High-throughput and simultaneous analysis of twelve cannabinoids in hemp oil using liquid chromatography with ultraviolet detection (LC-UV): Part I

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Abstract

In this study, the LC separation of twelve cannabinoids, including CBC, CBD, CBDA, CBDV, CBDVA, CBG, CBGA, CBN, Δ8-THC, Δ9-THC, THCA A, and THCV, has been systematically optimized using a Phenomenex Luna Omega 3 µm Polar C18 150 mm × 4.6 mm column with regard to the effects of the type of organic solvent, i.e. methanol and acetonitrile, the content of the organic solvent, and the pH of the mobile phase. The optimization has resulted in three LC conditions at 1.0 mL/minute able to separate the 12 cannabinoids: 1) a mobile phase consisting of water and methanol, both containing 0.1% formic acid (pH 2.69), with a gradient elution at 75% methanol for the first 3 minutes and then linearly increase to 100% methanol at 12.5 minutes; 2) a mobile phase consisting of water and 90% (v/v) acetonitrile in water, both containing 0.1% formic acid and 20 mM ammonium formate (pH 3.69), with an isocratic elution at 75% acetonitrile for 14 minutes; and 3) a mobile phase consisting of water and 90% (v/v) acetonitrile in water, both containing 0.03% formic acid and 20 mM ammonium formate (pH 4.20), with an isocratic elution at 75% acetonitrile for 14 minutes.

Introduction

Cannabis sativa L. is a cosmopolitan species that is widely distributed around the world, and this collective name is used to denote various botanical forms [1]. Two varieties have societal significance: *Cannabis sativa var. sativa* and *Cannabis sativa var. indica*, with the former being commonly referred to as industrial hemp and the latter being generally known as marijuana. The therapeutic, physiological, and psychological properties of cannabis are attributed to cannabinoids, compounds uniquely isolated from *Cannabis sativa* L. with a typical C_{21} terpenophenolic skeleton (see **Table 1**). To date, more than 120 cannabinoids have been isolated from cannabis plants. In the Federal Controlled Substance Act of 1970, cannabis was defined as a "Schedule 1" substance, i.e. no accepted medical use and high risk of addiction. This law made medical and recreational cannabis use illegal. It is noted that industrial hemp is subjected to this law and growing industrial hemp is restricted in the United States, despite the difference between hemp and marijuana. Nevertheless, Americans have long favored the use of marijuana. At present, thirty states and the District of Columbia have passed medical marijuana laws, albeit with considerable state-to-state variation in the specific provisions of the laws. In addition, ten states and the District of Columbia have passed recreational marijuana laws.

For the analysis of cannabinoids in products of *Cannabis sativa* L., early published methods often used GC coupled with either flame ion detector (FID) or mass spectrometry (MS). However, a serious problem with these GC methods was that acidic cannabinoids can be thermally decarboxylated to their neutral counterparts at the injection port [2]. Conversely, recently published methods often used LC coupled with either UV or MS because the LC separation can avoid thermal stress so that cannabinoids can be analyzed in their original forms. With regard to the LC/UV versus LC/MS methods, more LC/MS methods have been published recently because a large number of cannabinoids can be present in products of *Cannabis sativa* L. and a total separation of all cannabinoids is not absolutely necessary for the LC/MS methods. However, LC/MS methods demand expensive instruments that are commonly unavailable and usually inappropriate for routine analysis by the cannabis growers and commercial suppliers. Therefore, the development of a LC/UV method that is able to simultaneously analyze many cannabinoids in a short analytical time continues to be a viable research task, especially in consideration that LC/UV can readily meet the required limit of quantification (LOQ) by the analysis of cannabinoids in products of *Cannabis sativa* L. At present, although a few validated LC-UV methods have been published in the literature, a method able to simultaneously analyze more cannabinoids in a shorter run time is still in high demand.

