APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Cannabis Analysis: Potency Testing Identification and Quantification of THC and CBD by GC/FID and GC/MS

Introduction

Analysis of cannabis has taken on new importance in light of legalized marijuana in several states of the USA. Cannabis contains several different components classed as cannabinoids. Primary cannabinoids of interest are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). Positive identification and quantification of the THC/CBD ratio is a primary objective in the analysis of cannabis. Cannabis is analyzed for several different purposes. This application note will concentrate on the potency identification and quantification of THC and CBD in cannabis by Gas Chromatography. Other application notes will cover potency by HPLC, pesticide analysis and residual solvent analyses.

As of 2014, Cannabis has been legalized in 20 states in the USA and the District of Columbia. Possession is still illegal by Federal statutes. This can influence interstate transportation of cannabis products, but it can also influence laboratory possession of cannabis for testing purposes. Consult state regulatory agencies for proper licensing requirements.

For recreational marijuana, the psychoactive THC is the primary component of interest. Desirable traits of recreational cannabis would include high levels of THC and low levels of CBD and CBN. This information can be used to compare the relative strengths of the plant material based on THC content. Higher content THC plant material can demand higher prices. This information is valuable to growers, dispensaries and taxing authorities.

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Medical marijuana is often characterized by higher levels of CBD and lower levels of THC. The therapeutic CBD is desirable for medicinal effect but the psychoactive THC may be unnecessary and undesirable for some patients. This THC/CBD ratio information is of primary importance to the medical personnel prescribing cannabis for medicinal purposes. Some medicinal effects of cannabis could include reduced symptoms such as nausea, seizures, eye pressure and pain.

Cannabis can be administered and consumed in several forms. Plant material can be smoked, or added directly to baked food products. Extracted forms of cannabis could include edible wax used as butter in cooking, baking or as a liquid tincture used for direct ingestion of the oil product. Edible and tincture forms tend to offer longer lasting effects than smoked forms.

Multiple analyses are often required including:

- Macroscopic and Microscopic analysis
- Potency testing: THC/CBD ratio
- Safety: Mold/mildew/microbes/bacteria, Pesticide residue, Terpenoids, flavonoids, Residual solvent analysis of extracted concentrates and Heavy metals

More than 40 cannabinoids have been identified in cannabis plant material. Most are at trace level concentrations, but eight main cannabinoids of interest must be resolved to determine potency testing.

Cannabichromene (CBC)	
Cannabidiol (CBD)	primary therapeutic component
Δ8-tetrahydrocannabinol (Δ8THC)	
Δ9-tetrahydrocannabinol (Δ9THC)	primary psychoactive component
Cannabigerol (CBG)	
Cannabinol (CBN)	sample breakdown due to age or poor storage conditions
THC Acid (THCA) (carboxy THC)	Native form of THC in plant material, not detected by GC
Cannabidiolic Acid (CBDA) (Carboxy CBD)	Native form of CBD in plant material, not detected by GC

The major cannabinoid in the cannabis plant material is THC Acid (THCA) which is thermally labile and converts to THC by decarboxylation during heating of the sample in smoking, cooking or in the hot GC injector port. For this reason, THCA is not detected by gas chromatography but is detected by HPLC.

THCA has some therapeutic properties which may be desirable for some patients. Therefore some preparations may be intended for consumption to keep the THCA without conversion to the psychoactive THC. Edible preparations, tinctures and oils are useful forms to preserve the THCA.

Medicinal cannabis typically has a higher CBD and lower THC. Example: 21%: CBD and 1% THC

Recreational cannabis typically has higher THC and lower CBD. Example: 24 %: THC and 2% CBD

This THC level in modern cannabis is much higher than 4-6% THC from clandestine cannabis in the 1970's.

Typically, edibles and extracted liquids and solids do not have to be tested for pesticides, mold and mildew if the plant material used for extraction has been tested. But these extracts still must be tested for potency of THC and CBD. Extraction from the food matrix requires additional diligence.

