APPLICATION NOTE

Fast and Easy Achiral & Chiral Analysis of Cannabinoids

It has been repeatedly suggested that the effect of Δ9-Tetrahydrocannabinol (THC) or other single cannabinoids are not equal to that of whole cannabis preparations: some of the bio-activity observed for these preparations could be due to acidic cannabinoids. That is why a method which separates both neutral compounds such as THC as well as acidic cannabinoids such as THCA in plant material must be available. In Europe there is a growing interest in medicinal cannabis, which is used to treat a series of pathologies. Nonetheless, cannabis is a strictly controlled drug under specific regulations or is illegal even for therapeutic purposes in many European countries. Certified medicinal cannabis is currently used for the treatment of a number of pathologies, including multiple sclerosis, epilepsy, neuropathic pain, arthritis, depression, anxiety disorders, sleep disorders, psychosis, glaucoma and Tourette's syndrome. It is also used for the relief of symptoms such as nausea and vomiting as a result of chemotherapy, and appetite stimulation in patients suffering from HIV and AIDS.

The cannabinoids form a group of related compounds of which about 70 are known. Among the major cannabinoids in Cannabis sativa L. THC is generally accepted to be the compound that possesses the psychoactive properties.

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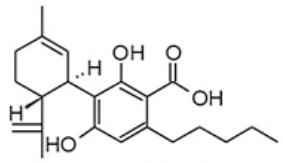
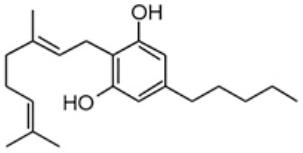
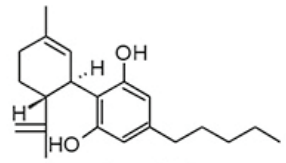
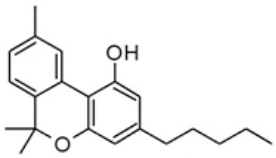
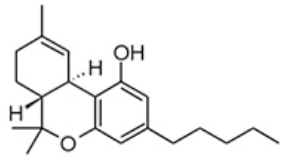
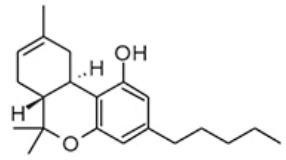
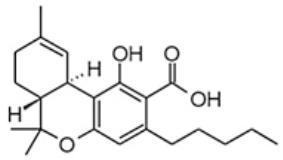
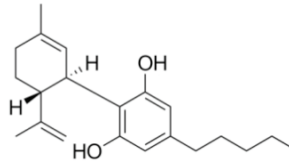
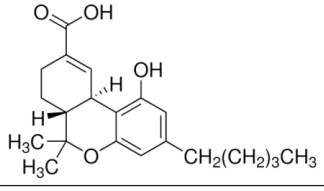
Substance	Abr.	Structure
Cannabidiolic acid	CBDA	
Cannabigerol	CBG	
Cannabidiol	CBD	
Cannabinol	CBN	
Δ9-Tetrahydrocannabinol	Δ9-THC	
Δ8-Tetrahydrocannabinol	Δ8-THC	
Δ9-Tetrahydrocannabinolic acid	THCA	
11-Hydroxy–Δ9-THC	11-hydroxy-THC	
11-nor-9-Carboxy-Δ9-THC	9-carboxy-THC	

The analysis of the original composition of plant material is necessary for such purposes as phenotyp determination and quality control of medical cannabis used for therapeutic treatment.

HPLC permits the determination of the original composition of plant cannabinoids by direct analysis. In this application note two fast methods are presented achieving high-performance in less than 9 min (see Figure 1, 2). The first application using YMC-Triart C18 is a dedicated UHPLC method. Secondly, a Core-Shell column, Meteoric Core C18, is used. A third also very quick method shows a solution for the chiral separation of THC isomers using a polysaccharide phase (see Figure 3).
In the following RP-application five cannabinoids were successfully separated with YMC-Triart C18 applying a gradient of water, methanol with formic acid as additive.