Experimental

LC Instrumental conditions

1. Instrument: Shimadzu Prominence LC-20 system
2. Column: Phenomenex Luna Omega 3µm Polar C_{18} 150 mm × 4.6 mm
3. Flow rate: 1 mL/min
4. Injection volume: 20 µL
5. Detection: UV at 230 nm

Results

Table 1. The 12 cannabinoids that are analyzed in this study.

Compound	Structure
Cannabichromene (CBC)	Cannabigerolic acid (CBGA)
Cannabidiol (CBD)	Cannabinol (CBN)
Cannabidiolic acid (CBDA)	Δ8-tetrahydrocannabinol (Δ8-THC)
Cannabidivarin (CBDV)	Δ9-tetrahydrocannabinol (Δ9-THC)
Cannabidivarinic acid (CBDVA)	Δ9-tetrahydrocannabinolic acid (THCA A)
Cannabigerol (CBG)	Tetrahydrocannabivarin (THCV)

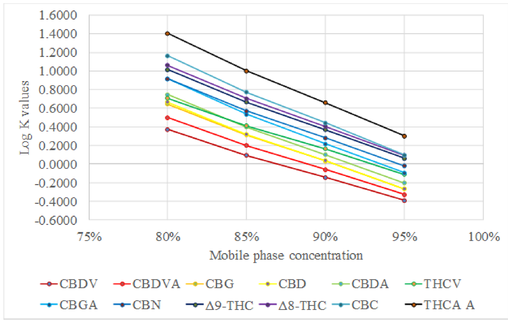

Figure 1. Log k values of 12 cannabinoids versus methanol concentration at pH 2.69 ($k = \frac{t_r - t_m}{t_m}$, where t_r and t_m are retention time and dead time, respectively).

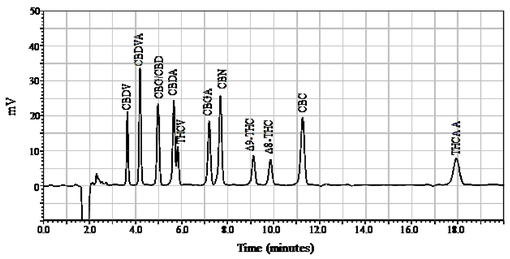

Figure 2. Chromatogram of 12 cannabinoids at 2 ppm using a mobile phase consisting of water (A) and methanol (B), both containing 0.1% formic acid (pH 2.69), with an isocratic elution at 85% B.

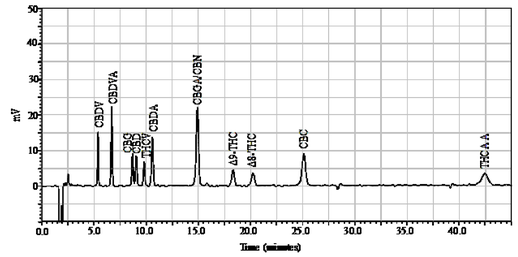

Figure 3. Chromatogram of 12 cannabinoids at 2 ppm using a mobile phase consisting of water (A) and methanol (B), both containing 0.1% formic acid (pH 2.69), with an isocratic elution at 80% B.

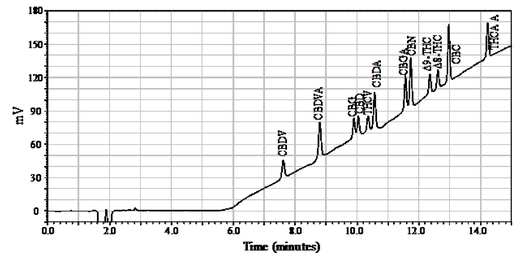

Figure 4. Chromatogram of 12 cannabinoids at 2 ppm using a mobile phase consisting of water (A) and methanol (B), both containing 0.1% formic acid (pH 2.69), with a gradient elution at 75% methanol for the first 3 minutes and then linearly increase to 100% methanol at 12.5 minutes.

