diplopia, Balance difficulty, Palpitations, Abdominal pain, Nausea, Drowsiness, Dizziness, Fever, Headache, Fatigue, Anorexia, Weight decrease, Dry mouth, Chills |

| First author, year | Country | Posology | Duration | Enrolled patients | Patients who have completed the trial | Mean age [yrs (SD)] | Sex [M/F (N)] | Outcomes | Reported adverse events |
|---|---|---|---|---|---|---|---|---|---|
| Zajicek, 2003 | UK | 2.5 mg | 15 weeks | 419 | 404 | placebo: 50.9 (7.6) active: 50.2 (8.2) | 141/278 | Effect of cannabinoids on spasticity and other symptoms in patients with MS | Bladder, Depression of anxiety, Dizzy of light-headedness, Dry mouth, Gastrointestinal tract, Improvement in symptoms, Infection, Miscellaneous, Numbness of paraesthesia, Pain, Sleep, Spasms of stiffness, Tremor of lack of coordination, Vision, Weakness of reduced mobility |
| Killestein, 2002 | The Netherlands | 2.5–5 mg bid | 4 weeks | 16 | 16 | placebo & active: 46 (7.9) | ND | Efficacy, safety, and tolerability effects of THC in MS patients | Emotional lability, Ataxia, Somnolence, Increased spasticity, Dizziness, Headache, Dry mouth, Other |

F: female, M: male, ND: no data, yrs: years, bid: twice a day, tid: three times daily.

**Table S2.** Summary of nabilone studies

| First author, year | Country | Posology | Duration | Enrolled patients | Patients who have completed the trial | Mean age [yrs (SD)] | Sex [M/F (N)] | Outcomes | Reported adverse events |
|---|---|---|---|---|---|---|---|---|---|
| Hermann, 2019 | Canada | 1-2 mg once a day | 14 weeks | 39 | 33 | placebo & active 87 (10) | 30/9 | Efficacy and safety of nabilone for agitation with moderate to severe Alzheimer's | Sedation (including lethargy, treatment limiting sedation, significant increase in NPS, myocardial infarction, bradycardia, rash, dizziness, lethargy |
| Kalliomäki 2012 | UK | 1–3 mg | 7 weeks & 5 days | 30 | 24 | placebo & active: 29.3 (no data) | 30/0 | Effect of nabilone on capsaicin-induced pain and hyperalgesia and on other CNS biomarkers | Somnolence, Postural dizziness, Tachycardia, Bradycardia, Dizziness, Headache, Fatigue, Dry mouth |
| Pooyania, 2010 | Canada | 0.5 mg once or bid | 10 weeks | 12 | 11 | placebo & active: 42.36 (no data) | 11/0 | Alleviation of spasticity in patients with spinal cord injury (SCI) | Ataxia, Drowsiness, Vertigo (mild), Lack of motivation, Headache, Asthenia, Dry mouth |

| First author, year | Country | Posology | Duration | Enrolled patients | Patients who have completed the trial | Mean age [yrs (SD)] | Sex [M/F (N)] | Outcomes | Reported adverse events |
|---|---|---|---|---|---|---|---|---|---|
| Redmond, 2008 | Canada | 0.5-1 mg | 3 visits with washout periods of at least one week | 20 | 17 | placebo & active: male: 22.5 (1.5) female: 23.2 (2.8) | 7/10 | Analgesic and antihyperalgesic properties of nabilone | Mild sedation, Euphoria, Feeling cold, Nausea, Dizziness, Headache, Increased appetite, Dry mouth |
| Skrabek, 2008 | Canada | 0.5-1 mg bid | 4 weeks | 40 | 33 | placebo: 50.11 (5.96) active: 47.6 (9.13) | 37/3 | Benefit of nabilone in pain management and QoL improvement in patients with fibromyalgia | Euphoria, Depression, Psychological high, Dissociation, Nightmares, Decreased concentration, Ataxia, Confusion, Hallucination, Orthostatic hypotension, Tachycardia, Sensory disturbance, Drowsiness, Lightheaded, Vertigo, Headache, Dysphoria, Anorexia, Dry mouth |
| Wissel, 2006 | Austria/Germany | 0.5 mg once or tid | 9 weeks | 13 | 11 | placebo & active: 44.85 (13.82) | 4/9 | Efficacy and safety of low dose nabilone in spasticity related pain | Dysphagia (slight), Drowsiness, Weakness in lower limbs (slight) |

F: female, M: male, ND: no data, yrs: years, bid: twice a day, tid: three times daily

**Table S3**. Adverse effects reported in case of nabilone

| Adverse effects | ICD | No. of studies reporting the AE | References | Classification |
|---|---|---|---|---|
| Ataxia | R2700 | 2 | Pooyania, 2010<br>Skrabek, 2008 | Central nervous system |
| Confusion | R4100 | 1 | Skrabek, 2008 | Central nervous system |
| Decreased concentration | F9900 | 1 | Skrabek, 2008 | Central nervous system |
| Depression | F32H0 | 1 | Skrabek, 2008 | Central nervous system |
| Dissociation | F44H0 | 1 | Skrabek, 2008 | Central nervous system |
| Euphoria | F31H0 | 2 | Redmond, 2008<br>Skrabek, 2008 | Central nervous system |
| Feeling cold | F3800 | 1 | Redmond, 2008 | Central nervous system |
| Hallucination | R4430 | 0 | Skrabek, 2008 | Central nervous system |
| Mild sedation | F1310 | 1 | Redmond, 2008 | Central nervous system |
| Nightmares | F5150 | 1 | Skrabek, 2008 | Central nervous system |
| Psychological high | F3800 | 1 | Skrabek, 2008 | Central nervous system |
| Sedation (Including Lethargy) | F1310 | 1 | Hermann, 2019 | Central nervous system |
| Significant increase in NPS | R7490 | 1 | Hermann, 2019 | Central nervous system |
| Somnolence | R4000 | 1 | Kalliomäki 2012 | Central nervous system |
| Treatment limiting sedation | F1310 | 1 | Hermann, 2019 | Central nervous system |
| Bradycardia | R0010 | 2 | Hermann, 2019<br>Kalliomäki 2012 | Cardiovascular |
| Myocardial infarction | I2100 | 1 | Hermann, 2019 | Cardiovascular |
| Orthostatic hypotension | I9510 | 1 | Skrabek, 2008 | Cardiovascular |
| Postural dizziness | I9510 | 1 | Kalliomäki 2012 | Cardiovascular |
| Tachycardia | R0000 | 2 | Kalliomäki 2012<br>Skrabek, 2008 | Cardiovascular |
| Anorexia | R6300 | 1 | Skrabek, 2008 | Miscellaneous |
| Asthenia | R5300 | 1 | Pooyania, 2010 | Miscellaneous |
| Dizziness | R4200 | 3 | Hermann, 2019<br>Kalliomäki 2012<br>Redmond 2008 | Miscellaneous |
| Drowsiness | R4000 | 3 | Pooyania, 2010<br>Wissel 2006<br>Skrabek, 2008 | Miscellaneous |
| Dry mouth | R6820 | 4 | Pooyania, 2010<br>Kalliomäki 2012<br>Redmond, 2008<br>Skrabek, 2008 | Miscellaneous |
| Dysphagia (slight) | R13H0 | 1 | Wissel 2006 | Miscellaneous |
| Dysphoria | R53H0 | 1 | Skrabek, 2008 | Miscellaneous |
| Fatigue | R5300 | 1 | Kalliomäki 2012 | Miscellaneous |
| Headache | R5100 | 4 | Pooyania, 2010<br>Kalliomäki 2012<br>Redmond, 2008<br>Skrabek, 2008 | Miscellaneous |

| Adverse effects | ICD | No. of studies reporting the AE | References | Classification |
|---|---|---|---|---|
| Increased appetite | R6320 | 1 | Redmond, 2008 | Miscellaneous |
| Lack of motivation | R4530 | 1 | Pooyania, 2010 | Miscellaneous |
| Lethargy | R5300 | 1 | Hermann, 2019 | Miscellaneous |
| Lightheaded | R4200 | 1 | Skrabek, 2008 | Miscellaneous |
| Nausea | R13H0 | 1 | Redmond, 2008 | Miscellaneous |
| Rash | R2100 | 1 | Hermann, 2019 | Miscellaneous |
| Sensory disturbance | R2000 | 1 | Skrabek, 2008 | Miscellaneous |
| Vertigo | R42H0 | 1 | Skrabek, 2008 | Miscellaneous |
| Vertigo (mild) | R42H0 | 1 | Pooyania, 2010 | Miscellaneous |
| Weakness in lower limbs (slight) | R6880 | 1 | Wissel 2006 | Miscellaneous |

