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Application News

No. SCA-190-051

High Performance Liquid Chromatography

Quantitation of Cannabinoids in Cannabis Dried Plant Materials, Concentrates and Oils based on AOAC Official Method 2018.11

Part I: PDA Analysis

User Benefits

Although Shimadzu provides with the Cannabis Analyzer an economical and robust solution for quantitative analysis of cannabinoids, some laboratories need/want to work based on AOAC methods. To address the needs of this laboratories this application note demonstrates that the official AOAC Method for quantitation of cannabinoids could be performed seamlessly on LC-40 series X3 systems with PDA detector.

This Application News is consisting of two parts. Part I describes PDA analysis and Part II describes LCMS/MS analysis.

▪ Introduction

Among other activities the Association of Official Agricultural Chemists (AOAC) works on various analysis methods within the Official Methods of Analysis^{SM} (OMA) program. Approved methods undergo rigorous, systematic scientific scrutiny to ensure they are highly credible and defensible - and can be used with confidence by industry, regulatory agencies, research organizations, testing laboratories and academic institutions.[1]

The AOAC published a method for quantitation of cannabinoids in cannabis dried plant materials, concentrates and oils[2]. This method was adopted for demonstration of analysis of CBD rich tea on Shimadzu Nexera X3* system.

▪ Experimental

The UHPLC system applied for this article was a modular Shimadzu LC system containing binary solvent delivery pump LC-40BX3 with integrated eluent degasser, thermostatic HPLC autosampler SIL-40X3, column thermostat CTO-40C and photodiode array detector SPD-M40 equipped with an UHPLC cell. The control of the instrument and data processing was performed by Shimadzu LabSolutions LCMS chromatography data system software, version 5.99 SP2. The analytical conditions are shown in Table 1.

Table 1: Analytical conditions

Column**	Shim-pack Velox C18 (150 mm x 2.1 mm I.D, 1.8 µm.; P/N 227-32007-04)
Column temperature	25 °C
Mobile phase A	20 mM aqueous NH_4HCO_3, pH 3.2 (adjusted with formic acid)
Mobile phase B	Acetonitrile (LCMS grade)
Flow rate	0.4 mL/min
Elution mode	Gradient
Gradient program	0.00 min 40% B, 12.00 min 95% B, 14.00 min 95% B, 14.01 min 40%, 17 min 40% B
Injection volume	3 µL
Autosampler temperature	10 °C
PDA acquisition rate	3.125 Hz
Quantification wavelength	240 nm
Quantification bandwidth	10 nm
Reference wavelength	360 nm
Reference bandwidth:	100 nm

Standard Preparation

The standard solutions containing a mixture of twelve cannabinoid standards (0.50 mg/L, 1.0 mg/L, 2.5 mg/L, 5.0 mg/L, 10 mg/L) were prepared according to the AOAC method in Table 2018.11A.

Sample Preparation

Because this application only should focus on the analytical part of AOAC method, samples (4 different kind of commercially available hemp tea leaves) have been prepared according the sample preparation protocol of the Shimadzu Cannabis Analyzer package.

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* Column back pressure and required resolution would also allow to perform the analysis on a *Nexera XS system*

** AOAC Method column: C18, 2.0 µm, 150 × 2.1 mm

Results and Discussion

Figure 1 shows the separation of twelve cannabinoids in a standard mixture. The USP requirement for the resolution R of critical pairs CBG and CBD as well as d9-THC and d8-THC (R ≥ 1) is satisfied by following results:

CBG and CBD:	USP resolution > 1.3
d9-THC and d8-THC:	USP resolution > 1.7

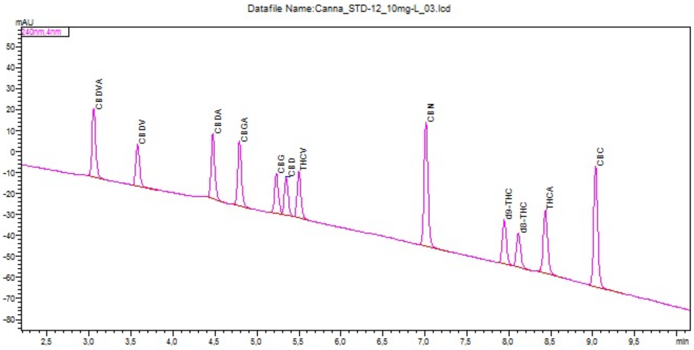


Figure 1: Example chromatogram of 12 cannabinoids from a standard mixture (each compound 10 mg/L dissolved in methanol)

Although pre-tests (see Fig. 1) have shown up to 10 % better response with comparable noise when acquiring data with 12.5 Hz, the frequency 3.125 Hz was selected for further measurements, because in the original AOAC Method a sampling frequency of 2.5 Hz was used.