Analytical Procedures: GC or HPLC

Two analytical techniques have been successfully used for the potency testing of cannabis: Gas chromatography (GC) and High Performance Liquid Chromatography (HPLC). There are advantages and disadvantages to each technique. Refer to any specific guidelines offered by the state regulations for suggested analytical technique.

- Total THC is of primary interest in cannabis potency testing along with the THC/CBD ratio for therapeutic value.
- Total THC = THC + THCA
- Total CBD = CBD + CBDA (each must be corrected for weight of the carboxylic acid groups)

HPLC can identify the acid components of THCA and CBDA before conversion to their corresponding free forms of THC and CBD. This is often preferred for edible materials and extracted tinctures. This procedure can also be used for the original plant material potency testing and cannabinoid ratio calculations.

GC does not detect THCA or CBDA directly. The carboxylic acids decarboxylate in the intense heat of smoking, baking or GC injector port. THCA decarboxylates to THC and CBDA decarboxylates to CBD.

GC converts the acid forms to the free cannabinoids by in-situ decarboxylation in the heat of the injector, but the conversion may be incomplete depending on temperature and injector considerations. Heating the sample before analysis can produce a more reliable conversion and may be worth the extra moments for more accurate reporting. GC generally mimics the conversion process during smoking of plant material. GC is generally considered faster and simpler than HPLC so it is often preferred. GC/FID is preferred for speed of analysis and simplicity in routine identification and quantification of cannabinoid concentrations. For positive identification of each cannabinoid, gas chromatography with a mass spectrometer would be preferred. A GCMS system with a second injector and a FID in a second channel makes for a versatile hardware configuration. The GCMS channel can use a small diameter capillary column for higher resolution and reduced flow rate. The MS will also be necessary for other cannabis testing such as terpenoids and pesticide analysis.

Using gas chromatography, cannabis potency is based on the concentration of decarboxylated THC and CBD.

Using HPLC, cannabis potency testing is based on sum of THC and THCA.

Experimental/Analytical

For THC/CBD analysis, leafy cannabis is extracted with organic solvent to dissolve oily resin on the surface of the plant material. Solvents that have been used successfully include methanol, isopropanol, ethyl acetate and others. The supernatant of the extract is injected into a gas chromatograph for separation and detected by either a flame ionization detector or by a mass spectrometer for positive identification.

Sample Handling:

To insure uniform sample, follow guidelines for random sampling of plant section to include leaf, bud, and flower if available.
Dry plant material two hours at 35 °C with forced air ventilation
Weigh approximately 100 mg dried plant material and grind the sample to a powder to pass through a 1 mm sieve
Add 30 ml organic solvent, sonicate 30 minutes, and filter
(Optional step to convert THCA to THC): Evaporate to dryness at 200 °C for 20 minutes, Reconstitute in organic solvent
Analysis by gas chromatography

GC Column Selection

Several types of analytical columns can be used for the separation of cannabinoids, but the most common columns are small diameter, thin filmed non-polar stationary phase columns. Non-polar columns such as 100 % dimethyl silicone or the popular 5 % diphenyl 95 % dimethyl silicone do not have sufficient polarity to separate the cannabidiol from cannabichromene. This is a critical separation in accurate THC/CBD ratio determination. An intermediate polarity column is preferred to resolve these important cannabinoids of interest. 35% diphenyl with 65% dimethyl silicone is sufficient for the resolution of these components and still has a high temperature limit to allow all cannabinoids to elute. Starting GC conditions for 9 minute analysis by GC/FID:

Column:	15 m X 530 u X 0.5 u Elite35
Carrier:	4 ml/min Hydrogen
Injector:	Capillary split injector, split liner with glass wool, 275 °C
Detector:	FID

Starting GC conditions for a slower 30 minute analysis by GCMS: includes terpenoids and pesticides (splitless)

Column:	30 m X 25 0u X 0.25 Elite35
Carrier:	1.3 ml/min Helium
Injector:	Capillary split injector, split liner with glass wool, 275 °C
Detector:	Mass Spectrometer, mass range: 50-400 da

Separation of cannabinoid standards can be fast at less than three minutes with a short, small diameter column. But real world samples have a huge Δ9THC that can overload a small bore column. Therefore a 530 u diameter wide bore column to the FID is preferred since it has much more sample capacity. Real world samples have several later eluting trace cannabinoids which requires sufficient time and temperature to elute at least six trace cannabinoids after CBN from the column before the next injection. Therefore a three minute chromatogram is not realistic. A nine minute FID chromatogram is more realistic.