**Table S4**. Adverse effects reported in case of dronabinol

| Adverse events (dronabinol) | ICD | No. of studies reporting the AE | Reference | Classification |
|---|---|---|---|---|
| "Loopy", foggy thinking | F3800 | 1 | Wong, 2012 | Central nervous system |
| Agitation | R4510 | 1 | van den Elsen, 2015 | Central nervous system |
| Ataxia | R2700 | 1 | Killestein, 2002 | Central nervous system |
| Bradykinesia | F4440 | 1 | van den Elsen, 2015 | Central nervous system |
| Cognitive disorder | F0670 | 1 | van den Elsen, 2015 | Central nervous system |
| Concentration problem | F9900 | 1 | Ahmed, 2014 | Central nervous system |
| Confusion | R4100 | 1 | Brisbois, 2011 | Central nervous system |
| Delirium | F0580 | 1 | van den Elsen, 2015 | Central nervous system |
| Disturbed mental concentration | F9900 | 1 | Esfandyari, 2006 | Central nervous system |
| Emotional lability | F6030 | 1 | Killestein, 2002 | Central nervous system |
| Euphoria | F31H0 | 2 | Svendsen, 2004 Ahmed, 2014 | Central nervous system |
| Euphoria/relaxed | F31H0 | 1 | Esfandyari, 2006 | Central nervous system |
| Euphoric mood | F31H0 | 1 | van den Elsen, 2015 | Central nervous system |
| Excitement | F3090 | 1 | Esfandyari, 2006 | Central nervous system |
| Feeling of drunkenness | F3800 | 1 | Svendsen, 2004 | Central nervous system |
| Hyperactivity | F9000 | 1 | Svendsen, 2004 | Central nervous system |
| Insomnia | G4700 | 1 | Schimrigk, 2017 | Central nervous system |
| Migraine | G4300 | 1 | Svendsen, 2004 | Central nervous system |
| Multiple sclerosis aggravated | G3500 | 1 | Svendsen, 2004 | Central nervous system |
| Nervousness | R4500 | 1 | Svendsen, 2004 | Central nervous system |
| Relaxation | R5300 | 1 | Ahmed, 2014 | Central nervous system |

| Adverse events (dronabinol) | ICD | No. of studies reporting the AE | Reference | Classification |
|---|---|---|---|---|
| Seizure | R5680 | 1 | Brisbois, 2011 | Central nervous system |
| Sleep difficulty | G4790 | 1 | Svendsen, 2004 | Central nervous system |
| Somnolence | R4000 | 2 | Killestein, 2002<br>van den Elsen, 2015 | Central nervous system |
| Speech disorders | F8010 | 1 | Svendsen, 2004 | Central nervous system |
| Troubles sleeping | G4790 | 1 | Brisbois, 2011 | Central nervous system |
| Visual hallucinations | R4410 | 1 | Ahmed, 2014 | Central nervous system |
| Bowel obstruction / constipation | K5660/K5900 | 1 | Brisbois, 2011 | Gastrointestinal |
| Diarrhoea | K5910 | 1 | Brisbois, 2011 | Gastrointestinal |
| Lose stools | K5910 | 1 | Malik, 2017 | Gastrointestinal |
| Stomach cramps | K3180 | 1 | Brisbois, 2011 | Gastrointestinal |
| Thrush | K1370 | 1 | Brisbois, 2011 | Gastrointestinal |
| Abdominal pain | R1040 | 1 | Svenden, 2004 | Miscellaneous |
| Anorexia | R6300 | 1 | Svenden, 2004 | Miscellaneous |
| Aphasia | R4700 | 1 | van den Elsen, 2015 | Miscellaneous |
| Apraxia | R4820 | 1 | van den Elsen, 2015 | Miscellaneous |
| Balance difficulty | H8190 | 1 | Svenden, 2004 | Miscellaneous |
| Balance disorder | H8190 | 1 | van den Elsen, 2015 | Miscellaneous |
| Bladder | N3280 | 1 | Zajicek, 2003 | Miscellaneous |
| Blurred vision | H5380 | 1 | Ahmed, 2014 | Miscellaneous |
| Chest pain | R0730 | 1 | van den Elsen, 2015 | Miscellaneous |
| Chills | R6880/R5500 | 1 | Svenden, 2004 | Miscellaneous |
| Chronic obstructive pulmonary diease | J4490 | 1 | van den Elsen, 2015 | Miscellaneous |
| Coordination disturbance | R2780 | 1 | Ahmed, 2014 | Miscellaneous |
| Decreased appetite | R6330 | 1 | van den Elsen, 2015 | Miscellaneous |
| Depression or anxiety | F32H0 | 1 | Zajicek, 2003 | Miscellaneous |
| Diplopia | H5320 | 1 | Svenden, 2004 | Miscellaneous |
| Dizziness | R4200 | 5 | Svenden, 2004<br>Schimrigk, 2017<br>Ahmed, 2014<br>Killestein, 2002<br>van den Elsen, 2015 | Miscellaneous |
| Dizziness/lightheadness | R4200 | 2 | Esfandyari, 2006<br>Wong, 2002 | Miscellaneous |
| Dizzy of lightheadedness | R4200 | 1 | Zajicek, 2003 | Miscellaneous |
| Drowsiness | R4000 | 3 | Svendsen, 2004<br>Ahmed, 2014<br>Esfandyari, 2006 | Miscellaneous |
| Drowsiness/tiredness | R4000 | 1 | Wong, 2012 | Miscellaneous |

| Adverse events (dronabinol) | ICD | No. of studies reporting the AE | Reference | Classification |
|---|---|---|---|---|
| Dry eye | H0410 | 2 | Ahmed, 2014<br>van den Elsen, 2015 | Miscellaneous |
| Dry mouth | R6820 | 6 | Svendsen, 2004<br>Schimrigk, 2017<br>Ahmed, 2014<br>Esfandyari, 2006<br>Killestein 2002<br>Zajicek, 2003 | Miscellaneous |
| Edema | R6090 | 1 | Brisbois, 2011 | Miscellaneous |
| Eye hemorrhage | H4480 | 1 | van den Elsen, 2015 | Miscellaneous |
| Fatigue | R5300 | 4 | Svendsen, 2004<br>Schimrigk, 2017<br>Malik, 2017<br>van den Elsen, 2015 | Miscellaneous |
| Fever | R5090 | 2 | Brisbois, 2011<br>Svendsen, 2004 | Miscellaneous |
| Flushing | R3200 | 1 | Esfandyari, 2006 | Miscellaneous |
| Gamma-glutamyltransferase increased | R7490 | 1 | van den Elsen, 2015 | Miscellaneous |
| Gastrointestinal tract | ? | 1 | Zajicek, 2003 | Miscellaneous |
| Headache | R5100 | 9 | Brisbois, 2011<br>Svendsen, 2004<br>Schimrigk, 2017<br>Malik, 2017<br>Ahmed, 2014<br>Esfandyari, 2006<br>Wong, 2012<br>Killestein, 2002<br>van den Elsen, 2015 | Miscellaneous |
| Hepatic enzyme increased | R7490 | 1 | van den Elsen, 2015 | Miscellaneous |
| Hives/rash | R21H0 | 1 | Brisbois, 2011 | Miscellaneous |
| Low blood count | R7990 | 1 | Brisbois, 2011 | Miscellaneous |
| Malaise | R5300 | 2 | Ahmed, 2014<br>van den Elsen, 2015 | Miscellaneous |
| Miosis | H5700 | 1 | van den Elsen, 2015 | Miscellaneous |
| Nausea | R13H0 | 5 | Svendsen, 2004<br>Schimrigk, 2017 Malik, 2017 Ahmed, 2014<br>Esfandyari, 2006 | Miscellaneous |
| Nausea/Vomiting | R13H0 | 1 | Brisbois, 2011 | Miscellaneous |

