Hair analysis for cannabidiolic acid (CBDA) and cannabidiol (CBD) – method validation, application to authentic samples and its implications for practitioners

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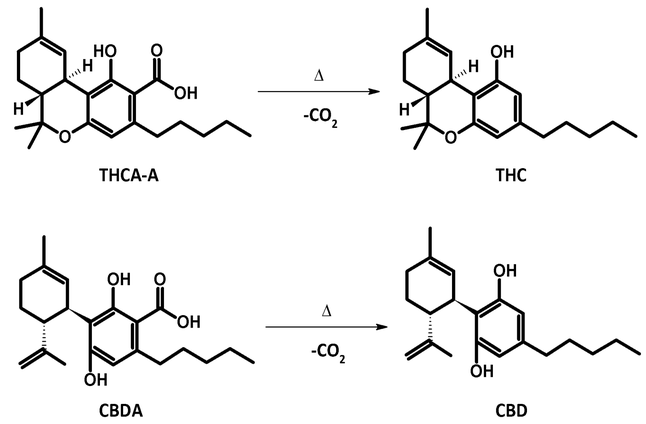
Introduction

In recent years, various new aspects in the context of hair analysis for cannabinoids arose which have to be considered when analyzing hair samples and interpreting the results [1]. In this context, a compound often analyzed as a plausibility check is cannabidiol (CBD). In analogy to THC, this analyte is not produced by the cannabis plant but derives from decarboxylation of a biogenetic precursor, cannabidiolic acid (CBDA). So far, the presence of CBDA in hair samples has not been investigated.
Applying methanolic extraction and LC-MS/MS for the analysis of CBD in hair samples, the analyte could not be detected in numerous hair samples [2]. This observation could be explained on the one hand by the fact that much of the seized marijuana does not contain relevant amounts of CBD. However, another explanation could be that, similar to THCA-A, much of the analyte might be present in the form of CBDA in hair. If the second would be the case, this may lead to the same analytical issues encountered with THCA-A and THC.
The aim of the present study was to develop a method for the sensitive detection of CBDA and CBD in hair and to assess, if CBDA is present in THC positive hair samples in relevant amounts.



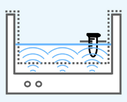
Formation of THC and CBD by decarboxylation of THCA-A and CBDA, respectively.

Methods

Hair sample preparation

washing with 4 ml water, 4 ml acetone, and 4 ml petroleum ether

20 mg hair + 1.5 ml methanol
+
20 µl internal standard (IS)



3 h ultrasonication

- evaporation to dryness
- reconstitution in mobile phase B
- LC-MS/MS analysis

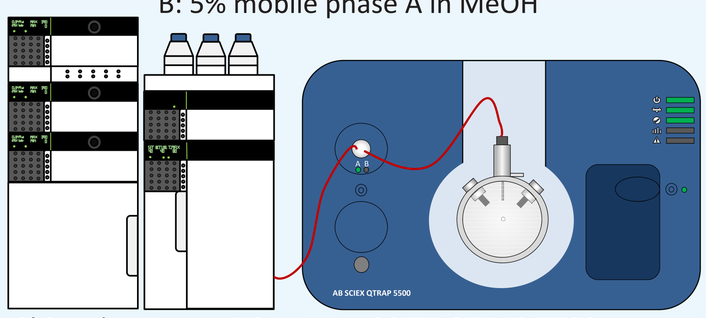
LC-MS/MS

An LC-MS/MS method covering CBDA, CBD, THCA-A, and THC was validated according to the guidelines of the GTFCh [3].

Gradient:

Time	% B
0 - 1 min	20
1 - 2 min	60
2 - 6 min	100
6 - 7.5 min	100
7.5 - 7.6 min	10
7.6 - 10 min	10

A: 0.1% HCOOH (v/v) in deionized water
B: 5% mobile phase A in MeOH



Shimadzu Nexera X2 SCIEX QTRAP 5500

Column:
Kinetex 2.6 µm XB-C_{18} column (100 x 2.1 mm)

