

Comparing CBD aroma oils with HPLC-UV

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SUMMARY

Seven different aroma oils from various manufacturers were tested using a validated cannabinoid analysis method and a KNAUER AZURA® HPLC system. The analysis method can be used to quantify 6 different cannabinoids and to qualify 16 cannabinoids within 22 min. Here it was demonstrated that this method can be used to verify the compliance of cannabis-derived products with German narcotics laws [1], meaning the method can be used for quality control purposes. In this case all of the tested aroma oils were below the critical limit of 0.2% total THC content, while three of the products had no THC content at all.

INTRODUCTION

Cannabidiol (CBD) in various products, such as tea, chocolate, gum, or oils has been a hot topic for several years now. Compared to Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) products containing the non-psychoactive CBD does not require a special authorisation via the Federal Institute for Drugs and Medical Devices (Bundesopiumstelle of the Bundesinstitut für Arzneimittelzulassung, BfArM) [2]. The legal situation regarding the sale of CBD-containing products is still not completely clarified. There are constant changes in the declaration of the products. For example, there are several requests to the Novel Food Regulation (EU 2015/2283) where

manufacturers are re-evaluating their foodstuffs to obtain approval of their products [3]. Nevertheless, to verify the products compliance quality control is required. This is especially the case when measuring the total THC content (Δ^9 -THC, Δ^8 -THC, Δ^9 -THCA), a critical parameter that may not be exceeded by 0.2%. For all foodstuffs different limits of THC content are set by the German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR). For example, alcoholic and non-alcoholic beverages should not exceed an amount of 5 $\mu\text{g}/\text{kg}$, whereas edible oils should fall below 5000 $\mu\text{g}/\text{kg}$ [4].



Additional
Information

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For continuous quality controls a High Pressure Liquid Chromatography system (HPLC) makes it possible to verify and monitor the quality of THC containing products to prevent product recalls, such as the one experienced by the discounter LIDL in August 2021 [5]. With this work an exemplary analysis of seven different CBD aroma oils was conducted to verify the cannabinoid content using the developed method from the Application Note [VPH0070](#) [6].

SAMPLE PREPARATION

50 mg of oil was extracted in 10 ml methanol for 15 min inside an ultra-sonic bath, with shaking onto a vortex device every 5 min. After filtrating over a 0.20 µm PTFE syringe filter the solution was measured with the HPLC system. Each oil was prepared and measured in a double determination.

RESULTS

Following the German pharmacopeia method (DAB) [7] six cannabinoids (CBD, CBDA, CBN, Δ^9 -THC, Δ^8 -THC, Δ^9 -THCA) were quantified and a qualification and assignment can be made with 16 different cannabinoids (VPH0070) [6]. **Fig. 1** shows the overlay of an analysed sample with a cannabinoid standard. **Fig. 2** shows a closer look of the qualified cannabinoids. The calculation for the total amount of THC and CBD was made using a factor of 0.877, which resulted from dividing the molar masses of the neutral (CBD) towards the acid (CBDA) form.

For example, the total amount of CBD with single values of 1.94 % CBDA and 3.28 % CBD is 4.98 %, following the equation from (1.1).

$$[CBD_{Total}] = [CBDA] \times 0.877 + [CBD]$$

(1.1)

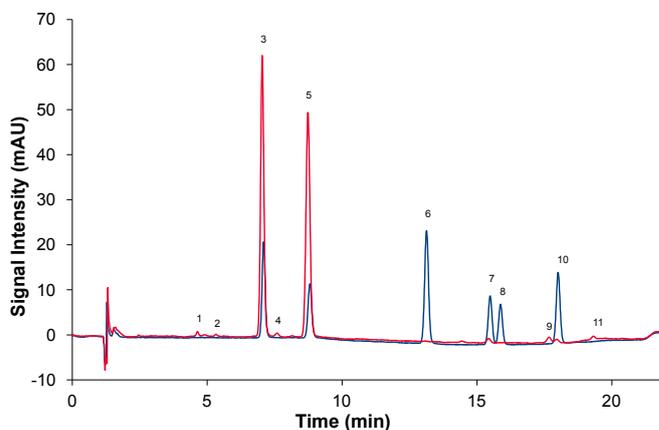


Fig. 1 Overlay of quantitative 10 µg/mL standard mix (blue) and sample 3, 1:10 dilution with MeOH (red) 1. CBDVA 2. CBDV 3. CBDA 4. CBGA 5. CBD 6. CBN 7. Δ^9 -THC 8. Δ^8 -THC 9. CBC 10. Δ^9 -THCA 11. CBLA

