Determination of Cannabidiol and Additional Cannabinoid Content in Hemp Tea

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1. Introduction

Cannabis contains more than 500 unique compounds, including over 80 chemical alkaloids known as cannabinoids. Numerous health benefits have been reported that are attributed to their pharmacological characteristics, which allow for use as medical treatment. They can affect physiological processes, such as inflammation, pain perception and seizures, which is a reason for the growing interest in “Medical Cannabis” [1, 2]. While use of cannabis for medicinal purposes is still subject to a lot of debate in Europe, hemp products containing < 0.3 % of the psychoactive compound d9-tetrahydrocannabinol (THC) have always been legal in most countries. With the growing interest in the cannabis plant, the market for cannabidiol (CBD) containing food and cosmetics products is also increasing.

Quantification of cannabinoids is essential for the accurate labelling of hemp products, for quality control, as well as to establish legality with regards to THC content.

In this work, High Performance Liquid Chromatography (HPLC) is the method of choice for analysis of cannabinoid content in different CBD rich hemp tea samples. The HPLC-UV method used provides good linearity, low limit of detection, as well as high precision of retention time and peak area for the cannabinoids under investigation.

2. Materials and Methods

2.1 Analytical Conditions

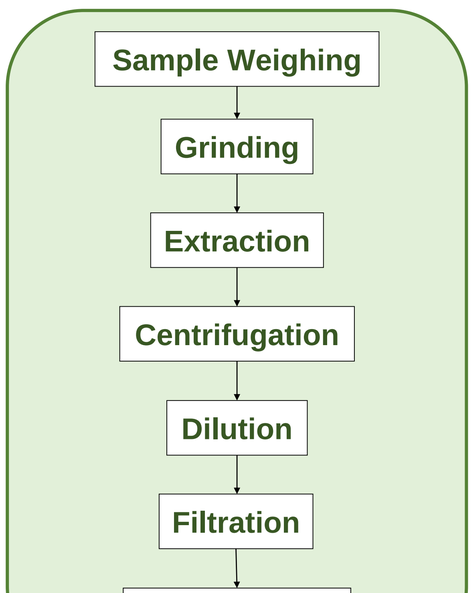
Simultaneous analysis of cannabinoids was performed using a Shimadzu Prominence-i HPLC system equipped with an UV-Vis detector. Chromatographic separations were carried out using a C18 modified separation column. Analytical conditions are further specified in Table 1.

Tab. 1: Analytical conditions.

LC system:	Prominence-i – LC-2030C Plus
Column:	Shim-pack XR-ODS II, 75 mm L x 3.0 mm, 2.2 µm with guard column
Mobil phase:	H_2O / MeOH / H_3PO_4
Flow rate:	1 mL/min
Elution mode:	Gradient
Oven temperature:	50 °C
Injection volume:	5 µL
Detection:	UV at 220 nm

2.2 Sample Preparation

Sample pre-treatment is illustrated in Figure 1 (left).



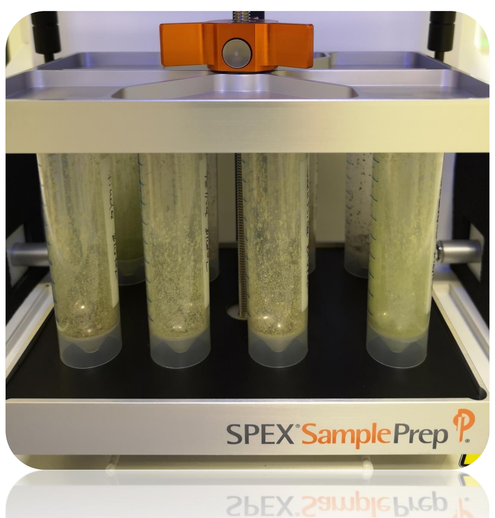


Fig. 1: (left) Sample pretreatment procedure; (right) ground hemp tea samples

i. Hemp tea samples were weighed
ii. Samples were placed in centrifuge tubes with two steel balls
iii. Samples were homogenized using a 2010 Geno/Grinder from SPEX® SamplePrep (Fig. 1 (right))
iv. Cannabinoids were extracted into methanol
v. Extract mixture was centrifuged
vi. Supernatant was diluted with methanol
vii. Diluted samples were filtered
viii. Filtered samples were analyzed by HPLC

3. Results

3.1 Calibration curves and precision of retention time and peak area

Calibration curves in a concentration range 0.5 to 50 mg/L for each compound demonstrated high linearity R^2 > 0.9999. The relative standard deviation (RSD %) for retention time and each peak area from six consecutive analyses were ≤ 0.018 % and 0.197 %, respectively (Table 2). Furthermore, resolution R according to European Pharmacopeia for psychoactive cannabinoids Δ^9-THC and Δ^8-THC was obtained with R = 2.6.

