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Determination of the relative percentage distribution of THCA and Δ^9-THC in herbal cannabis seized in Austria – Impact of different storage temperatures on stability

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ABSTRACT

Cannabis is globally by far the most widespread illicit drug of abuse. Especially since its legalization in some of the US, controversies about the legal status of cannabis for recreational and medical use have come up.

Δ^9-Tetrahydrocannabinol (Δ^9-THC), which is the major active ingredient in cannabis products, is mainly responsible for the psychoactive effects. Its inactive biosynthetic precursor tetrahydrocannabinolic acid (THCA) is present in different quantities in fresh and undried cannabis plants. Under influence of drying, temperature and UV exposure it decomposes to Δ^9-THC.

In this study, a quantification of Δ^9-THC and THCA was carried out to check the stability of cannabis samples. The determination of the degradation of THCA to Δ^9-THC in 29 cannabis products seized in Austria was monitored by HPLC-UV. Mobile phase consisted of a 25 mM triethylammoniumphosphate buffer (pH 3.0) and acetonitrile (36:64). A common LiChrospher® 100 RP-18 column was utilized as stationary phase. To check the influence of low as well as high temperature on the degradation process of the cannabinoid THCA to Δ^9-THC, samples were stored in a freezer or in a drying cabinet for a specified time period. It was shown successfully that high storage temperatures led to a more rapid and complete decomposition of THCA to Δ^9-THC while at low temperatures only slight or no changes of the percentage distribution were determined.

1. Introduction

Cannabis is a genus of plant that belongs like the hop (*Humulus lupulus*) to the family of *Cannabaceae*. The genus cannabis is botanically divided into three species: *Cannabis sativa* L., *Cannabis indica* Lam. and *Cannabis ruderalis* J. Originally it is indigenous in Central Asia, but it also grows in West and North Africa, Afghanistan and the Carribean. Due to optimal possibilities of hydroponic cultivation in nutrient solutions, artificial lighting and heating cannabis is commonly grown in professionally equipped illict indoor plantations all over the world.

The flowering parts of the female plant contain the highest concentration of various cannabinoids. The most important cannabinoids are Δ^9-tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG) and cannabichromene (CBC). Δ^9-THC is mainly responsible for the psychoactive effects while the other cannabinoids show no or only less psychotropic impacts. The majority of Δ^9-THC in the plant is present in the pharmacological inactive form tetrahydrocannabinolic acid (THCA). Through heating, for example during smoking, baking or cooking the precursor is decomposed to the active compound Δ^9-THC. CBD and CBN are non-psychoactive and are present in some cannabis species. They possess biological effects like modulation of immune responses [1], anti-inflammatory [2] and antibacterial activity.

THCA must not be mixed up with tetrahydrocannabinol carboxylic acid (THC-COOH, 11-COOH-THC), a major metabolite of Δ^9-THC which can be used as an indicator of cannabis abuse in urine and blood tests.

Because of the therapeutic benefits of cannabis, licensed dronabinol products are available on the pharmaceutical market. They reduce vomiting and nausea in cases of cancer and chemotherapy or the lack of appetite of people who suffer from HIV/Aids. Moreover, in Austria the oral spray Sativex®, which contains Δ^9-THC and CBD is applied for symptomatic improvements of multiple sclerosis patients.

Based on the therapeutic effects and the recent legalization of cannabis in some countries discussions about the general legal status have come up.

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Besides alcohol, tobacco and caffeine cannabis is worldwide one of the most misused drugs. On the European drugs market the two distinct products "marijuana" (herbal cannabis) and "hashish" (cannabis resin) are mainly traded. According to the European Drug Report 2014, 73.6 million (21.7%) adults (15–64) used cannabis in their lifetime, while 18.1 million (5.3%) adults (15–64) and 14.6 million (11.2%) young adults (15–34) consumed cannabis in the last year. The prevalence of the misuse of these herbal products is pronounced due to the fact that over 80% of the seizures in Europe are cannabis [3].

In literature, several articles dealing with the analyses and determination of cannabinoids in plant material [4–6], urine [7,8], blood [8,9], oral fluid [8,10,11] as well as hair [12] using various chromatographic methods are available [13].

However, gas chromatography is one of the most common used techniques for analysis of cannabinoids [11,14–20].

In 1973, Turner and his group carried out stability tests of cannabinoids in plant material by GC coupled with hydrogen flame-ionization detectors. Plant material was stored at various temperatures for 104 weeks [21].

Also, the influence of long-term storage conditions at 4 °C and 22 °C on the stability of cannabinoids was reported by Trofin et al. [22,23].

In 2014, Ambach et al. succeeded in simultaneous quantification of Δ^9-THC, THCA, CBD and CBN in seized drugs using HPLC-DAD [24].