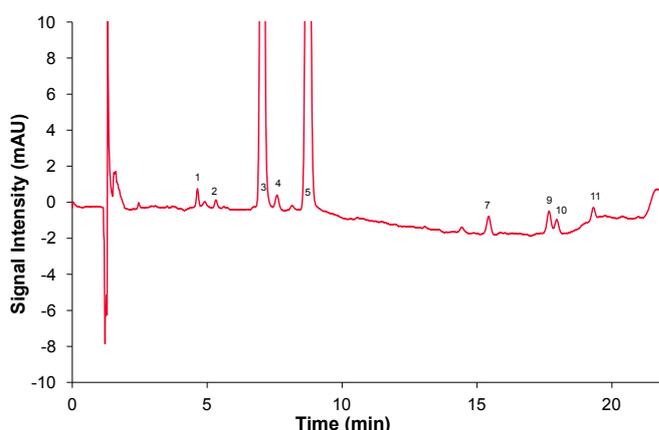


Fig. 2 Exemplary chromatogram from Sample 3, 1:10 dilution with MeOH, 1. CBDVA 2. CBDV 3. CBDA 4. CBGA 5. CBD 7. Δ^9 -THC 8. Δ^8 -THC 9. CBC 10. Δ^9 -THCA 11. CBLA

Using the, previously validated, method according to Guideline ICHQ2R1 the measurement of seven different CBD aroma oils was performed - the results of this analysis are shown in **Tab. 1**. These results are compared to the manufacturer's declared specification in **Tab. 2**. By means of total amount of THC or CBD the specification is fulfilled with the measurement. Only the deviation of 4.99 % towards 5.35 % of total CBD with sample 5 is not comparable.

Tab. 1 Results of the six validated cannabinoids

Summary			CBDA	CBD	CBN	Δ^9 -THC	Δ^8 -THC	Δ^9 -THCA
Repeatability	Time	RSD (%)	0.08	0.07	-	0.07	-	0.03
	Area		0.63	0.65	-	0.75	-	0.75
	Height		0.42	0.43	-	0.99	-	0.51
Calibration	R		0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
LOD*	-	($\mu\text{g/ml}$)	0.05	0.14	0.12	0.25	0.24	0.18
LOQ*	-		0.14	0.41	0.37	0.76	0.72	0.55
Recovery	Blank	(%)	101	99	99	99	101	101
	Spiked Sample		-	-	99	95	103	98

*LOD = Limit of Detection LOQ = Limit of Quantification

Tab. 2 Results in comparison with specification declared by manufacturer. Values are mean values by double determination

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Content (%)							
CBDA	0.07	0.01	2.24	0.01	0.03	0.03	0.05
CBD	5.09	7.20	3.85	5.20	4.96	5.02	5.77
Sum CBD	5.15	7.21	5.81	5.20	4.99	5.05	5.81
CBN	< LOQ	< LOQ	< LOQ	0.02	< LOQ	< LOQ	< LOQ
Δ^9 -THC	0.08	0.03	0.08	0.16	< LOQ	< LOQ	< LOQ
Δ^8 -THC	< LOQ	< LOQ	< LOQ				
Δ^9 -THCA	< LOQ	< LOQ	0.04	< LOQ	< LOQ	< LOQ	< LOQ
SumTHC	0.08	0.03	0.12	0.16	< LOQ	< LOQ	< LOQ
Specification							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Sum CBD	-	> 7 %	Min. 5 %	-	53.5 mg/g	5.03 %	-
Sum THC	< 0.2 %	0.1 %	< 0.2 %	< 0.2 %	< 0.05 mg/g	0.00 %	< 0.01 %

CONCLUSION

In summary, it was shown that the method used is appropriate for the qualification and quantification of different cannabinoids in CBD aroma oils. All given specifications from the manufactures were fulfilled, whereas the only deviation was with sample 5. A potential reason for

this deviation could be the use of a different sample preparation method or analytical determination. Most importantly, the legal limit of THC content below 0.2 % was undercut in every sample.