Analytical Standards

Commercial standards are available at a typical concentration of 1000 ug/ml. Another common standard available is a mixture of three cannabinoids (CBD, Δ9THC and CBN) each at 1000 ug/ml. There is no commercially available standard to conform to the expected ratios of THC/CBD. A suitable standard can be prepared from commercially available standards.

Typical sample preparation will take 100 mg dried plant material in 30 ml solvent. Recreational cannabis will have an expected concentration of 20 % THC. This calculates to 20 mg THC in 30 ml solution or 666.7 ug/ml which is the same order of magnitude as the standard. Use additional dilution of the sample as necessary to bring the THC into the linear range of calibration.

Other cannabinoids will be trace level to approximately 1% level. Example: 990 ul of Δ9THC (1000 ug/ml) + 10 ul of 3 cannabinoid standard (1000 ug/ml) will make a suitable calibration standard for recreational cannabis. This will be a standard for typically high THC and low CBD. Similar dilutions can be used for higher CBD and lower THC calibration standards. Use additional dilution of the sample as necessary to bring the THC into the linear range of calibration. Samples are reported in weight/weight % of THC to plant material and CBD to plant material.

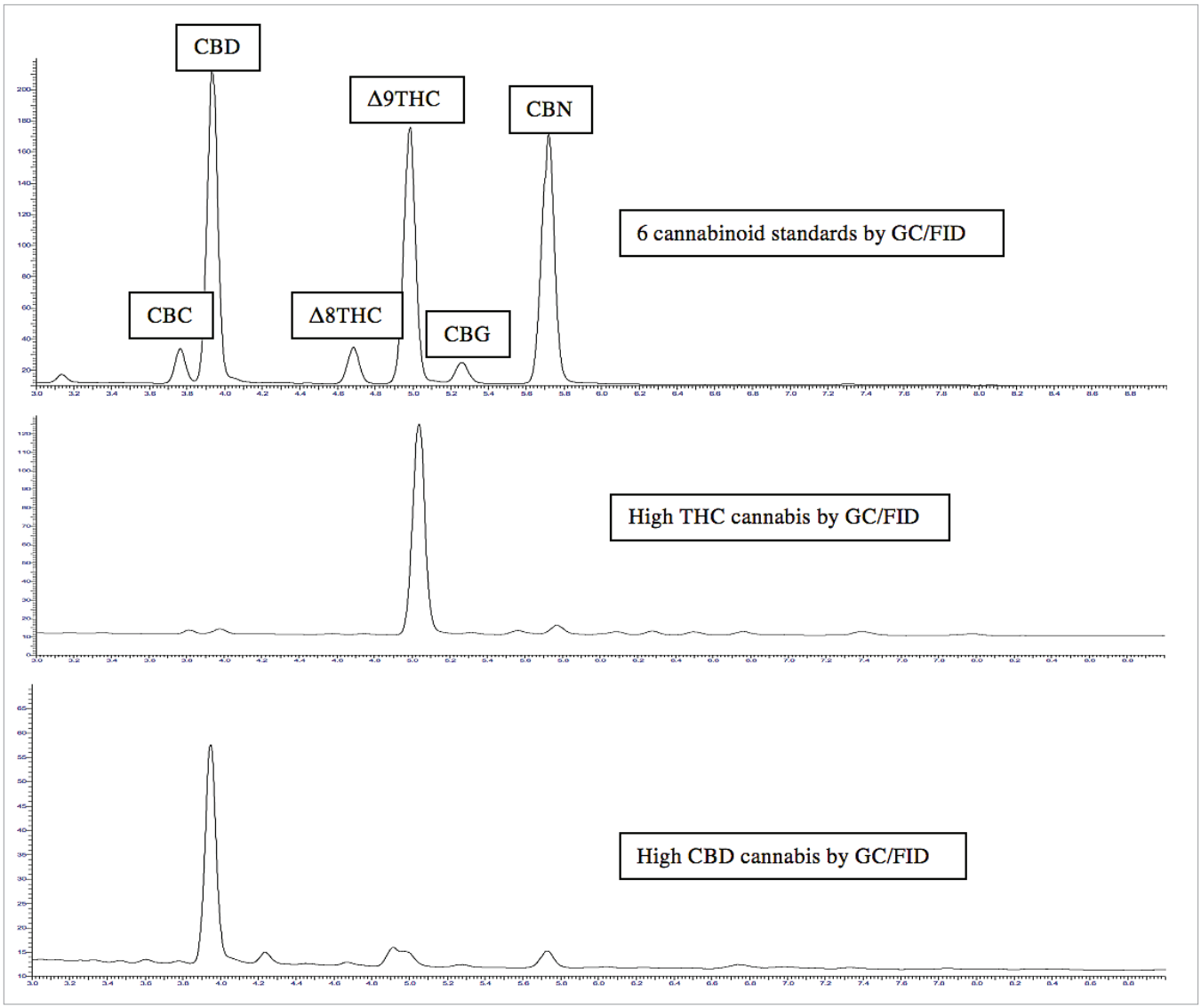

Figure 1. Cannabinoids by GC/FID.

Typical Testing
Refer to individual jurisdiction for actual testing requirements.

Macroscopic/Microscopic Examination
Color consistency, debris, stems, seeds, contaminants and adulterants. Added adulterants to plant materials have been documented in many locations to improve the appearance and weight. To the naked eye, finely powered sand shimmers like the oily resin of the plant material and adds weight. Finely powdered lead adds significant weight.

THC/CBD Potency
Without specific methods of regulation from the individual states, procedures could follow the DEA guidelines (Drug Enforcement Administration). The primary analytical method is gas chromatography. Report is in %THC relative to original plant material (w/w).

