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Simultaneous quantification of delta-9-THC, THC-acid A, CBN and CBD in seized drugs using HPLC-DAD☆

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ABSTRACT

An HPLC–DAD method for the quantitative analysis of Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid-A (THCA-A), cannabidiol (CBD), and cannabinol (CBN) in confiscated cannabis products has been developed, fully validated and applied to analyse seized cannabis products. For determination of the THC content of plant material, this method combines quantitation of THCA-A, which is the inactive precursor of THC, and free THC. Plant material was dried, homogenized and extracted with methanol by ultrasonication. Chromatographic separation was achieved with a Waters Alliance 2695 HPLC equipped with a Merck LiChrospher 60 RP-Select B (5 μm) precolumn and a Merck LiChroCart 125-4 LiChrospher 60 RP-Select B (5 μm) analytical column. Analytes were detected and quantified using a Waters 2996 photo diode array detector. This method has been accepted by the public authorities of Switzerland (Bundesamt für Gesundheit, Federal Office of Public Health), and has been used to analyse 9092 samples since 2000. Since no thermal decarboxylation of THCA-A occurs, the method is highly reproducible for different cannabis materials. Two calibration ranges are used, a lower one for THC, CBN and CBD, and a higher one for THCA-A, due to its dominant presence in fresh plant material. As provider of the Swiss proficiency test, the robustness of this method has been tested over several years, and homogeneity tests even in the low calibration range (1%) show high precision (RSD ≤ 4.3%, except CBD) and accuracy (bias ≤ 4.1%, except CBN).

1. Introduction

An HPLC-method for the quantitative detection of Δ^9-tetrahydrocannabinolic acid-A (THCA-A), Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) in confiscated cannabis products has been developed, fully validated and applied to analyse seized cannabis products. In *Cannabis sativa*, THCA-A is the non-psychoactive precursor of THC. In fresh plant material, about 90% of the total THC is available as THCA-A. When heated (smoked or baked), THCA-A is to a major extent converted to THC.

Methods for the analysis of THC and THCA-A in cannabis plant material [1–3], vegetable oils [4] as well as biological matrices, such as urine [5], serum [5,6], and hair [7] and also together with CBD and CBN [8] have been reported. For forensic analysis the total THC content must be determined. Standardized methods for the determination of cannabinoids in cannabis material rely on conversion of THCA-A to THC by either thermal conversion prior to HPLC or GC analysis or by in-situ conversion by injection into a heated GC injection port [1]. Differences in decarboxylation of THCA-A can yield different results, if THC is analysed by HPLC, and differences in efficiency of decarboxylation have been found by Dussy et al. [2]. Too drastic reaction conditions during decarboxylation (temperature and time) may result in ring-opening of THC or in oxidation, thus producing a loss in total THC. In contrast to practical thermal decarboxylation, the presented method combines quantitation of both analytes, THCA-A and THC without decarboxylation, thus avoiding the aforementioned problems.

The ratio of CBN to THC can be used as an indicator for sample deterioration [9] due to oxidative aromatization, e.g. by prolonged storage of cannabis products exposed to air and in the production process of hashish [10]. As an additional criterion besides the total THC content itself, the CBD-to-THC ratio can be used for the distinction of fibre/industrial hemp from drug-grade cannabis. In fibre hemp, CBD is the major cannabinoid and the CBD content in these cases is generally higher than the total THC content [11]. CBD has also been reported to modify the effects of THC and to have anti-anxiety and anti-psychotic effects [12,13].