| Adverse events (dronabinol) | ICD | No. of studies reporting the AE | Reference | Classification |
|---|---|---|---|---|
| Numbness | R2080 | 1 | Esfandyari, 2006 | Miscellaneous |
| Other | R6880 | 1 | Killestein, 2002 | Miscellaneous |
| Pain | R5200 | 1 | Brisbois, 2011 | Miscellaneous |
| Palpitations | R0020 | 1 | Svendsen, 2004 | Miscellaneous |
| Presyncope | R5500 | 1 | van den Elsen, 2015 | Miscellaneous |
| Restlessness | R4510 | 1 | van den Elsen, 2015 | Miscellaneous |
| Sensory loss | R4480 | 1 | van den Elsen, 2015 | Miscellaneous |
| Shortness of breath / fluid on lungs | R0600 | 1 | Brisbois, 2011 | Miscellaneous |
| Skin disorder, not otherwise specified | R2380 | 1 | van den Elsen, 2015 | Miscellaneous |
| Syncope | R5500 | 1 | van den Elsen, 2015 | Miscellaneous |
| Tired / drowsy | R5300 | 1 | Brisbois, 2011 | Miscellaneous |
| Unsteady feet | H8190 | 1 | Brisbois, 2011 | Miscellaneous |
| Vasovagal | R5500 | 1 | Esfandyari, 2006 | Miscellaneous |
| Vertigo | R42H0 | 1 | Schimrigk, 2017 | Miscellaneous |
| Weight decrease | R6340 | 1 | Svendsen, 2004 | Miscellaneous |
| Back pain | M5480 | 1 | van den Elsen, 2015 | Musculoskeletal |
| Increased spasticity | M6290 | 1 | Killestein, 2002 | Musculoskeletal |
| Muscle spasms | M6290 | 1 | van den Elsen, 2015 | Musculoskeletal |
| Muscle weakness | M6280 | 2 | Svendsen, 2004 van den Elsen, 2015 | Musculoskeletal |
| Myalgia | M7910 | 1 | Svendsen, 2004 | Musculoskeletal |
| Pain in extremity | M7960 | 1 | van den Elsen, 2015 | Musculoskeletal |
| Nasopharyngitis | J0000 | 1 | van den Elsen, 2015 | Respiratory |
| Pneumonia | J1890 | 2 | Brisbois, 2011 van den Elsen, 2015 | Respiratory |
| Upper airway infection | J0690 | 1 | Svendsen, 2004 | Respiratory |
| Hot flushes | N9580 | 2 | Svendsen, 2004 Wong, 2012 | Urogenital |
| Renal impairment | N1900 | 1 | van den Elsen, 2015 | Urogenital |
| Urge incontinence | N3940 | 1 | van den Elsen, 2015 | Urogenital |
| Vaginal discharge | N8980 | 1 | Brisbois, 2011 | Urogenital |

**Table S5.** Distribution of CBD product at various temperatures in an oxidative atmosphere

| No. | Compound | $t_R$ (min) | $M_w$ (g/mol) | O 250 °C | O 300 °C | O 400 °C | O 500 °C |
|---|---|---|---|---|---|---|---|
| | | | | Mean±SD (%) | Mean±SD (%) | Mean±SD (%) | Mean±SD (%) |
| 1 | Unidentified monoterpene | 11.62 | 134 | n.d. | n.d. | 0.42±0.07 | n.d. |
| 2 | *p*-Mentha-1,3,8-triene | 12.76 | 134 | 0.94±0.15 | 1.37±0.65 | 0.64±0.18 | n.d. |
| 3 | *p*-Mentha-1,5,8-triene | 13.17 | 134 | 1.00±0.04 | 1.16±0.52 | 1.70±0.07 | 3.64±0.62 |
| 4 | *p*-Cymene | 13.32 | 134 | n.d. | n.d. | n.d. | 2.87±0.45 |
| 5 | *p*-Cymenene | 14.83 | 132 | n.d. | n.d. | 0.47±0.19 | 1.61±0.69 |
| 6 | *p*-Mentha-1,4,8-triene | 14.92 | 134 | n.d. | n.d. | 1.30±0.41 | 3.60±1.09 |
| 7 | 6-Pentylbenzofuran-4-ol | 25.99 | 204 | n.d. | 0.28±0.11 | n.d. | n.d. |
| 8 | Olivetol | 26.46 | 180 | n.d. | 0.21±0.07 | 0.76±0.21 | n.d. |
| 10 | Cannabicyclol | 29.74 | 314 | 1.08±0.27 | 0.21±0.07 | 0.64±0.13 | 0.44±0.10 |
| 12 | Unidentified A* | 29.98 | 312 | 0.30±0.04 | 0.41±0.03 | 1.02±0.29 | 0.33±0.11 |
| 14 | 6-Methyl-3-pentyl-9-(propan-2-ylidene)-5a,6,7,8,9,9a-hexahydro-dibenzo[*b*,*d*]furan-1-ol | 30.28 | 314 | 0.23±0.05 | 0.34±0.04 | 0.68±0.17 | n.d. |
| 16 | 5-Pentyl-2-(4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-yl)benzene-1,3-diol | 30.51 | 314 | 0.11±0.02 | n.d. | 0.72±0.42 | n.d. |
| 17 | Unidentified | 30.54 | 312 | 0.19±0.04 | 0.78±0.10 | 1.75±2.40 | n.d. |
| 18 | Unidentified B* | 30.61 | 312 | 0.26±0.05 | 0.58±0.16 | 0.19±0.05 | n.d. |
| 19 | Cannabichromene | 30.73 | 314 | 5.03±0.95 | 5.74±0.59 | 5.32±0.67 | 2.36±1.05 |
| 20 | CBD | 30.81 | 314 | 62.02±5.09 | 61.58±2.95 | 52.19±6.56 | 64.41±9.98 |
| 21 | Unidentified C* | 31.10 | 314 | 0.74±0.13 | 1.04±0.14 | 1.27±0.29 | n.d. |
| 22 | Unidentified D* | 31.15 | 314 | n.d. | 0.52±0.07 | 0.72±0.18 | n.d. |
| 23 | $\Delta^8$-THC | 31.25 | 314 | 3.57±0.74 | 2.54±0.23 | 3.38±0.73 | 2.10±0.66 |
| 25 | $\Delta^9$-THC | 31.48 | 314 | 16.09±3.96 | 12.85±0.86 | 13.45±2.38 | 8.55±3.77 |
| 26 | Cannabielsoin | 31.50 | 330 | 3.44±0.14 | 3.83±0.50 | 2.56±0.27 | 2.89±0.68 |
| 27 | Unidentified E* | 31.81 | 314 | n.d. | n.d. | 0.39±0.02 | 1.63±0.56 |
| 28 | Cannabinol | 32.41 | 310 | 5.00±0.34 | 6.56±0.49 | 10.44±0.82 | 5.59±2.55 |