Table 2: Results of determination of precision (RSD [%], n = 6) of retention time and peak area for a 10 mg/L standard mixture

Cannabinoid	Retention Time	Peak Area
Canabdivarin (CBDV)	0.018	0.131
Tetrahydrocannabdivarin (THCV)	0.015	0.108
Cannabidiol (CBD)	0.012	0.098
Cannabigerol (CBG)	0.009	0.099
Cannabidiolic acid (CBDA)	0.009	0.119
Cannabigerolic acid (CBGA)	0.009	0.113
Cannabinol (CBN)	0.008	0.138
Δ^9-Tetrahydrocannabinol (Δ^9-THC)	0.007	0.108
Δ^8-Tetrahydrocannabinol (Δ^8-THC)	0.007	0.113
Cannabichromene (CBC)	0.007	0.104
Δ^9-Tetrahydrocannabinolic acid (THCA)	0.009	0.197

3.2 Sample Analysis

Five different samples were analyzed for CBD and CBDA content. Figure 2 shows an overlay of chromatograms for samples and a standard mixture with 10 mg/L for each compound.

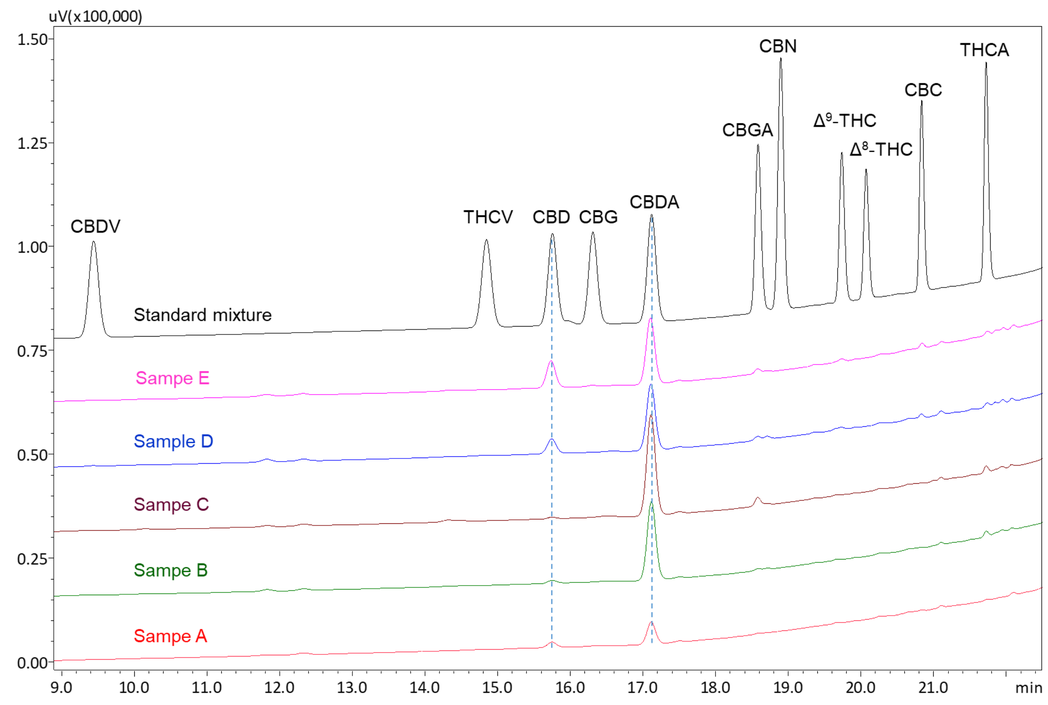


Figure 2: Chromatograms of the 10 mg/L standard mixture and hemp tea samples.

The CBD concentration in all samples was low or under LOD (Sample C) compared to CBDA. The total CBD content was calculated taking into account decarboxylation factor according to:

$$\text{Total CBD [wt.\%]} = [\%\ \text{CBD}] + 0.877 \times [\%\ \text{CBDA}]$$

Furthermore, a small amount of CBGA (< 0.08%) was determined in bud samples C, D and E. Psychoactive Δ^9-THC was not determined or was under limit of quantification.

Table 3: Concentration of CBD, CBDA and total CBD content in hemp tea samples

Sample	Label Claim [%] CBD	Dry weight [%] CBD	Dry weight [%] CBDA	Total CBD [%]
A (leaves)	not specified	0.11	0.41	0.47
B (leaves)	not specified	0.06	1.47	1.35
C (buds)	0.014	---	1.87	1.64
D (buds)	3.7	0.31	1.22	1.38
E (buds)	0.014	0.57	1.24	1.67

4. Conclusion

The presented method for analysis of cannabinoids was suitable for analysis of hemp tea from leaves and buds. The total content of CBD found in the dry teas samples was in the range 0.47 - 1.67 %, with % CBDA significantly higher than CBD. The results showed a discrepancy between determined content and label claim. However, the label stated cannabinoid content in brewed tea, not in dry sample. In a follow up experiment, cannabinoid content will be determined in tea brewed according to label instruction (1.5 g in 200 ml hot water).

References

[1] Perry G. Fine, Mark J. Rosenfeld, Rambam Maimonides Medical Journal, October 2013, Volume 4, Issue 4.
[2] Klein TW, Newton CA, Adv Exp Med Biol. 2007;601:395-413.