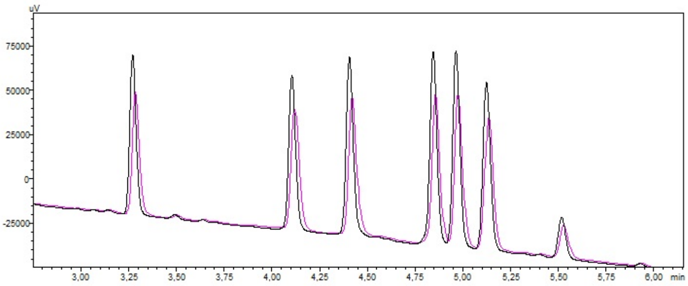


Figure 2: Data comparison of cannabinoid standard mix (pink trace: 3.125 Hz sampling frequency; black trace: 12.5 Hz sampling frequency)

Calibration

A five-level calibration using cannabinoid standard mixtures according the Table 2 was performed. The data overlay of the five standards (see Fig. 3) shows good system precision.

Table 2: Compound table

Compound	RT [min]	Level 1 Conc. [mg/L]	Level 2 Conc. [mg/L]	Level 3 Conc. [mg/L]	Level 4 Conc. [mg/L]	Level 5 Conc. [mg/L]
CBDVA	3,060	0.5	1	2.5	5	10
CBDV	3,578	0.5	1	2.5	5	10
CBDA	4,477	0.5	1	2.5	5	10
CBGA	4,794	0.5	1	2.5	5	10
CBG	5,234	0.5	1	2.5	5	10
CBD	5,349	0.5	1	2.5	5	10
THCV	5,501	0.5	1	2.5	5	10
CBN	7,016	0.5	1	2.5	5	10
d9-THC	7,948	0.5	1	2.5	5	10
d8-THC	8,115	0.5	1	2.5	5	10
THCA	8,439	0.5	1	2.5	5	10
CBC	9,033	0.5	1	2.5	5	10

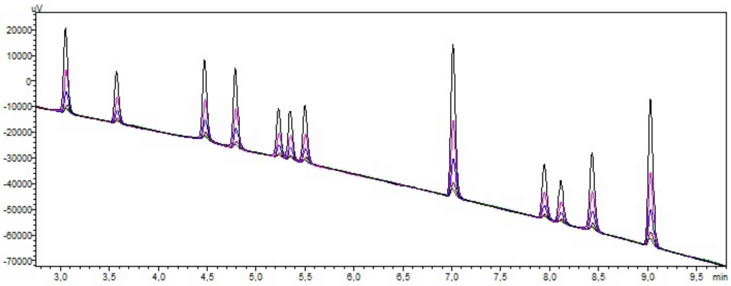
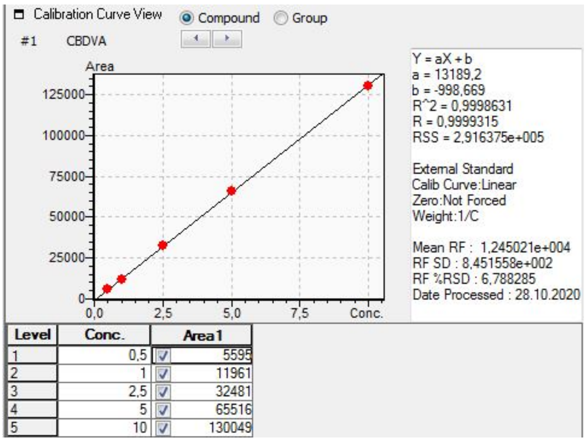


Figure 3: Overlay of chromatograms for analysis of 0.5, 1.0, 2.5, 5 and 10 mg/L twelve-compound mixture of cannabinoids standards.

Excellent linearity with determination coefficient $R^2 > 0.999$ was achieved for all analysed compounds (AOAC requirement $R^2 > 0.995$) as it is shown in the example in Figure 4 for CBDVA.



Y = aX + b
a = 13189,2
b = -998,669
R^2 = 0,9998631
R = 0,9999315
RSS = 2,916375e+005

External Standard
Calib Curve:Linear
Zero:Not Forced
Weight:1/C

Mean RF : 1,245021e+004
RF SD : 8,451558e+002
RF %RSD : 6,788285
Date Processed : 28.10.2020

Level	Conc.		Area1
1	0,5	☑	5595
2	1	☑	11961
3	2,5	☑	32481
4	5	☑	65516
5	10	☑	130049

Figure 4: Example calibration curve of CBDVA

Sample analysis

Four kind of different commercially available CBD hemp tea brands have been prepared according to the *Cannabis Analyzer Package* protocol which is, like the AOAC method, a liquid extraction method (Figure 5).