* Unidentified compounds appear under both inert and oxidative conditions

n.d.: below detection limit; $t_R$: retention time; $M_W$: molecular weight

**Table S6.** Distribution of CBD product at various temperatures in an inert atmosphere

| No. | Compound | $t_R$ (min) | $M_w$ (g/mol) | I 250 °C | I 300 °C | I 400 °C | I 500 °C |
|---|---|---|---|---|---|---|---|
| | | | | Mean ±SD (%) | Mean ±SD (%) | Mean ±SD (%) | Mean ±SD (%) |
| 2 | *p*-Mentha-1,3,8-triene | 12.88 | 134 | n.d. | n.d. | 1.08±0.35 | n.d. |
| 3 | *p*-Mentha-1,5,8-triene | 13.23 | 134 | n.d. | n.d. | 1.34±0.05 | 1.70±0.36 |
| 4 | *p*-Cymene | 13.31 | 134 | n.d. | n.d. | n.d. | 1.93±0.13 |
| 5 | *p*-Cymenene | 14.78 | 132 | n.d. | n.d. | n.d. | 0.48±0.14 |
| 6 | *p*-Mentha-1,4,8-triene | 14.88 | 134 | n.d. | n.d. | n.d. | 1.83±0.48 |
| 8 | Olivetol | 26.43 | 180 | n.d. | n.d. | 0.50±0.19 | 2.25±0.45 |
| 9 | 2,2-Dimethyl-7-pentyl-2*H*-chromen-5-ol | 27.21 | 246 | n.d. | n.d. | n.d. | 1.00±0.51 |
| 10 | Cannabicyclol | 29.74 | 314 | 0.12±0.02 | n.d. | 0.29±0.11 | 0.38±0.01 |
| 11 | Unidentified | 29.83 | 314 | 0.15±0.04 | n.d. | 0.12±0.02 | 0.22±0.08 |
| 12 | Unidentified A* | 29.98 | 312 | 0.14±0.03 | n.d. | 0.30±0.08 | 1.97±0.25 |
| 13 | Unidentified | 30.08 | 314 | n.d. | n.d. | n.d. | 0.59±0.06 |
| 14 | 6-Methyl-3-pentyl-9-(propan-2-ylidene)-5a,6,7,8,9,9a-hexahydro-dibenzo[*b*,*d*]furan-1-ol | 30.28 | 314 | 0.39±0.06 | 0.15±0.03 | 0.33±0.12 | 0.75±0.07 |
| 15 | Unidentified | 30.45 | 312 | n.d. | n.d. | n.d. | 0.44±0.03 |
| 16 | 5-Pentyl-2-(4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-yl)benzene-1,3-diol | 30.51 | 314 | 0.50±0.04 | n.d. | 0.40±.0.23 | 1.42±0.11 |
| 18 | Unidentified B* | 30.62 | 312 | n.d. | 0.18±0.07 | 0.33±0.17 | 0.32±0.07 |
| 19 | Cannabichromene | 30.73 | 314 | 7.00±0.49 | 3.17±0.94 | 5.64±1.76 | 2.35±0.54 |
| 20 | CBD | 30.82 | 314 | 48.20±5.13 | 74.62±4.40 | 62.11±9.53 | 67.66±5.51 |
| 21 | Unidentified C* | 31.10 | 314 | 0.56±0.06 | 0.62±0.18 | 0.70±0.12 | 1.07±0.16 |
| 22 | Unidentified D* | 31.17 | 314 | 0.45±0.02 | n.d. | 0.46±0.10 | 0.88±0.22 |
| 23 | $\Delta^8$-THC | 31.25 | 314 | 4.94±0.67 | 1.64±0.32 | 2.73±0.68 | 2.76±0.63 |
| 24 | $\Delta^{6a,10a}$-THC | 31.39 | 314 | n.d. | n.d. | n.d. | 1.36±0.29 |
| 25 | $\Delta^9$-THC | 31.49 | 314 | 33.94±4.07 | 17.95±2.96 | 20.17±4.42 | 5.88±1.57 |
| 27 | Unidentified E* | 31.82 | 314 | n.d. | n.d. | n.d. | 0.53±0.07 |
| 28 | Cannabinol | 32.41 | 310 | 3.64±0.07 | 1.66±0.33 | 3.49±1.49 | 2.25±0.21 |

* Unidentified compounds appear under both inert and oxidative conditions
n.d.: below detection limit; $t_R$: retention time; $M_W$: molecular weight

**Table S7.** Characteristics, CBD content, and recommended daily intake of analyzed products based on label information

| No | Type | Ingredients | The claimed CBD content | Recommended daily intake | Measured CBD content | Maximum recommended CBD intake | Labeling |
|---|---|---|---|---|---|---|---|
| CB1 | CBD oil | phytocannabinoid extract, MCT oil | 30 mg/mL | 2 x 20 drops (2 x 0.67 mL) | 23.80±0.25 mg/mL | 31.9 mg | over labeled (79.3%) |
| CB2 | CBD oil | hemp flower extract in hemp seed oil min. 500 mg CBD – full-spectrum; other cannabinoids: CBDA, CBG, CBN, THC<0,2% | 50 mg/mL | 2 x 5 drops | 45.44±2.66 mg/mL | 22.7 mg | accurately labeled (90.9%) |
| CB3 | CBD oil | hemp seed oil, $CO_2$ whole plant hemp extract – full-spectrum | 41.66 mg/mL | 2 x 1 drop | 51.57±4.45 mg/mL | 2.6 mg | under labeled (123.8%) |
| CB4 | CBD oil | hemp oil, CBD (2%), THC (0%) other cannabinoids in traces | 19 mg/mL | 1 drop | 13.50±0.90 mg/mL | 0.7 mg | over labeled (71.1%) |
| CB5 | CBD oil | olive oil, hemp seed extract, supercritical extract $CO_2$ from organic hemp – supposedly full-spectrum | 30 mg/mL | ND | 20.06±0.24 mg/mL | ND | over labeled (66.9%) |
| CB6 | e-cigarette liquid | glycerol 80%, propylene glycol/propane-1,2-diol 20%, CBD 1% (100 mg) other natural terpenes *CBD was made by alcoholic extract from industrial hemp | 10 mg/mL | ND | <LOQ | ND | over labeled (0.0%) |
| CB7 | hemp seed oil | hemp seed oil from organic farming | ND | 2 mL | <LOD | 0.0 mg | NA |
| CB8 | CBD oil | hemp oil (91%), hemp extract (9% full-spectrum) | 25 mg/mL | 0.12 mL | 12.87±0.23 mg/mL | 1.5 mg | over labeled (51.5%) |
| CB9 | CBD oil | CBD (55% CBD distillate, CBD-enriched full-spectrum extract), sterilized hemp seed oil | 20 mg/mL | 1 mL | 19.38±0.26 mg/mL | 19.4 mg | accurately labeled (96.9%) |
| CB10 | hemp seed oil | cold-pressed hemp seed oil | ND | 2 mL | <LOD | 0.0 mg | NA |
| CB11 | hemp seed oil | cold-pressed hemp seed oil | ND | ND | <LOD | ND | NA |

| No | Type | Ingredients | The claimed CBD content | Recommended daily intake | Measured CBD content | Maximum recommended CBD intake | Labeling |
|---|---|---|---|---|---|---|---|
| CB12 | hemp seed oil | cold-pressed hemp seed oil | ND | ND | <LOD | ND | NA |
| CB13 | hemp seed oil | cold-pressed hemp seed oil | ND | 1-2 x 15 mL | <LOD | 0.0 mg | NA |
| CB14 | hemp seed oil | cold-pressed hemp seed oil | ND | 2 x 5 mL | <LOD | 0.0 mg | NA |
| CB15 | CBD capsule | hemp oil (91%), hemp extract (9%) – full-spectrum | 12.5 mg/capsule | 2–4 capsules | <LOQ | 0.0 mg | NA |
| CB16 | CBD oil | full-spectrum hemp oil, *Cannabis sativa* L. supercritical extract | 50 mg/mL | 2–10 drops | 47.42±1.99 mg/mL | 23.7 mg | accurately labeled (94.8%) |
| CB17 | CBD oil | hemp oil, CBD (300 mg), Omega-3 (1.2 g), Omega-6 (4 g), Vitamin E (8 mg) - | 30 mg/mL | ND | 25.92±1.16 mg/mL | ND | over labeled (86.4%) |
| CB18 | Capsule | supercritical extract – full-spectrum, 400 mg CBD/CBDa | 40 mg/mL | 1 drop | 24.71±1.18 mg/mL | 1.2 mg | over labeled (61.8%) |
| CB19 | CBD oil | 100% organic cold-pressed hemp seed oil, hemp extract - full-spectrum (CBG: ~0.18%, CBC: 0.2%) | 50 mg/mL | ND | 50.51±3.85 mg/mL | ND | accurately labeled (101.0%) |
| CB20 | CBD oil | organically grown phytocannabinoid-rich hemp oil – broad-spectrumbroad-spectrum, grape seed oil, hemp seed oil, orange oil | 33 mg/mL | 1–3 x 5–15 drops | 31.67±2.34 mg/mL | 71.3 mg | accurately labeled (96.0%) |
| CB21 | CBD oil | full-spectrum plant extract, MCT plant oil-RSPO | 50 mg/mL | 2-3 x 5 drops | 26.62±1.00 mg/mL | 20.0 mg | over labeled (53.2%) |
| CB22 | CBD oil | hemp $CO_2$ extract 2740 mg (27%) – supposedly full-spectrum, hemp seed oil 7260 mg (73%) of which CBDA/CBD~500 mg | 50 mg/mL | 1–3 x 2–10 drops | 19.58±0.35 mg/mL | 29.4 mg | over labeled (39.2%) |
| CB23 | CBD oil | organic hemp oil, cannabidiol extract – full-spectrum boosted with CBD distillate | 50 mg/mL | 2 x 1–5 drops | 43.18±0.70 mg/mL | 21.6 mg | over labeled (86.4%) |

| No | Type | Ingredients | The claimed CBD content | Recommended daily intake | Measured CBD content | Maximum recommended CBD intake | Labeling |
|---|---|---|---|---|---|---|---|
| CB24 | CBD oil | CBD hemp extract – broad-spectrumbroad-spectrum, MCT coconut oil, natural flavoring (wild cherry) | 20 mg/mL | 20 drops (10 mg CBD) gradually increased to 70 mg | 22.76±0.24 mg/mL | 70.0 mg | under labeled (113.8%) |
| CB25 | CBD oil | olive oil, hemp extract, terpenes | 50 mg/mL | 3 x 3-4 drops | 54.09±0.14 mg/mL | 32.5 mg | accurately labeled (108.2%) |
| CB26 | CBD oil | hemp seed oil, hemp extract (of which 300 mg CBD) – broad-spectrumbroad-spectrum, natural tocopherols (E3016) | 30 mg/mL | 2 x 6 drops | 29.58±0.23 mg/mL | 17.7 mg | accurately labeled (98.6%) |
| CB27 | CBD oil | BIO hemp seed oil, CBD (5%) | 50 mg/mL | 1 drop | 50.10±0.91 mg/mL | 2.5 mg | accurately labeled (100.2%) |

CBDA: cannabidiolic acid, CBG: cannabigerol, CBN: cannabinol, THC: tetrahydrocannabinol, ND: no data, NA: not applicable, LOD: limit of detection, LOQ: limit of quantification. The measured CBD content values are presented as mean ± SD (n=3).

