the Cannabis Scientist®

Application Book

Your guide to cutting-edge methods in cannabis and cannabinoid analysis

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Cannabinoid Potency in Cannabis Oil and Medical Marijuana by GC-FID

HPLC Method to Differentiate Four THC Stereoisomers Formed From Δ9-THC Degradation

Headspace SPME-GC/MS Analysis of Terpenes in Cannabis

Simultaneous Analysis of Synthetic Cannabinoids in Urine

(C)an(n)alyze: determination of 16 cannabinoids inside flowers, oils and seeds

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Cannabinoid Potency in Cannabis Oil and Medical Marijuana by GC-FID

By Lindsy Jansen

A GC-FID method for the identification and quantification of neutral cannabinoids in cannabis oil and medical marijuana

The use of cannabinoid-based products and medical marijuana is growing in response to increased legalization in multiple countries. With an increasing variety of products available, the control of cannabinoid content is becoming popular in modern day manufacturing and testing laboratories.

Street marijuana usually contains high levels of Δ9-THC and a lower level of CBD, with medical marijuana and even hemp containing higher levels of CBD and lower levels of Δ9-THC. The primary psychoactive component of all cannabis products is Δ9-THC, whilst CBD is the primary therapeutic component. Consumer hemp generally comes in the form of hemp oil, primarily used for medical purposes, whilst street and medical marijuana are often smoked.

The analysis of cannabinoid potency is vital not only for quality control, but also toxicology purposes. In this application, SCION Instruments developed a quick and easy method for the identification and quantitation of neutral cannabinoid content by Gas Chromatography – Flame Ionisation Detection (GC-FID). The method was tested and validated using both hemp oil and medical marijuana samples.

For the identification and quantitation of the acidic cannabinoids – primarily THCA, CBDA, CBGA and CBCA – derivatization is required during the sample preparation stage. During sample introduction to the GC, the heat from the injector causes decarboxylation of the carboxyl groups of acidic cannabinoids, resulting in identification/quantitation of neutral cannabinoids. Derivatizing with BSTFA prevents decarboxylation from occurring, thus allowing full identification/quantification of both acidic and neutral cannabinoids.

The demonstrated method is applicable to various sample types, with only adjustment to the sample preparation needed. The following application note also highlights excellent linearity and recovery of neutral cannabinoids, along with limit of detection/quantification for each of the target cannabinoids.

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Application Notes
Scion Instruments

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HPLC Method to Differentiate Four THC Stereoisomers Formed From Δ^9-THC Degradation

Discover a robust, chiral HPLC stationary phase method to separate Δ^{10}-THC and $\Delta^{6a,10a}$-THC isomers produced from Δ^9-THC degradation

By Jeffrey B. Williams, Kathleen B. Calati, Kirk W. Hering, Roxanne E. Franckowski, Weston J. Umstead and Donna M. Iula

Degradation of Δ^9-THC to Δ^{10}-THC and $\Delta^{6a,10a}$-THC isomers provide a challenging separation in Cannabis-derived products. The use of an immobilized cellulose chiral column under normal-phase liquid chromatography conditions provides an analytical method to fully separate these four THC isomers for identification and accurate determination of potency.

As the primary phytocannabinoid associated with psychoactive properties, Δ^9-THC is crucial to the determination of potency for extracts, edibles, and other Cannabis-derived products. The additional processing required for providing these Cannabis products may result in their degradation, forming isomers that can be misidentified and provide invalid potency claims. Δ^9-THC can isomerize to two diastereomers, (6aR,9S)-Δ^{10}-THC and (6aR,9R)-Δ^{10}-THC. These two distinct stereoisomers may undergo additional isomerization to form a pair of enantiomers, providing 9(S)-$\Delta^{6a,10a}$-THC and 9(R)-$\Delta^{6a,10a}$-THC, respectively (Figure 1).

The stereochemical similarities between the Δ^{10}-THC and $\Delta^{6a,10a}$-THC isomers are particularly challenging to fully resolve under typical reversed-phase liquid chromatography. Additionally, the $\Delta^{6a,10a}$-THC enantiomers are not separable without the use of a chiral stationary phase.

A chiral column under normal-phase liquid chromatography (NPLC) conditions fully separated these THC isomers (Figure 2). Chiral column CHIRALPAK® IB N-3 (250 x 4.6 mm, 3 µm) under NPLC conditions, controlled at 30°C, was used. Elution was accomplished with mobile phase 95:5 Hexane:Isopropyl Alcohol (IPA) at 0.85 ml/min for 15 minutes. A 1 µl injection of a 1 mg/ml solution in IPA was monitored at 228 nm. The neat materials were formulated into certified reference material (CRM) solutions. To provide CRMs with optimal stability, each of the solutions were prepared as a 1 mg/ml solution in acetonitrile. Although acetonitrile and hexane are immiscible, the inclusion of IPA to the mobile phase maintains the baseline resolution of the analytes despite the diluent. This method may be used to develop testing methods to resolve and accurately quantify ingredients in Cannabis products.

