Forensic Science International 254 (2015) 167–171

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

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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ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 12 June 2015
Accepted 7 July 2015
Available online 17 July 2015

This work is dedicated to Austrian Public Prosecution.

Keywords:
Δ^9-Tetrahydrocannabinol (Δ^9-THC)
THCA
Content determination
Stability test under temperature influence
High-performance liquid chromatography

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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http://dx.doi.org/10.1016/j.forsciint.2015.07.019
0379-0738/

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

Table 1
Percentage distribution of Δ^9-THC and THCA in fresh plant material after drying. The absolute percentage (%-absol.) represents the actual concentrations of Δ^9-THC and THCA, whereas the relative percentage (%-rel.) of both cannabinoids describes the ratio with respect to their sum (100%).

Sample	% Δ^9-THC absol.	% THCA absol.	% Δ^9-THC rel.	% THCA rel.
1	1.32	11.64	10.19	89.81
2	1.99	7.62	20.71	79.29
3	3.60	11.37	24.05	75.95
4	4.11	13.76	23.00	77.00
5	2.64	11.94	18.11	81.89
6	1.95	18.78	9.41	90.59
7	3.17	12.33	20.45	79.55
8	1.02	15.82	6.06	93.94
9	1.66	9.74	14.56	85.44
10	2.17	12.2	15.10	84.90
11	2.21	13.32	14.23	85.77
12	2.11	16.18	11.54	88.46
13	1.50	19.77	7.05	92.95
14	3.36	10.14	24.89	75.11
15	3.71	12.23	23.27	76.73
16	1.54	5.03	23.44	76.56
17	4.47	13.74	24.55	75.45
18	5.23	17.42	23.09	76.91
19	3.87	20.98	15.57	84.43
20	0.48	21.56	2.18	97.82
21	2.90	16.01	15.34	84.66
22	1.82	7.16	20.27	79.73
23	3.18	17.09	15.69	84.31
24	3.79	20.30	15.73	84.27
25	1.11	18.14	5.77	94.23
26	0.90	17.77	4.82	95.18
27	1.32	16.13	7.56	92.44
28	5.22	16.11	24.47	75.53
29	1.62	20.48	7.33	92.67
			% THC rel.	% THCA rel.
Average			15.46 ± 7.08	84.54 ± 7.08

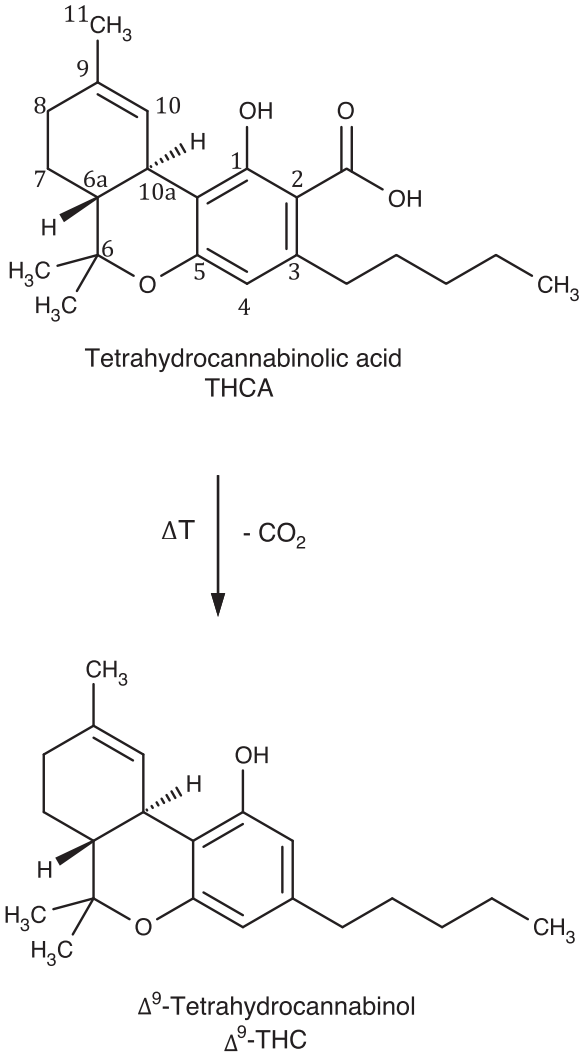

Fig. 1. Chemical structures of tetrahydrocannabinolic acid and Δ^9-tetrahydrocannabinol. Biosynthetic decomposition of tetrahydrocannabinolic acid to Δ^9-tetrahydrocannabinol through heating (ΔT).

percentage of Δ^9-THC was about 15.46% and 84.54% of THCA. These results demonstrate that the theoretical average percentage distribution would enable a different prosecution. Therefore, the analysis of each seized plant material is advisable as concentrations of Δ^9-THC and THCA vary from factors such as storage temperature, UV exposure or cannabis species. These factors may also explain the obtained error bars.