# SUPPLEMENTARY MATERIALS – FIGURES

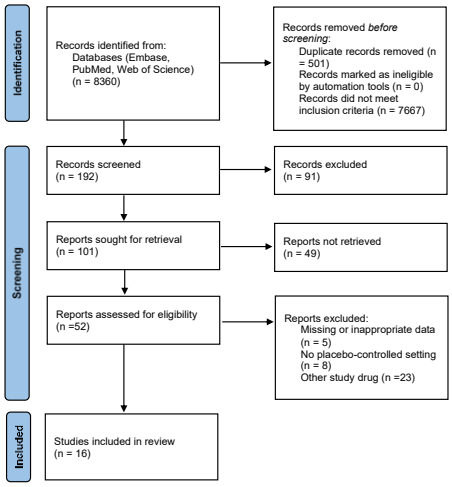


**Figure S1.** PRISMA flow diagram for the meta-analysis

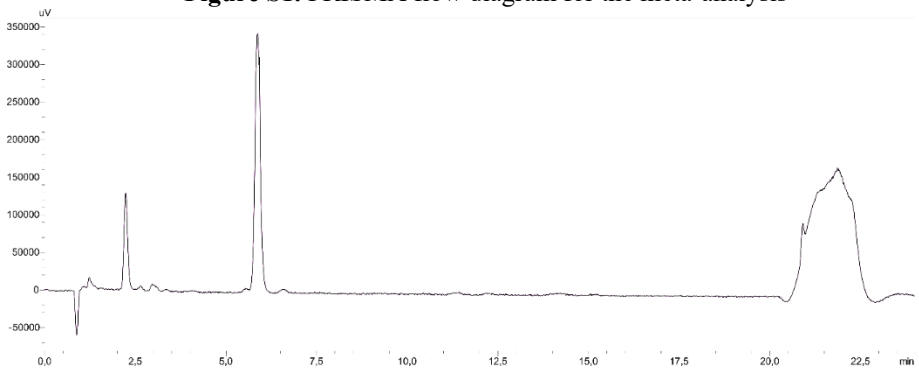


**Figure S2.** UHPLC chromatogram of product CB1 recorded at 210 nm.

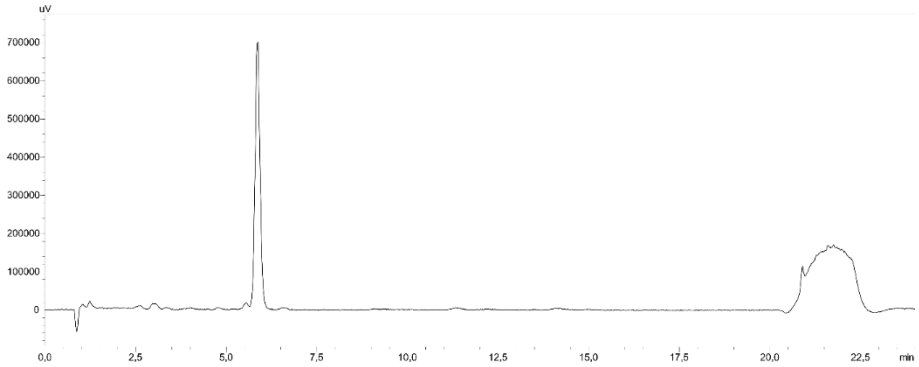


**Figure S3.** UHPLC chromatogram of product CB2 recorded at 210 nm.

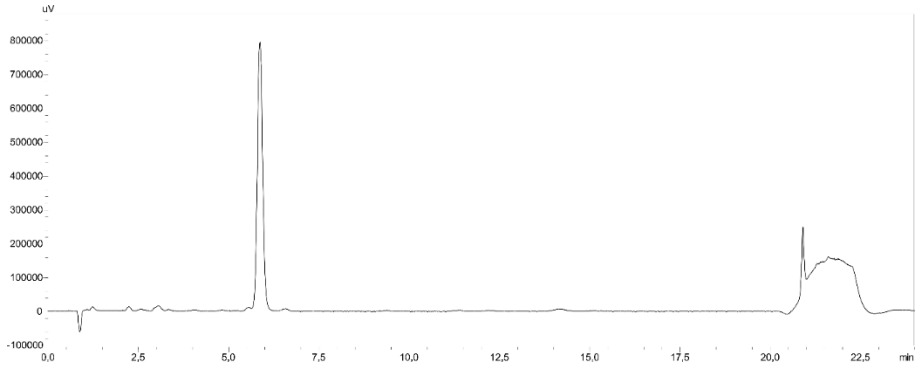


**Figure S4.** UHPLC chromatogram of product CB3 recorded at 210 nm.

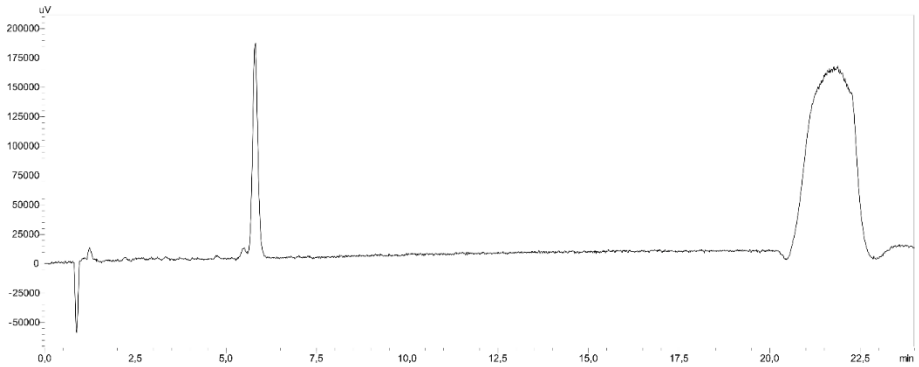


**Figure S5.** UHPLC chromatogram of product CB4 recorded at 210 nm.

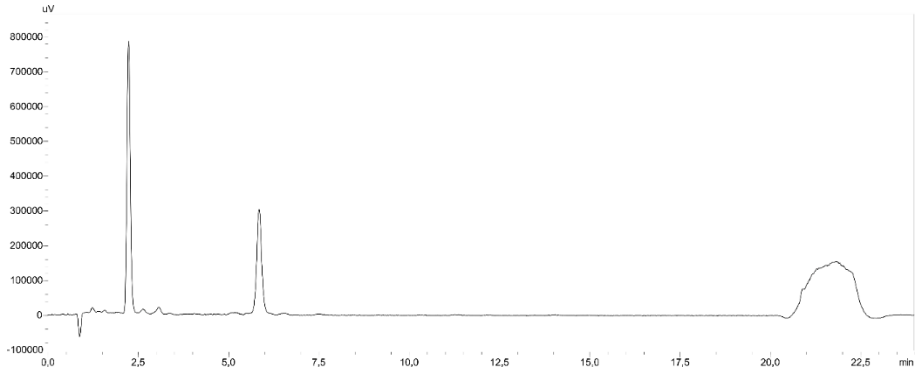


**Figure S6.** UHPLC chromatogram of product CB5 recorded at 210 nm.

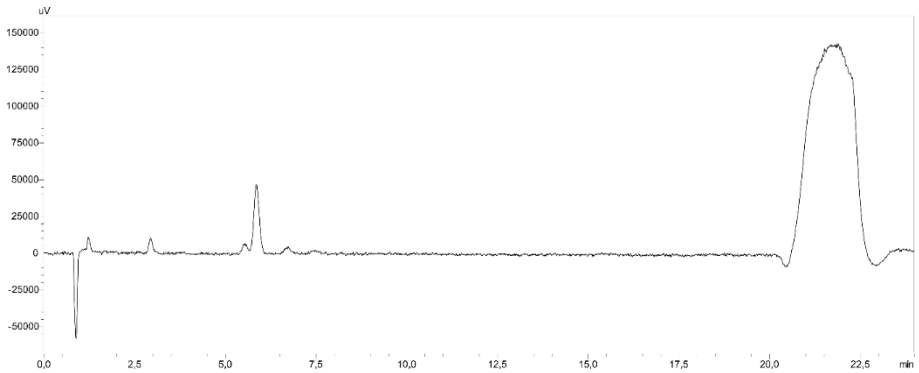


**Figure S7.** UHPLC chromatogram of product CB6 recorded at 210 nm.

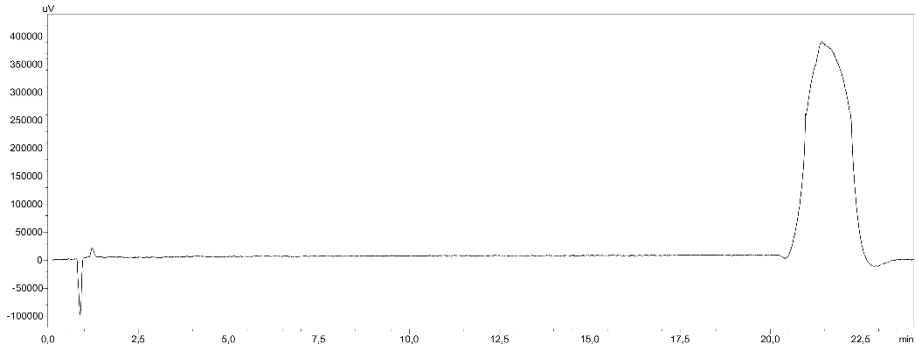