Analyte	Q1 mass [Da]	Q3 mass [Da]	CE[V]	CXP [V]	assigned IS
THCA-A	357	**313**	-34	-7	THCA-A-D_3
		245	-43	-5	
CBDA	357	**339**	-29	-15	THCA-A-D_3
		179	-30	-15	
THC	315	**193**	34	5	THC-D_3
		259	28	5	
CBD	315	**193**	34	5	CBD-D_3
		259	28	5	
THCA-A-D_3	360	316	-34	-7	
THC-D_3	318	196	34	5	
CBD-D_3	318	196	34	5	

Entrance potential was set to +/-8 V
Data in bold are ion transitions used for quantification
CE: Collision energy, CXP: Collision cell exit potential, IS: internal standard

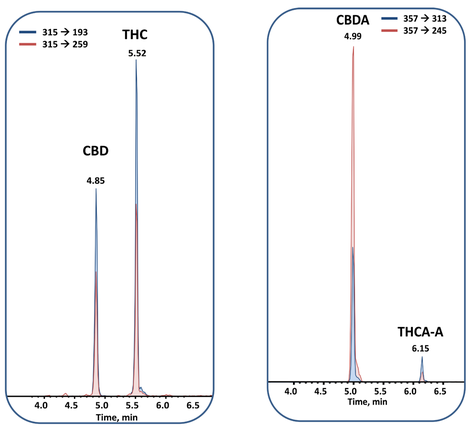
Hair samples

1.) Authentic forensic case work hair samples previously tested positive for THC
2.) One volunteer rubbed hash into his hair to simulate external contamination. One week later head hair samples were collected.

Results and discussion

Method development

A sufficient chromatographic separation is essential for an unambiguous identification of the respective compounds, as the isobars THC and CBD show an almost identical fragmentation pattern and the isobars THCA-A and CBDA a very similar fragmentation pattern.



Validation

The method was successfully validated according to the guidelines of the GTFCh.

Lower limit of quantification:
CBDA: 9 pg/mg
CBD: 15 pg/mg
THCA-A: 14 pg/mg
THC: 19 pg/mg

Linearity:
CBDA: 10 - 1000 pg/mg
CBD: 20 - 1000 pg/mg
THCA-A: 10 - 1000 pg/mg
THC: 20 - 1000 pg/mg

Authentic samples

Sample	Length [cm]	THCA-A [pg/mg]	THC [pg/mg]	CBDA [pg/mg]	CBD [pg/mg]
#1	17.0	27	< LOQ	< LOQ	n.d.
#2	11.0	47	< LOQ	< LOQ	< LOQ
#3	11.0	133	32	12	< LOQ
#4	36.0	34	< LOQ	12	< LOQ
#5	13.0	1018	559	37	370
#6	30.0	795	373	66	41
#7	15.0	27	n.d.	< LOQ	< LOQ

External contamination

a.) Methanolic extraction under ultrasonication (see above)

Length [cm]	THCA-A [pg/mg]	THC [pg/mg]	CBDA [pg/mg]	CBD [pg/mg]
3	62	< LOQ	36	54

b.) Alkaline hydrolysis
- 10 min 90°C in 1M NaOH
- LLE extraction with n-hexane:ethylacetate (9:1)
- LC-MS/MS analysis (see above)

Length [cm]	THCA-A [pg/mg]	THC [pg/mg]	CBDA [pg/mg]	CBD [pg/mg]
3	< LOQ	42	< LOQ	97

Conclusion

CBDA, the biogenetic precursor of CBD in the cannabis plant, was detected for the first time in hair samples. Similar as shown for THCA-A and THC, alkaline hydrolysis leads to an artefactual elevation of CBD concentration in hair samples, explaining former findings of relatively low CBD concentrations after methanolic extraction. Analysis for CBDA and THCA-A in hair can be useful to differentiate medicinal use of cannabis products (e.g. Dronabinol, Sativex) from illicit cannabis use.

References

[1] Moosmann et al. Finding cannabinoids in hair does not prove cannabis consumption. Sci Rep 5, 14906. DOI: 10.1038/srep14906, **2015**

[2] Roth et al. Development and validation of an LC-MS/MS method for quantification of Δ9-tetrahydrocannabinolic acid A (THCA-A), THC, CBN and CBD in hair. J Mass Spectrom, 48(2), p. 227-233, **2013**

[3] GTFCH, Toxichem Krimtech, Volume 76, Booklet 3, p. 209-216, **2009**

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