3.2. Influence of the storage temperature on the decomposition process of THCA and Δ^9-THC

To check the impact of the storage temperature on the decarboxylation process of THCA to Δ^9-THC, samples were stored at various temperatures for a specified time period.

Fig. 1 shows the decomposition process of THCA to Δ^9-THC due to decarboxylation during influence of high temperature in case of smoking or baking, for instance.

Stability tests were performed at 50 °C, 100 °C and 150 °C. For these experiments, samples were stored in a drying cabinet for 24 h. Samples stored at 50 °C were taken at intervals of 2 h for 8 h. During the analyses at 100 °C and 150 °C, samples were drawn hourly for 8 h to precisely monitor the degradation process of the two cannabinoids. After 24 h final samples were drawn for each temperature program to calculate the final concentrations. To ensure accuracy of the results, double determinations of the cannabis samples were performed. In Fig. 2, decomposition of THCA at 50 °C, 100 °C and 150 °C is represented graphically. Storage of plant material at 50 °C showed only slight influence on the decarboxylation process, since concentration changed from 12.21% to 11.69%. This caused an increase of Δ^9-THC from 1.51% to 2.12% (Fig. 3). Comparing these results with those of 100 °C and 150 °C, a remarkable decarboxylation of THCA was detected. Storage at these two temperatures led to an accelerated and complete decomposition of THCA, which was decarboxylated within 2 h and 1 h, respectively (Fig. 2). In comparison to Δ^9-THC, after 3 h at 100 °C storage temperature the maximum concentration of 12.28% was achieved, which decreased finally to 4.79% after 24 h. Using 150 °C, the maximum of 12.77% Δ^9-THC was already obtained after 1 h and the final concentration was about 0.19% after 24 h, indicating also the decomposition process of Δ^9-THC after intense thermal treatment. It is known that also decomposition of Δ^9-THC takes place at higher temperatures, as it is the case at analysis by GC. As a consequence, GC measurements show a slightly lower value of the sum of Δ^9-THC/THCA than obtained by HPLC analysis.

When baking hemp cookies faster decomposition of THCA is expected as baking temperatures are usually above 170 °C.

Additionally, cannabis samples stored at −25 °C for about 4 months were analyzed again to check stability of the two cannabinoids. In this case, percentage distribution of THCA and Δ^9-THC remained stable, which is in accordance with the study of Turner et al. [21]. They stored cannabis plant material at −18 °C for over 100 weeks. Samples were stable for about 30 weeks. Between 50 and 60 weeks changes in concentration took place.

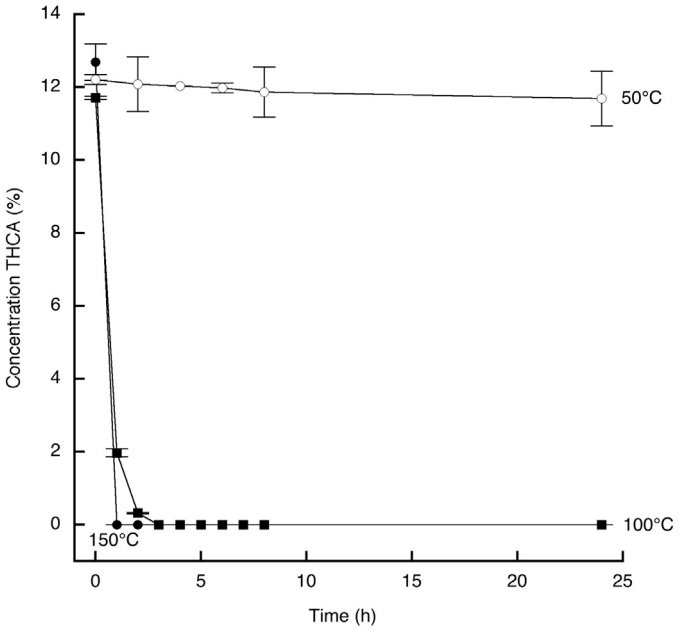

Fig. 2. Decomposition of tetrahydrocannabinolic acid in plant material over 24 h at 50 °C, 100 °C and 150 °C storage temperature in a drying cabinet. The graph shows decrease of concentration of tetrahydrocannabinolic acid versus time.