**Figure S8.** UHPLC chromatogram of product CB7 recorded at 210 nm.

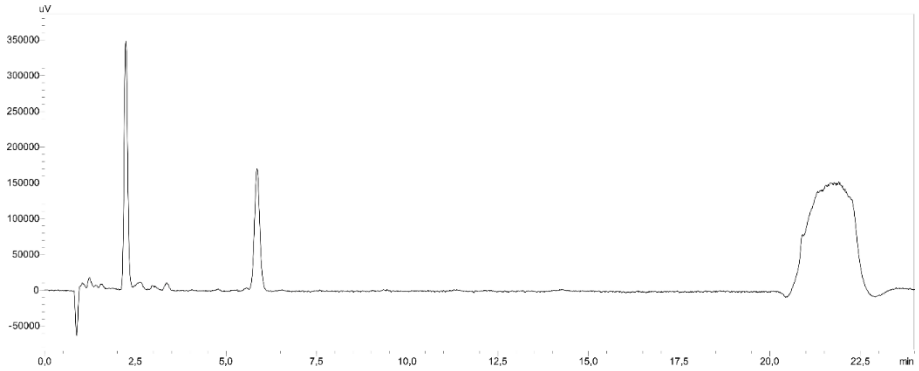


**Figure S9.** UHPLC chromatogram of product CB8 recorded at 210 nm.

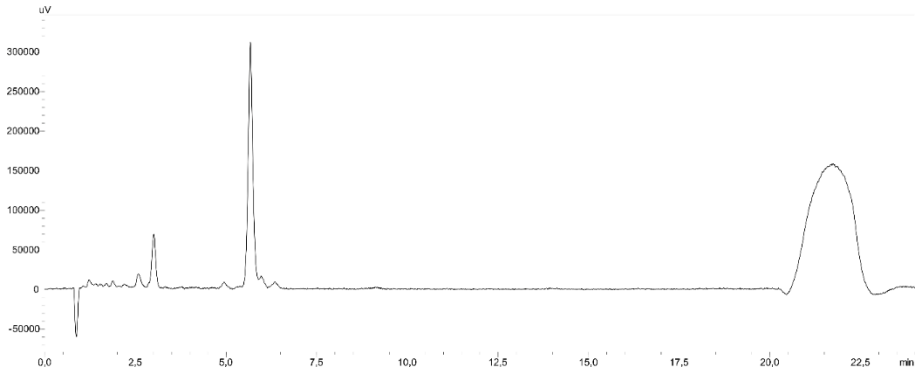


**Figure S10.** UHPLC chromatogram of product CB9 recorded at 210 nm.

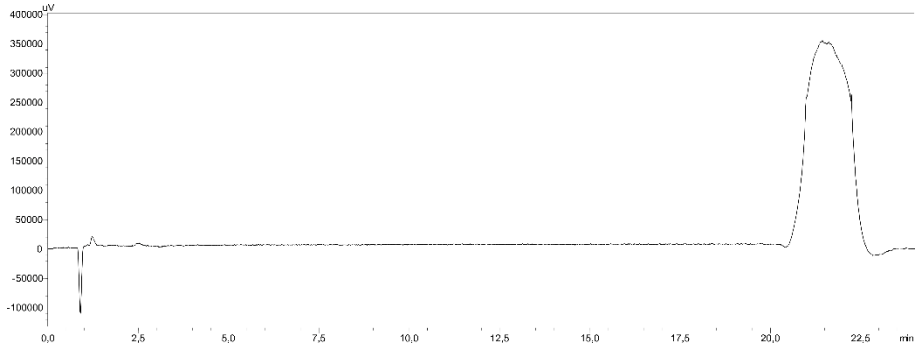


**Figure S11.** UHPLC chromatogram of product CB10 recorded at 210 nm.

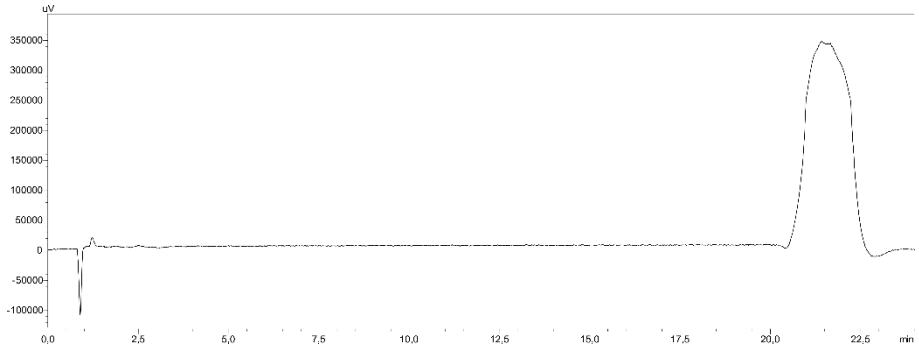


**Figure S12.** UHPLC chromatogram of product CB11 recorded at 210 nm.

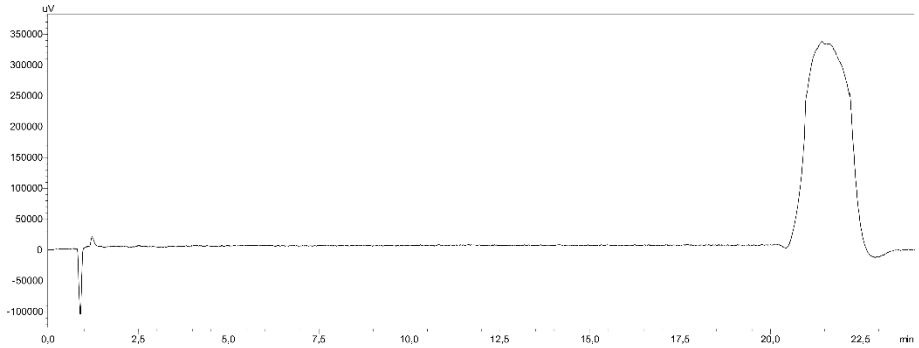


**Figure S13.** UHPLC chromatogram of product CB12 recorded at 210 nm.

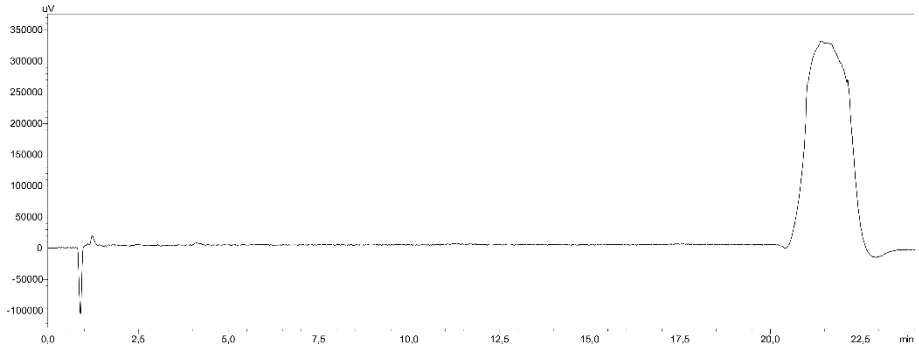


**Figure S14.** UHPLC chromatogram of product CB13 recorded at 210 nm.

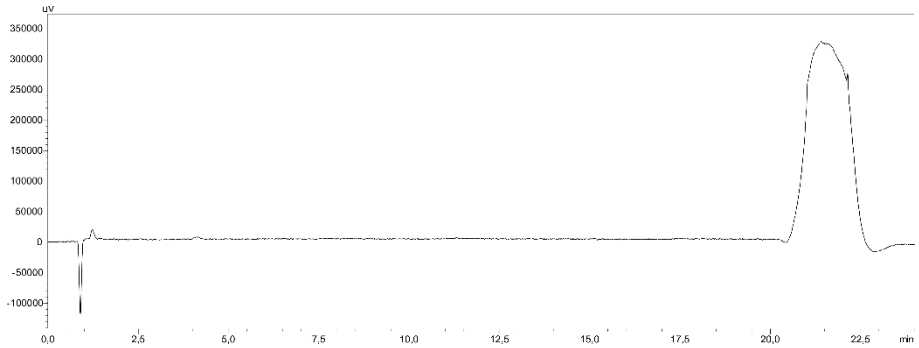