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MATERIALS AND METHODS

System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1l (LPG), with 10 ml pump head (stainless steel), degasser and mixer (200 µl)	APH34EA
Autosampler	AZURA AS 6.1l analytical HPLC autosampler, 700 bar	AAA00AA
Detector	AZURA MWD 2.1l multiwavelength detector	ADB01
Flow cell	Flow Cell Cartridge 10 mm path length, 10 µl, 300 bar	AMC38
Thermostat	AZURA CT 2.1 for up to 8 HPLC columns	ATC00
Column	Eurospher II 100-3 C18P, 150 x 4.6 mm	15VE182E2G
Software	ClarityChrom 8.3.	A1670

Method parameters

Column temperature	25 °C
Injection volume	10 µl
Injection mode	Partial loop, no headspace pressure, no air segment
Detection	228 nm, 306 nm
Data rate	10 Hz, 0.01 sec

Pump parameters

Eluent A	Water, HPLC grade, pH 2.2 with H ₃ PO ₄		
Eluent B	Acetonitril gradient grade		
Gradient	Time	A (%)	B (%)
	0	25	75
	7	25	75
	17	10	90
	19	10	90
	20	25	75
22	25	75	

Abbreviations

Cannabichromene	CBC
Cannabichromene acid	CBCA
Cannabidivarin	CBDV
Cannabidivarinic acid	CBDVA
Cannabidiol	CBD
Cannabidiolic acid	CBDA
Cannabigerol	CBG
Cannabigerolic acid	CBGA
Cannabinol	CBN
Cannabinol acid	CBNA
Cannabicyclol	CBL
Cannabicyclolic acid	CBLA
Δ ⁸ -Tetrahydrocannabinol	Δ ⁸ -THC
Δ ⁹ -Tetrahydrocannabinol	Δ ⁹ -THC
Δ ⁹ -Tetrahydrocannabinolic acid	Δ ⁹ -THCA
Δ ⁹ -Tetrahydrocannabivarin	THCV
Δ ⁹ -Tetrahydrocannabivarinic acid	THCVA



REFERENCES

- [1] Gesetz über den Verkehr mit Betäubungsmitteln (BtMG) Anlage I (zu § Abs.1) (nicht verkehrsfähige Betäubungsmittel), 2001.
- [2] Gesetz über den Verkehr mit Betäubungsmitteln „Betäubungsmittelgesetz in der Fassung der Bekanntmachung vom 1. März 1994 (BGBl. I S. 358), das zuletzt durch Artikel 1 der Verordnung vom 2. Juli 2018 (BGBl. I S. 1078) geändert worden ist“. BtMG, 1981
- [3] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015, 2015.
- [4] Bundesinstitut für Risikobewertung, BgVV empfiehlt Richtwerte für THC (Tetrahydrocannabinol) in hanfhaltigen Lebensmitteln, 07.2000.
- [5] J. Borsch, THC in Hanf Produkten: Wie viel ist erlaubt? DAZ, 26.08.2021
- [6] L. Loxterkamp, S. Stephan, K. Monks, (C)an(n)alyze: determination of 16 cannabinoids inside flowers, oils, and seeds, VPH0070, KNAUER Wissenschaftliche Geräte GmbH, 2019.
- [7] L. Loxterkamp, K. Monks, Analyzing cannabis flowers according to the German Pharmacopeia - monograph 2018, VPH0072, KNAUER Wissenschaftliche Geräte GmbH, 2019.

RELATED KNAUER APPLICATIONS

[VPH0070](#) - (C)an(n)alyze: determination of 16 cannabinoids inside flowers, oils and seeds

[VPH0072](#) - Analyzing cannabis flowers according to the German Pharmacopeia - monograph 2018