Hazekamp's group reported on an evaluation of the cannabinoid composition of cannabis tea. In their study preparation parameters, effects of solubilizers as well as the storage and the stability of tea samples were described [25].

Besides analytical studies about determination and quantification of cannabis, many papers report about the therapeutic effects of cannabinoids [26–30].

In Austria there are thresholds for Δ^9-THC (20 g with respect to the total quantity) and THCA (40 g with respect to the total quantity) cited according to the Narcotic Substances Limit Quantities Decree (BGBl. II Nr. 371/2014), which are crucial for the criminal proceeding of the defendant. Public prosecution may request a determination of the entire Δ^9-THC content of the seized plant material by gas chromatography (decarboxylation of THCA to Δ^9-THC due to heating) or analysis of the original content of the two controlled constituents by HPLC. Based on the paper of De Backer et al. [31], who reported also on cannabinoid concentrations in different types of herbal cannabis products, a theoretical existence of 92% of THCA and 8% Δ^9-THC has often been used as basis for the theoretical content calculation in favor of the defendant. To verify this given ratio, a large number of seized plant material should be determined and the percentage distribution of the two cannabinoids should be calculated. Additionally, the impact of different temperatures on the decomposition of THCA as well as the expected time to its full degradation should be elucidated.

Therefore, the aim of our research was to determine the distribution as well as the influence of different temperatures on the decomposition process of THCA to Δ^9-THC.

2. Materials and methods

2.1. Chemicals and solutions

All chemicals were of analytical grade.

Acetonitrile, ethyl acetate and *n*-hexane were obtained from Carl Roth (Karlsruhe, Germany). Triethylamine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphoric acid (85%) was bought from VWR (Darmstadt, Germany). Dronabinol (Δ^9-THC) and THCA standards were from THC Pharm GmbH (Frankfurt/Main, Germany). Δ^9-THC and THCA standards had a purity of >95% and 97.3%, respectively. Millipore water was prepared in our laboratory (Millipore, Darmstadt, Germany).

Cannabis samples were seized by Austrian Police.

Mobile phase was prepared by mixing triethylammoniumphosphate buffer pH 3.0 (25 mmol/l in nanopure water) and acetonitrile in the required ratio of 36:64. Afterwards the solution was degassed in the ultrasonic bath for 2 min.

2.2. Chromatographic conditions

Content determination was performed using a HP Hewlett Packard Series II, 1090, Liquid Chromatograph, equipped with an auto sampler and a diode array detector. Measurements were carried out under isocratic conditions at 40 °C with a flow rate of 1.5 ml/min and an injection volume of 25 μl. UV-detection was performed at 210 nm.

Data were evaluated with Chemstation Rev. A. 0903 (Agilent Technologies, Waldbronn, Germany) software.

A LiChrospher® 100 RP-18 (5 μm) LiChroCART® 125-4 from Merck KGaA (Darmstadt Germany) served as stationary phase.

2.3. Sample preparation/extraction procedure

Freshly seized cannabis samples were first air-dried at ambient temperature in the laboratory fume hood. However, mostly seized plant material was already dry. Afterwards herbal material was ground with a Kenwood CG100 coffee grinder (Kenwood Ltd, Havant, UK). Samples of marijuana (50 mg) were extracted in 25 ml *n*-hexane/ethyl acetate (9:1) for 20 min in the ultrasonic bath. Then extracts were filtered and 2 ml were transferred into a 10 ml volumetric flask. Solvent was removed under a gentle nitrogen stream and the dry residues were dissolved in mobile phase. Dissolving process was accelerated by an ultrasonic bath.

2.4. Calculation of THCA and Δ^9-THC content

Calibration curves of both THCA and Δ^9-THC were prepared by diluting stock solutions. Stock solution of each compound contained 1 mg/ml standard, which was diluted 1:10 (=100 μg/ml). Then, calibration points from 70 μg/ml to 10 μg/ml in intervals of ten were made. Additionally 5 μg/ml, 3 μg/ml and 1 μg/ml were prepared. Correlation coefficients (R) for THCA and Δ^9-THC calibration curves were 0.99998 and 0.99994, respectively.

3. Results and discussion

We carried out stability experiments and content determinations of the two cannabinoids, Δ^9-THC and THCA by HPLC-UV using a mobile phase presented by Ambach et al. [24], who validated their method.

3.1. Definition of the percentage distribution of Δ^9-THC and THCA in plant material

For the determination of the relative percentage distribution of Δ^9-THC and THCA in fresh plant material, 29 seized cannabis samples were analyzed. The majority of this plant material was already dried. When we received the samples, we attached importance on immediate analysis. In Table 1, the obtained results are presented. Obviously it is demonstrated that plant material is not homogenous and therefore considerable variations in content concentration may occur. Relative percentages of Δ^9-THC ranged between 2% and 25% while the values of THCA were between 75% and 98%, respectively. As it is shown in Table 1, the average relative