**Figure S15.** UHPLC chromatogram of product CB14 recorded at 210 nm.

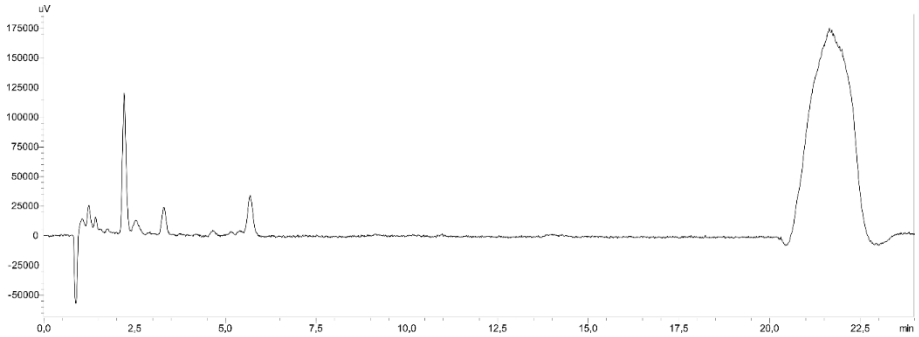


**Figure S16.** UHPLC chromatogram of product CB15 recorded at 210 nm.

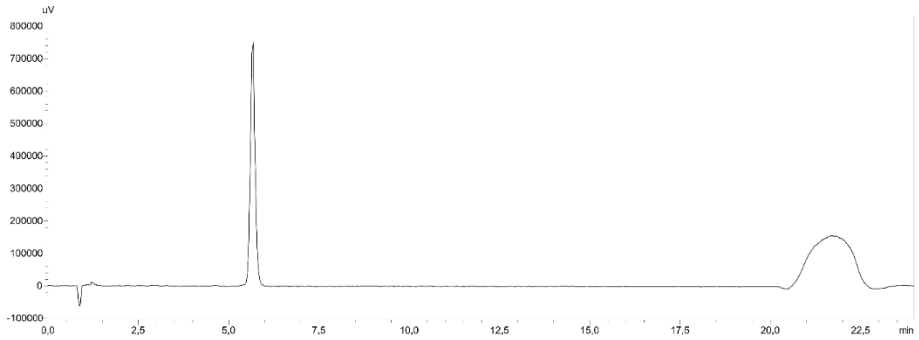


**Figure S17.** UHPLC chromatogram of product CB16 recorded at 210 nm.

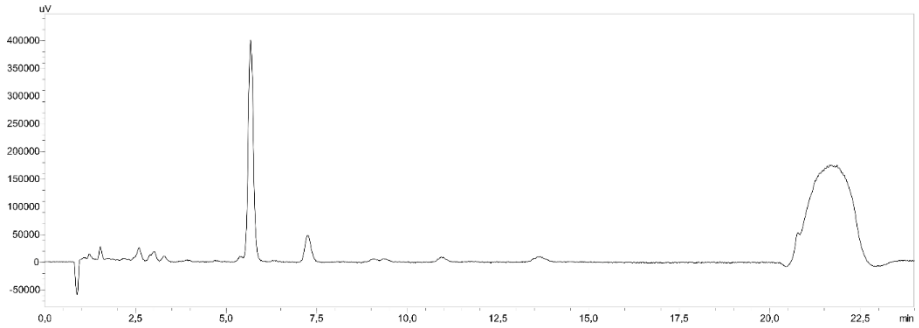


**Figure S18.** UHPLC chromatogram of product CB17 recorded at 210 nm.

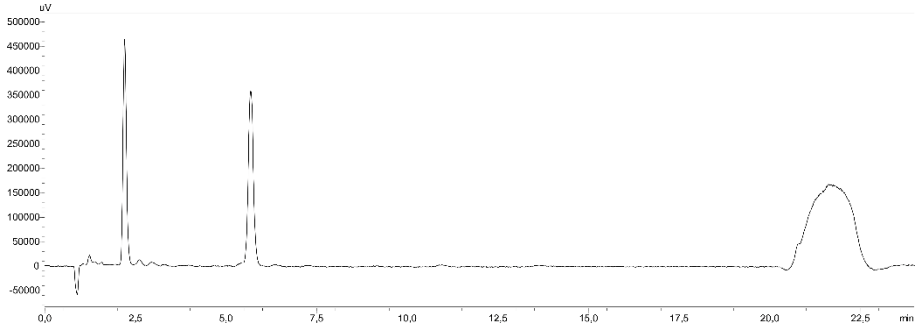


**Figure S19.** UHPLC chromatogram of product CB18 recorded at 210 nm.

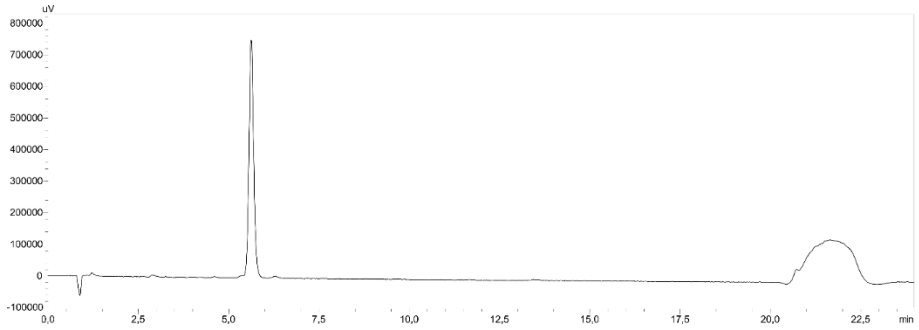


**Figure S20.** UHPLC chromatogram of product CB19 recorded at 210 nm.

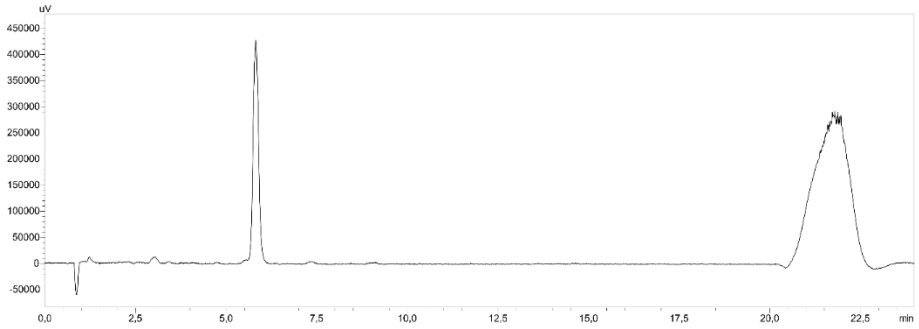


**Figure S21.** UHPLC chromatogram of product CB20 recorded at 210 nm.

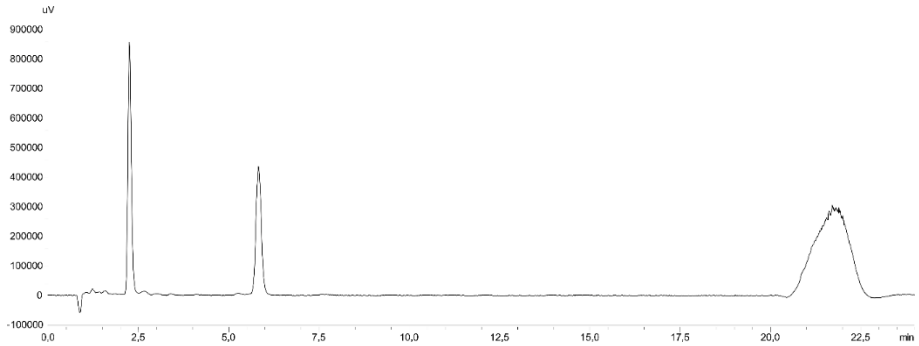