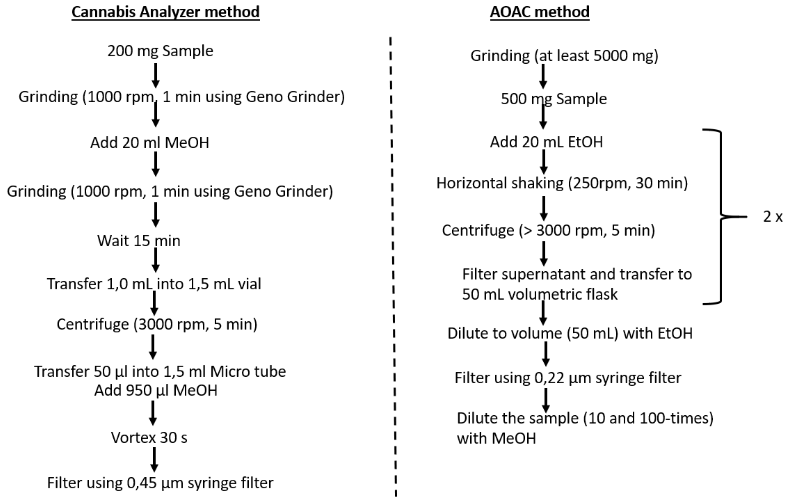


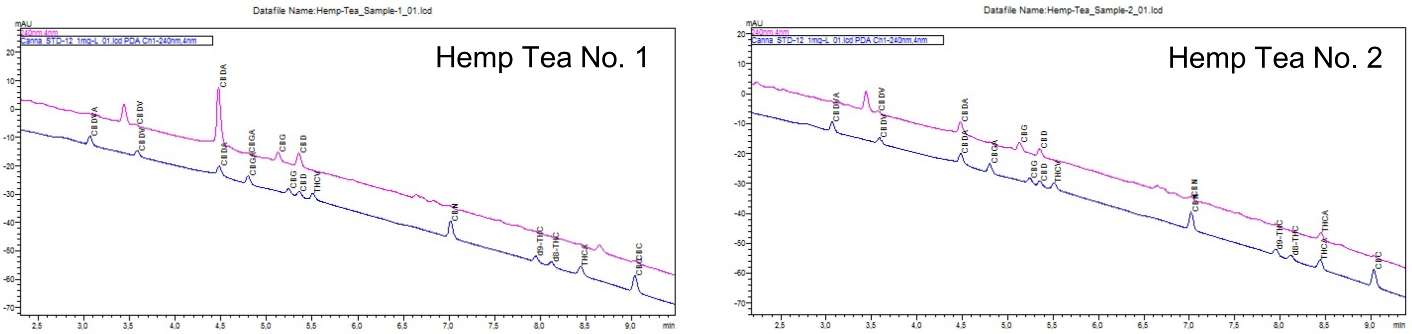
Figure 5: Brief description of Shimadzu Cannabis Analyzer Package- and AOAC sample pre-treatment protocol

Calculation of total CBD and total THC content

Figures 6 and 7 show chromatograms and related quantitative results of analysed hemp tea samples. For potency determination, the CBD and THC content was calculated under considering a decarboxylation factor of 0.887. The procedure allows to calculate theoretically maximal concentration of CBD and THC after their virtual conversion from acidic (CBDA or THCA) to neutral form:

% of THC $_{\text{Total}}$ = % of THC + (% of THCA x 0.877)

The same formula was applied for the calculation of the percentage of total CBD concentration. From all samples, only sample no. 3 contains total THC, but only in its acidic form THCA. The psychoactive form THC was not identified in all analysed samples in this study.



Name	Peak#	Ret. Time [min]	Resolution (USP)	Conc. [mg/L]
CBDVA	No peak detected	---	---	---
CBDV	No peak detected	---	---	---
CBDA	2	4.48	11.3	1.37
CBGA	3	4.79	3.6	0.05
CBG	4	5.13	3.9	0.30
CBD	5	5.35	2.4	0.56
THCV	No peak detected	---	---	---
CBN	No peak detected	---	---	---
d9-THC	No peak detected	---	---	---
d8-THC	No peak detected	---	---	---
THCA	No peak detected	---	---	---
CBC	8	9.03	4.1	0.03

Total CBD: 1.76 % (17.63 mg/g)