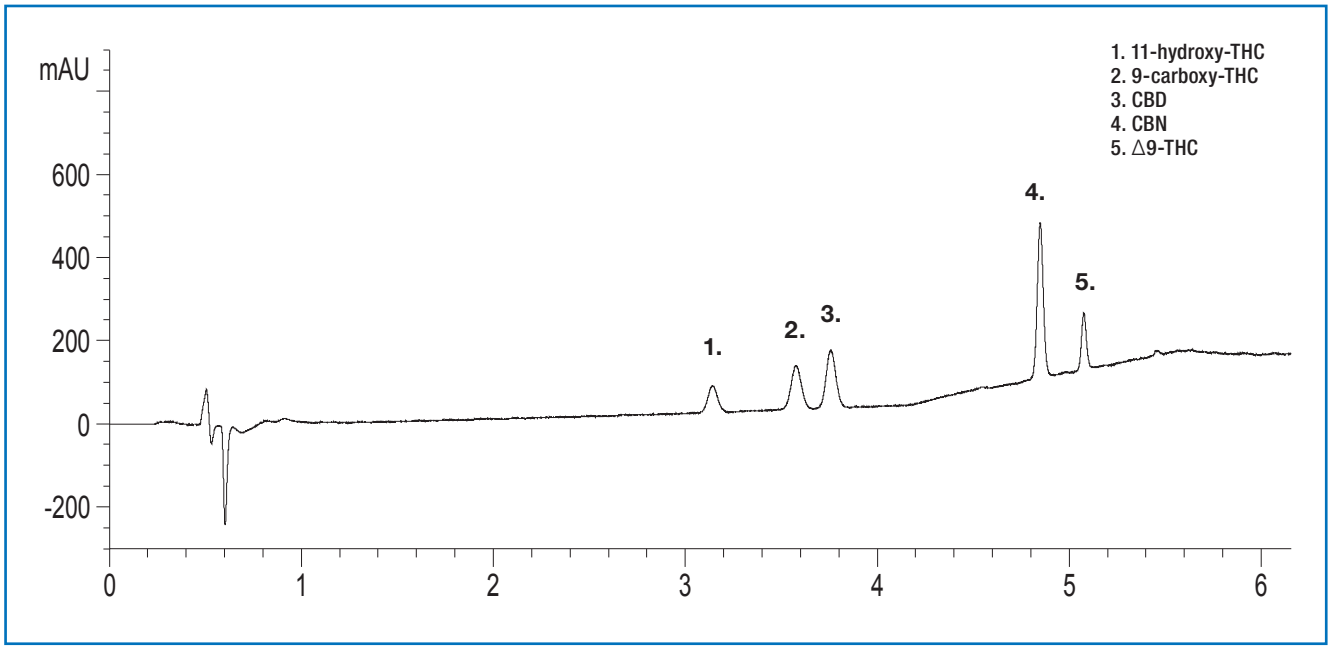


Figure 1: UHPLC separation of five cannabinoids using YMC-Triart C18 with a particle size of 1.9 µm.

Table 1: Method details

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 100 x 2.0 mm ID
Part No.:	TA12SP9-1002PT
Eluent:	A) water/formic acid (100/0.1) B) methanol/formic acid (100/0.1)
Gradient:	75-82 % B (0-3.5 min), 82-100 % B (3.6-5.0 min), 100 % B (5.0-8.0 min)
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	UV at 220 nm
Injection:	2 µL (17 µg/mL)

The Core-Shell column Meteoric Core C18 is a highly efficient phase, which enables distinction between CBG and CBD. These two compounds have previously presented challenging co-elution effects.

This RP-method uses isocratic conditions with water + formic acid (0.1 %) and acetonitrile (27/73) and allows separations in under 9 min.