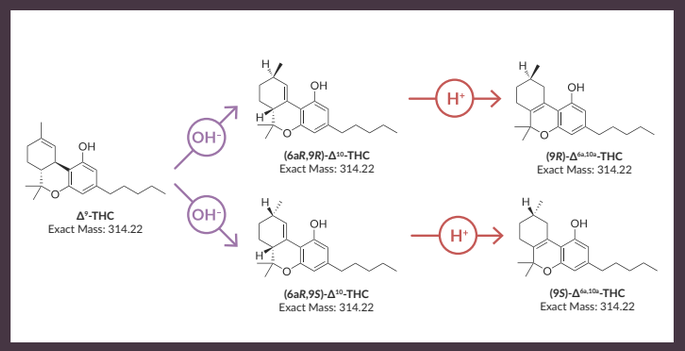
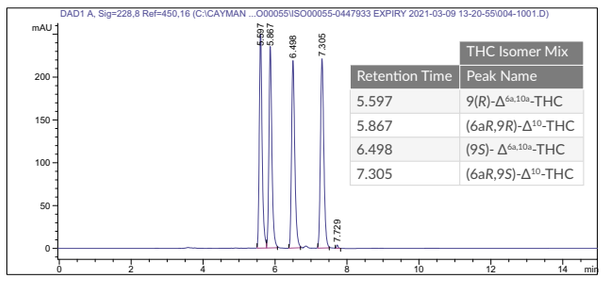


Figure 1. Isomerization of Δ9-THC under first basic (OH-) and then acidic (H+) conditions can lead to several structurally similar isomers



Retention Time	THC Isomer Mix Peak Name
5.597	9(R)-$\Delta^{6a,10a}$-THC
5.867	(6aR,9R)-Δ^{10}-THC
6.498	(9S)- $\Delta^{6a,10a}$-THC
7.305	(6aR,9S)-Δ^{10}-THC

NPLC Conditions

Instrument	Agilent 1100 Series
Column	CHIRALPAK® IB N-3 (250 x 4.6 mm, 3 µm)
Mobile Phase	A: 95:5 Hexane:Isopropyl Alcohol
Flow Rate	0.85 ml/min
Column Temp.	30°C
Wavelength	UV monitored at 228 nm

Figure 2. A co-injection of all four isomers in acetonitrile with the NPLC method

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Webinar
Phytocannabinoid Known Unknowns

Wall Poster
Phytocannabinoid Lab Guide

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Headspace SPME-GC/MS Analysis of Terpenes in Cannabis

A rapid method to identify cannabis terpenes for forensic and organoleptic applications

Cannabis sativa (cannabis or marijuana) contains over 100 different terpenes and terpenoids, including mono, sesqui, di and tri, as well as other miscellaneous compounds of terpenoid origin (1).Terpenes give the plant distinct organoleptic properties and produce characteristic aromas when the buds are heated or vaporized (2). Although the terpene profile does not necessarily indicate geographic origin of a cannabis sample, it can be used in forensic applications to determine the common source of different samples (3). In addition, different cannabis strains have been developed which have distinct aromas and flavors, a result of the differing amounts of specific terpenespresent (4).

Experimental

The dried cannabis sample was obtained courtesy of Hari H. Singh, Program Director at the Chemistry and Physiological Systems Research Branch of the United States National Institute on Drug Abuse at the National Institute of Health. Terpenes were isolated using headspace solid phase microextraction (SPME) followed by chromatographic separation on an Equity®-1 capillary GC column. Peak identifications were assigned using MS spectral matching against reference spectra in the Wiley and NIST libraries. Confirmatory identification was done based on retention index, which was calculated for the compounds identified in each sample using an n-alkane standard analyzed under the same GC conditions. This data was compared with published values and peak identifications were assigned (5,6,7).

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Application Note
Analysis of Pesticide Residues in Cannabis using QuEChERS and HPLC

Application Note
ICP-MS Analysis of Heavy Metals in Cannabis sativa

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Simultaneous Analysis of Synthetic Cannabinoids in Urine

Solid-phase extraction and LC-MS/MS method for analysis of 19 synthetic cannabinoids in urine. Specific compounds selected based on lab positivity rates.

By Abderrahim Abdelkaoui, Ritesh Pandya, Brian Kinsella and Michael Telepchak

Simple SPE procedure for the analysis of 19 synthetic cannabinoids in urine using UCT's Styre Screen® HLD highly crosslinked polymeric SPE cartridges

Newly identified synthetic cannabinoids pose a significant threat to public health and safety, as their implications in drug overdose and adverse events continue to rise in the United States and around the world. The diverse chemical structures of synthetic cannabinoids have a significantly high impact on their potency and side effects. The 19 synthetic cannabinoids included in this study were previously unreported in forensic toxicology casework in the United States. Currently, there are few published methods available for the analysis of these novel compounds. However, identifying and extracting these compounds from various biological matrices is becoming more critical for accurate forensic investigations and clinical diagnostics. This application note will outline a solid-phase extraction (SPE) and LC-MS/MS method to analyze 19 synthetic cannabinoids in urine.