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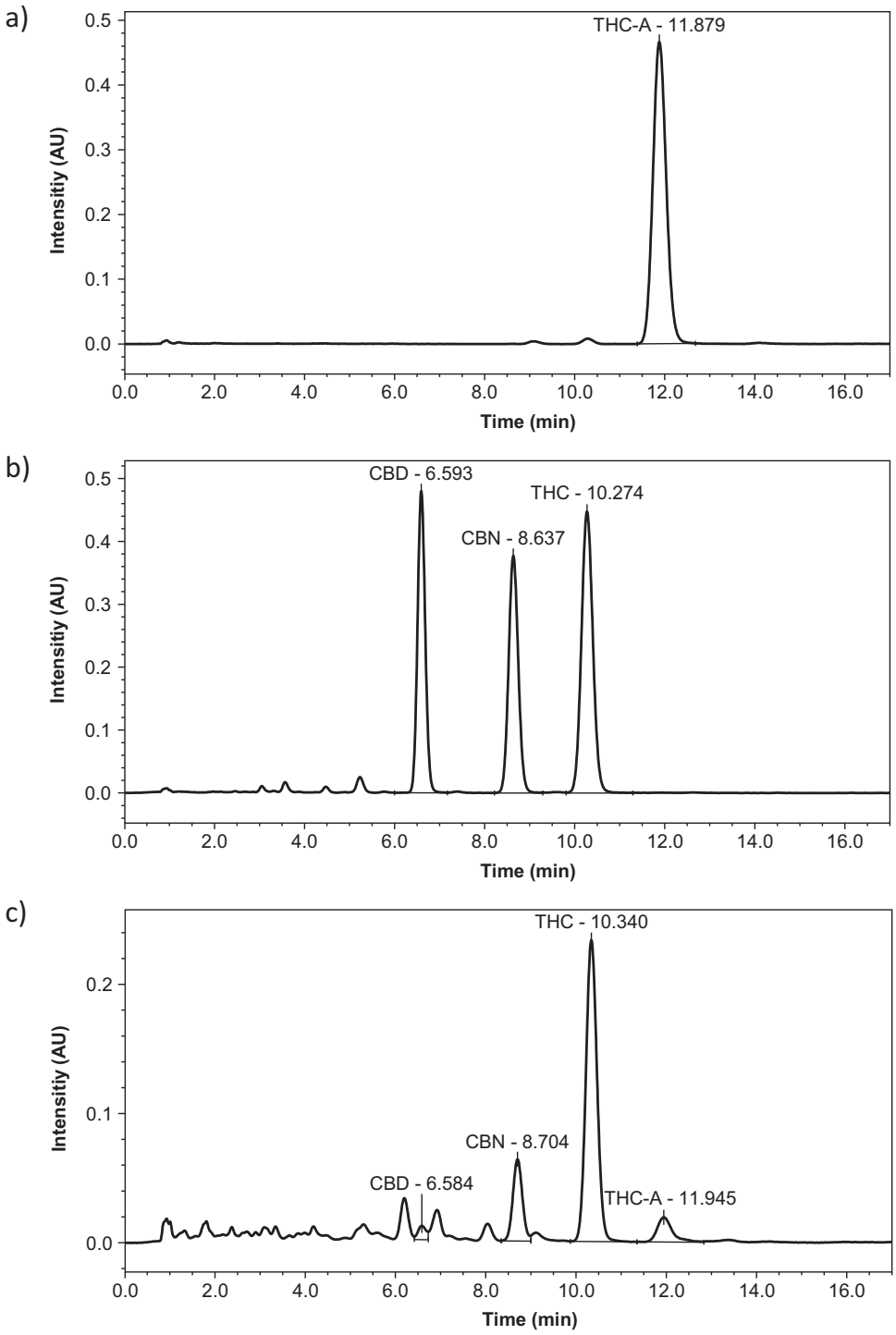

Fig. 1. Typical chromatograms for the reference standards CBD, CBN and THC (a) and THC-Acid A (b) which are measured in two separate runs, since concentrations are different in the calibration sets (run time 18 min). (c) Typical chromatogram of an authentic sample.

Due to the availability of certified reference standards, the development of this method became possible. This method has been accepted by the public authorities of Switzerland (Bundesamt für Gesundheit), and since no thermal decarboxylation of THCA-A is necessary, the method is highly reproducible for different cannabis materials. Validation data are presented, also precision data from a homogeneity test of proficiency test material supplied by our laboratory for an annual national proficiency test program.

2. Material and methods

Materials used: *n*-Hexane p.a., methanol (LiChrosolv), acetonitrile gradient grade, Milli-Q-water (ultra-pure water type1), triethylammoniumphosphate 1 M (TEAP), stored in refrigerator 5–8 °C, reference standards: THC, CBD and CBN as solutions 1 mg/mL, and THCA-A 10 mg powder used for preparation of stock solution (supplied by Lipomed, Weil, Germany)