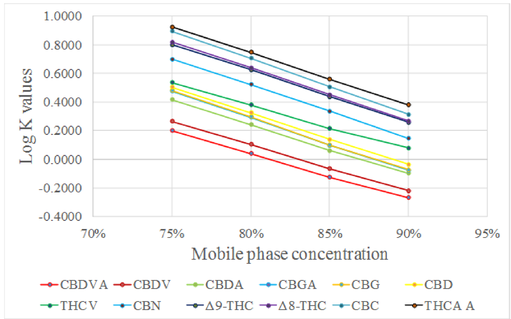

Figure 5. Log k values of cannabinoids versus acetonitrile concentration at pH 2.69.

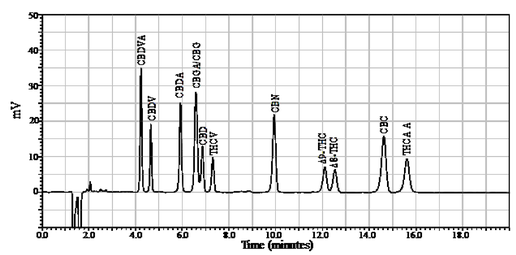

Figure 6. Chromatogram of 12 cannabinoids at 2 ppm using a mobile phase consisting of water (A) and acetonitrile (B), both containing 0.1% formic acid (pH 2.69), with an isocratic elution at 75% B.

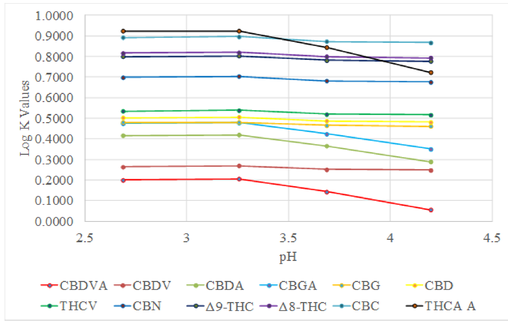

Figure 7. Log k values of cannabinoids versus pH at 75% acetonitrile concentration.

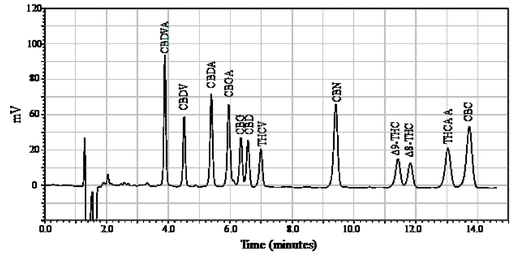

Figure 8. Chromatogram of 12 cannabinoids at 2 ppm using a mobile phase consisting of water and 90% (v/v) acetonitrile in water, both containing 0.1% formic acid and 20 mM ammonium formate (pH 3.69), with an isocratic elution at 75% acetonitrile.

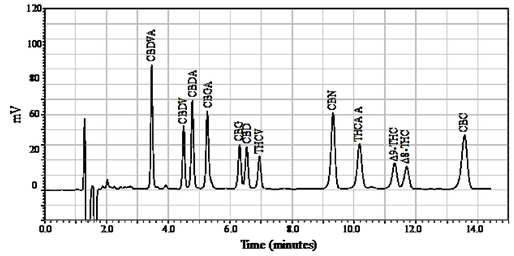

Figure 9. Chromatogram of 12 cannabinoids at 2 ppm using a mobile phase consisting of water and 90% (v/v) acetonitrile in water, both containing 0.03% formic acid and 20 mM ammonium formate (pH 4.20), with an isocratic elution at 75% acetonitrile.

Conclusions

- The LC separation of twelve cannabinoids has been systematically optimized with regard to the effects of the type of organic solvent, i.e. methanol and acetonitrile, the content of the organic solvent, and the pH of the mobile phase.
- Three fast LC separations of twelve cannabinoids have been successfully achieved. They can be used with either UV or MS detection for high-throughput and simultaneous analysis of cannabinoids in the products of *Cannabis sativa* L.

References

1. Zuk-Golaszewska, K., Golaszewski, J. *J. Elem.* 23, 971-984 (2018).
2. Dussy, F.E., Hamberg, C., Luginbuhl, M., Schwerzmann, T., Briellmann, T.A. *Forensic Sci.Int.* 149, 3-10 (2005).

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