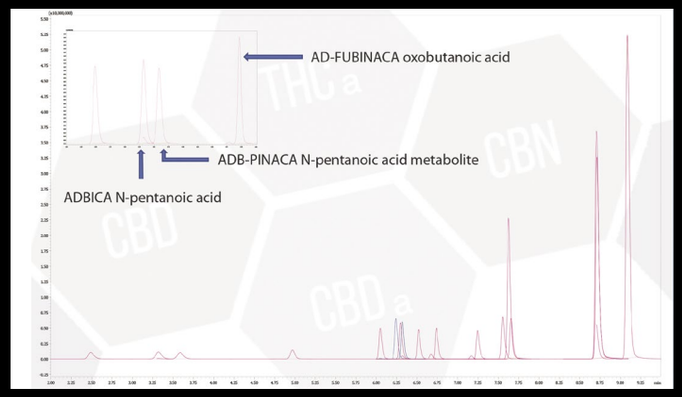

Figure 1. Chromatogram of 25 ng/mL extracted sample demonstrating the isobaric separation of ADBICA N-pentanoic acid and ADB-PINACA N-pentanoic acid metabolites

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Application Note
Simultaneous Analysis of Synthetic Cannabinoids in Urine

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Produced by the Cannabis Scientist

(C)an(n)alyze: determination of 16 cannabinoids inside flowers, oils and seeds

Summary

As research within cannabinoids for pharmaceutical purposes increases, so does the number of substances to be tested. Therefore, compared to the German pharmacopoeia (Deutsches Arzneibuch; DAB), an increase of 10 cannabinoids was performed for qualification and quantification with this work. The aim of the method development was to decrease the runtime and optimize the gradient program in comparison to the DAB method (1). To ensure accuracy of the method, a validation was performed according to ICH Guidelines Q2 R12. Parameters for the validation were selectivity, linearity, repeatability and the recovery rate. The given specification was derived from the Association of Official Analytical Chemists (AOAC) with the given standard method performance requirements for cannabis flowers and oils (3).

Introduction

Cannabis sativa L. is one of the oldest agricultural and medicinal plants, which produces a variety of compounds such as terpenoids, flavonoids and cannabinoids (4). The interaction of cannabinoids with the body's own cannabinoid receptors, which occur in a variety of brain cells for coordination, memory processing and spatial orientation, enables new pharmacological and psychological treatment options (5). Probably the most psychoactive cannabinoid of the four different isomers of Δ9-THC is the (-)-Δ9-trans-tetrahydrocannabinol, also known as dronabinol. In Germany, Δ9-THC is controlled by the narcotics law (Betäubungsmittelschutzgesetz; BtMG) due to its psychoactive properties. Since March 2017, the regulations changed by the amendment of article 1 BtMG. The amendment of annexes II and III of the BtMG now allows cannabis such as marijuana plants and plant parts to be marketed and prescribed. Thereby, cannabis was authorised for medical purposes as ready-to-use medicinal products (6). Production of cannabis products must be conducted and monitored in accordance with good manufacturing practice guidelines (GMP) to guarantee accurate labelling of medicinal products, food and cosmetics.

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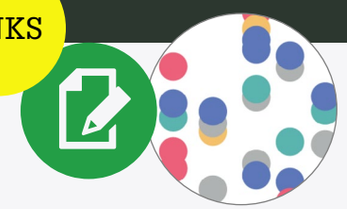

Article
Superior Size Separation

Article
Step Up For Cannabis Processing

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Streamline Cannabinoid Analyses with Restek's New Neutrals Mix

Restek's new nine-component cannabinoid mix streamlines cannabinoid analyses. By combining nine compounds into one ampul, calibration complexity is simplified, enabling labs to minimize errors, save time, and reduce cost. A high concentration of 1000 µg/mL adds additional flexibility in constructing calibration curves and lowers solvent spiking volume for labs assessing potency recoveries.

The cannabinoid neutrals mix (cat.# 34132) is a certified reference material, manufactured and QC-tested in Restek's ISO-accredited labs. Verified composition and stability, with two independently produced lots available, help satisfy your ISO requirements.

Compounds

- Cannabichromene (CBC)
- Cannabicyclol (CBL)
- Cannabidiol (CBD)
- Cannabidivarin (CBDV)
- Cannabigerol (CBG)
- Cannabinol (CBN)
- d8-Tetrahydrocannabinol (d8-THC)
- d9-Tetrahydrocannabinol (d9-THC)
- Tetrahydrocannabivarin (THCV)

For cannabinoids and other reference standards, sample preparation products, and the expert consultation your cannabis lab needs, turn to www.restek.com/cannabis

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Article
Cannabinoid Neutrals Mix (9 components)

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