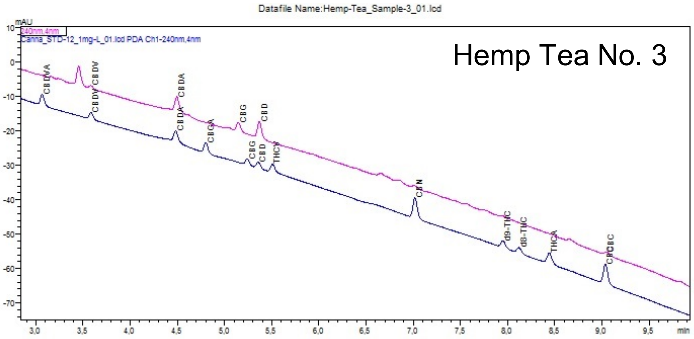
Total THC: Not detected

Name	Peak#	Ret. Time [min]	Resolution (USP)	Conc. [mg/L]
CBDVA	No peak detected	---	---	---
CBDV	3	3.57	1.5	0.07
CBDA	4	4.48	10.5	0.25
CBGA	No peak detected	---	---	---
CBG	5	5.13	6.9	0.27
CBD	6	5.35	2.4	0.29
THCV	No peak detected	---	---	---
CBN	No peak detected	---	---	---
d9-THC	No peak detected	---	---	---
d8-THC	No peak detected	---	---	---
THCA	8	8.45	18.0	0.16
CBC	No peak detected	0	0	0

Total CBD: 0.51 % (5.09 mg/g)

Total THC: 0.14% (1.39 mg/g)

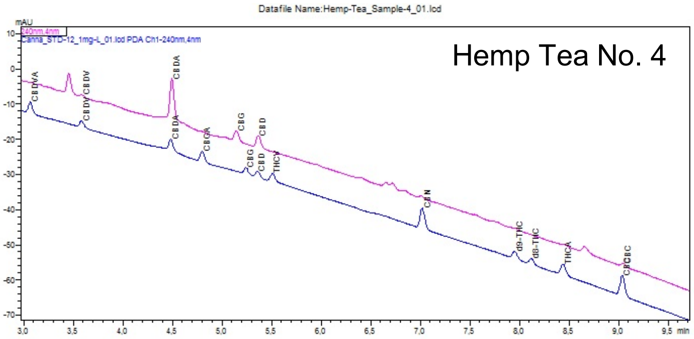
Figure 6: Quantitation of cannabinoids hemp tea samples no. 1 and no. 2 (blue trace: standards; pink trace: samples)



Name	Peak#	Ret. Time [min]	Resolution (USP)	Conc. [mg/L]
CBDVA	No peak detected	---	---	---
CBDV	5	3.58	1.5	0.07
CBDA	6	4.50	10.6	0.29
CBGA	No peak detected	---	---	---
CBG	7	5.15	6.9	0.25
CBD	8	5.37	2.4	0.54
THCV	No peak detected	---	---	---
CBN	No peak detected	---	---	---
d9-THC	No peak detected	---	---	---
d8-THC	No peak detected	---	---	---
THCA	No peak detected	---	---	---
CBC	9	9.04	41.5	0.04

Total CBD: 0,80 % (7,97 mg/g)

Total THC: Not detected



Name	Peak#	Ret. Time [min]	Resolution (USP)	Conc. [mg/L]
CBDVA	No peak detected	---	---	---
CBDV	No peak detected	---	---	---
CBDA	3	4.49	11.5	0.76
CBGA	No peak detected	---	---	---
CBG	4	5.14	6.9	0.27
CBD	5	5.37	2.4	0.38
THCV	No peak detected	---	---	---
CBN	No peak detected	---	---	---
d9-THC	No peak detected	---	---	---
d8-THC	No peak detected	---	---	---
THCA	No peak detected	---	---	---
CBC	7	9.04	4.0	0.04

Total CBD: 1,05 % (10,45 mg/g)

Total THC: Not detected

Figure 7: Quantitation of cannabinoids hemp tea samples no. 3 and no. 4 (blue trace: standards; pink trace: samples)

▪ Summary

This work demonstrated that AOAC official Method 2018.11 for quantitation of Cannabinoids can be performed well and reliably on Shimadzu Nexera X3 system. Due to the larger pressure generated by the smaller particle diameter column an UHPLC system of at least 70 MPa is required. Because of the spectral similarity of several cannabinoids of interest, the PDA does not provide a real advantage against standard UV detector.

▪ References

[1] About AOAC INTERNATIONAL, AOAC INTERNATIONAL, accessed 7 January 2021, <https://www.aoac.org/about-aoac-international/>

[2] Vaclavik, Lukas, et al. "Quantitation of Cannabinoids in Cannabis Dried Plant Materials, Concentrates, and Oils Using Liquid Chromatography–Diode Array Detection Technique with Optional Mass Spectrometric Detection: Single-Laboratory Validation Study, First Action 2018.11." Journal of AOAC International 102.6 (2019): 1822-1833.

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