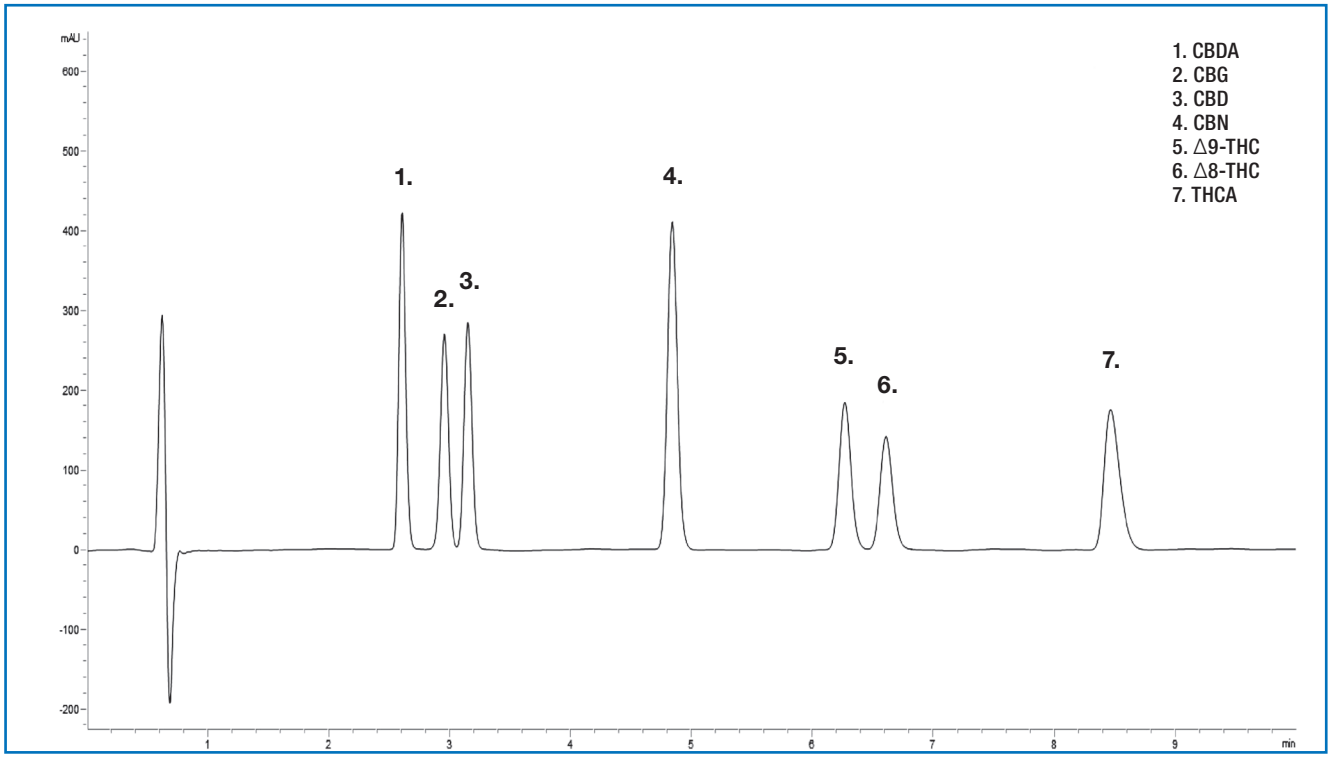


Figure 2: Fast separation of THC and related compounds using 2.7 µm Core-Shell based column, Meteoric Core C18.

Table 2: Method details

Column:	Meteoric Core C18 (2.7 µm) 100 x 4.6 mm ID
Part No.:	CAS08SQ7-1046PT
Eluent:	water+formic acid (0.1%) / acetonitrile (27/73)
Flow rate:	1.25 ml/min
Detection:	UV at 220 nm
Injection:	25 µL (0.05 mg/mL)
Temperature:	35 °C

Application Data by courtesy YMC America, Inc.

In many applications the separation of THC's enantiomers is required. In the following NP-application (+)trans-Δ9-THC and (-)trans-Δ9-THC were successfully separated with a resolution of 8.5. A separation is achieved within 3 min by using a 3 µm coated polysaccharide phase, CHIRAL ART Amylose-C. n-Heptane and 2-propanol were used as eluents under isocratic conditions (see Figure 3).

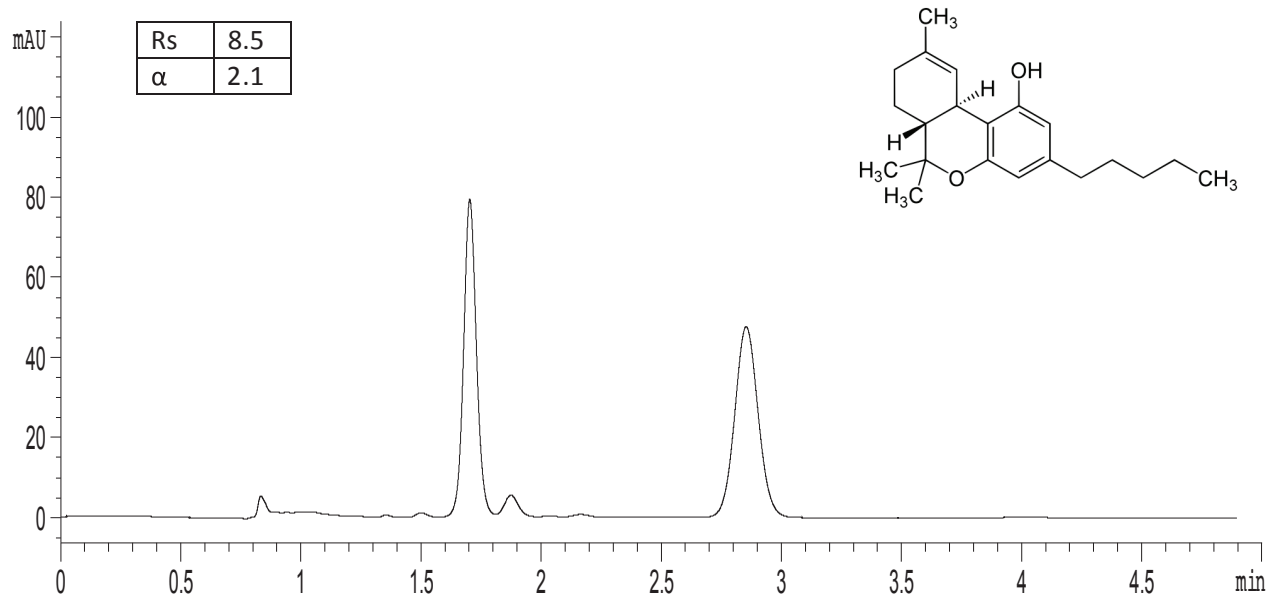


Figure 3: Chiral separation of tetrahydrocannabinol enantiomers using CHIRAL ART Amylose-C.

Table 3: Method details

Column:	CHIRAL ART Amylose-C (3 µm) 150 x 3.0 mm ID
Part No.:	KAN99S03-1503WT
Eluent:	n-heptane / 2-propanol (92/8)
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	UV at 228 nm
Injection:	10 µL (50 µg/mL)