**Figure S22.** UHPLC chromatogram of product CB21 recorded at 210 nm.

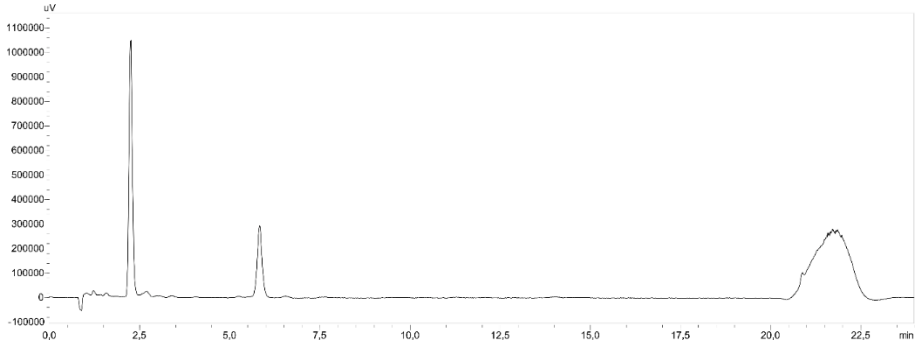


**Figure S23.** UHPLC chromatogram of product CB22 recorded at 210 nm.

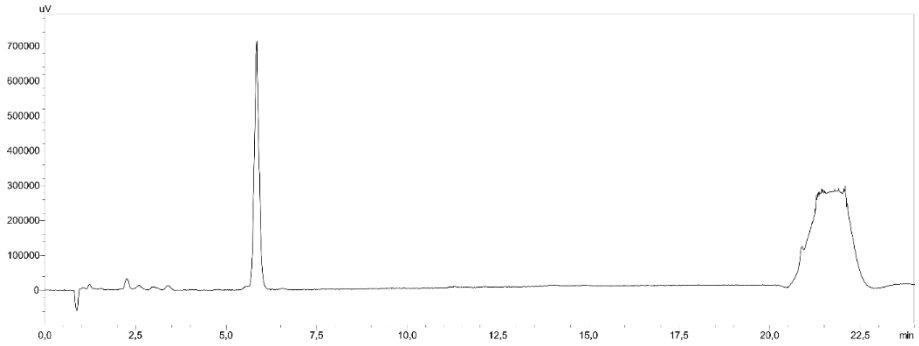


**Figure S24.** UHPLC chromatogram of product CB23 recorded at 210 nm.

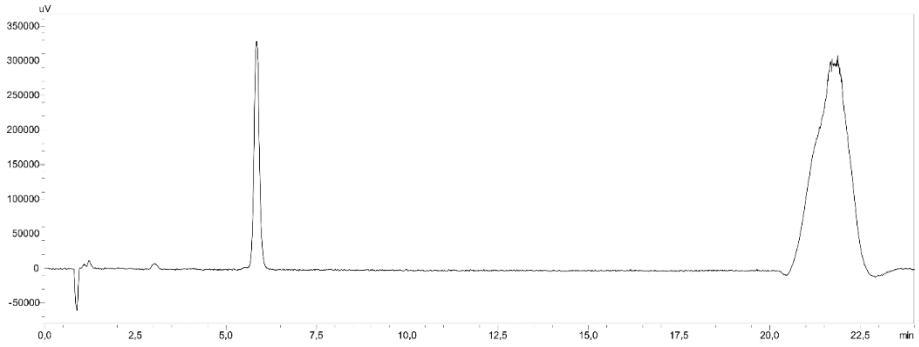


**Figure S25.** UHPLC chromatogram of product CB24 recorded at 210 nm.

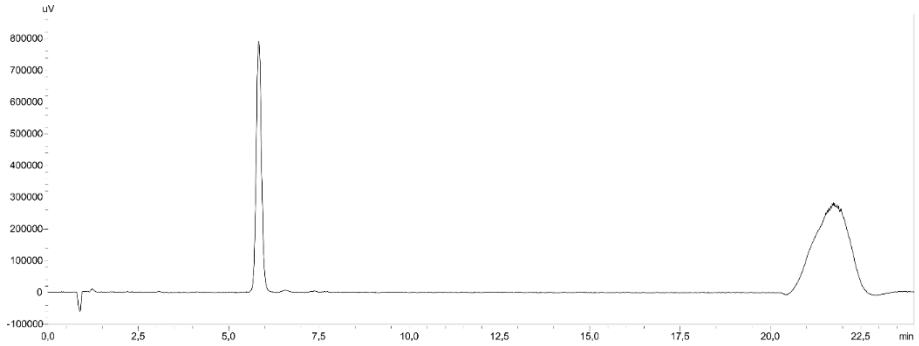


**Figure S26.** UHPLC chromatogram of product CB25 recorded at 210 nm.

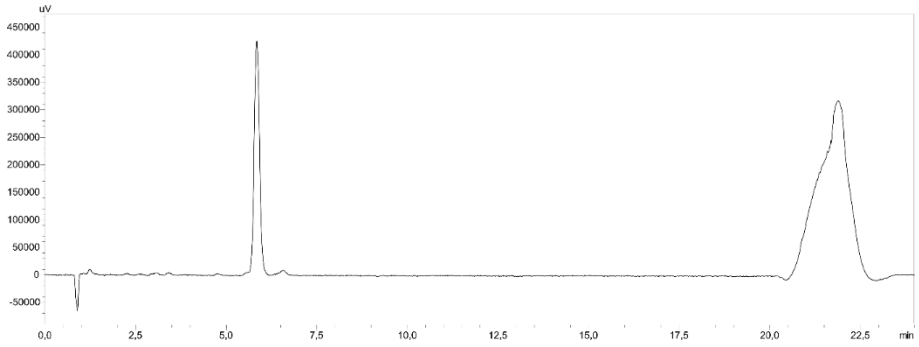


**Figure S27.** UHPLC chromatogram of product CB26 recorded at 210 nm.

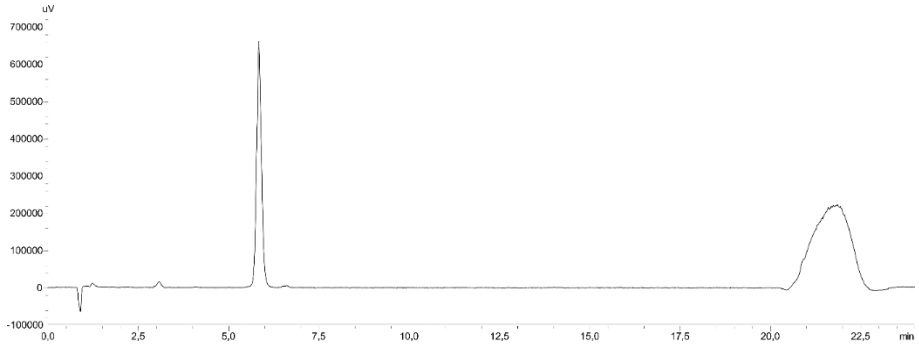


**Figure S28.** UHPLC chromatogram of product CB27 recorded at 210 nm.

# SUPPLEMENTARY MATERIALS – PUBLICATIONS RELATED TO THE THESIS