Moreover, 4 samples were stored protected from light at 25 °C for 12 months. Afterwards they were extracted and measured as described previously. As in Table 2 presented, only slight changes of the percentage distribution were obtained. These results are in good accordance with the work of Turner's group [21], however, they stored their samples at 22 °C.

In this study, it was shown successfully that the average percentage ratio of Δ^9-THC and THCA was $15.46 \pm 7.08\%$ to $84.54 \pm 7.08\%$, respectively. Furthermore, stability tests proved that only temperature higher than 50 °C led to an accelerated and complete decarboxylation of THCA. The concentration of Δ^9-THC

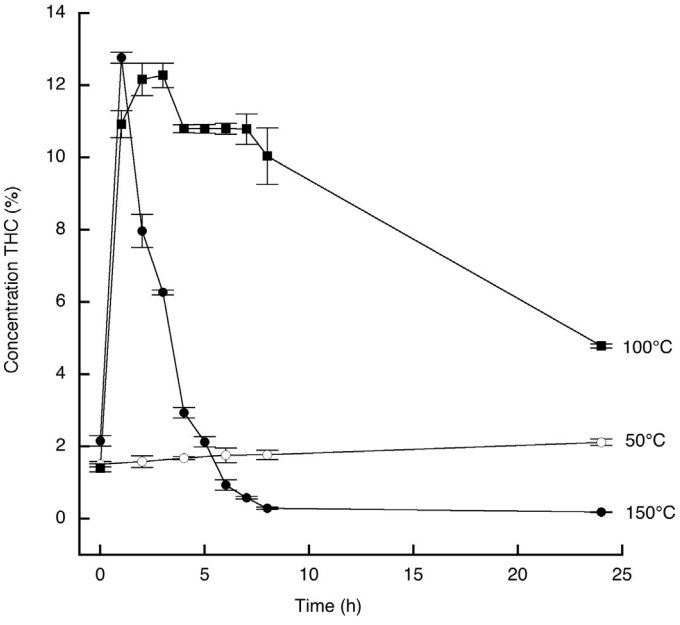

Fig. 3. Determination of Δ^9-tetrahydrocannabinol content in plant material at three different storage temperatures (50 °C, 100 °C and 150 °C) for 24 h. The graph shows changes of Δ^9-tetrahydrocannabinol concentration versus time.

Table 2
Comparison of the absolute percentage of Δ^9-THC and THCA of freshly dried cannabis and after 12 months storage at room temperature protected from light.

Sample	% Δ^9-THC		% THCA	
	Freshly dried	After 12 months storage at 25 °C	Freshly dried	After 12 months storage at 25 °C
1	2.00	5.55	12.95	10.70
2	1.22	3.23	12.59	11.03
3	1.15	2.60	9.40	7.96
4	1.40	3.54	6.63	7.58

suffered from high temperature and was therefore decreased as well. The obtained results underline the fact that storage of seized plant material in a freezer did not influence the decomposition of THCA to Δ^9-THC. Storage at ambient temperature caused only slight concentration changes.

4. Conclusion

Cannabis is doubtless the most abused and controversially discussed illicit drug worldwide. In many studies it is confirmed as a gateway drug [32,33], which is increasingly misused regardless of age and social classes.

In the presented study, the relative percentage distribution of the two cannabinoids Δ^9-THC and THCA in 29 herbal cannabis samples was determined. Additionally, stability tests concerning low as well as high storage temperatures were performed. It was shown that stress tests only at 100 °C and 150 °C led to a complete decarboxylation of THCA within short time. Storage in the freezer for some months did not influence the decomposition process considerably while storage at ambient temperature led to slight changes of Δ^9-THC/THCA ratio.

The presented data should bring more insight to clarify time-depending and temperature-depending storage of cannabis. This study should be helpful for public prosecutors.

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