Pesticides/Fungicides
Without specific methods of regulation from the individual states, procedures follow the EPA (Environmental Protection Agency) guidelines for pesticide residue analysis. Extraction is typically by SPE (Solid Phase Extraction) or QuEChers extraction followed by GC/MS/SIM analysis. HPLC analysis is required for the carbamate pesticides. Positive pesticide by some regulatory agencies has been defined as 0.1 ppm of any pesticide. Individual state regulations will undoubtedly be modified as this cannabis trend continues.

Pesticide classes of interest typically include:
- Chlorinated hydrocarbons, organophosphates, or pyrethroids by GC/MS
- Carbamate pesticides by HPLC or HPLC/MS

Terpenoids
Terpenoids are responsible for the aroma of cannabis while the cannabinoids themselves are odorless. Several terpenoids are present in cannabis plant material and would include α-pinene, β-pinene, β-myrcene, β-carophylene and others. Analysis is typically accomplished by GC/MS.

Mold/Mildew/Microbes/Fungus/Bacteria
Microscopic examination and petri dish culture for Aspergillus spp., Escherichia coli (E. coli) and salmonella.

Residual Solvents

Extracted concentrates of cannabis are formulated into hash oil, wax, butter (budder) and other forms. Extraction takes place with any several types of solvents such as carbon dioxide, butane, propane, ethanol, isopropanol, acetone and others. For safety purposes, solvent must be removed from the final product before consumption. Residual solvent is measured by headspace with gas chromatography and flame ionization detection (HS-GC-FID). Without specific methods of regulation from the individual states, procedures follow the ICH guidelines (International Council of Harmonization) for residual solvents in botanical preparations.

Heavy Metals

Without specific methods of regulation from the individual states, procedures follow the FDA (Food and Drug Administration) guidelines for heavy metals in food products. Analysis is by ICP-MS for trace level contaminants such as lead, mercury, arsenic, cadmium, chromium and others.

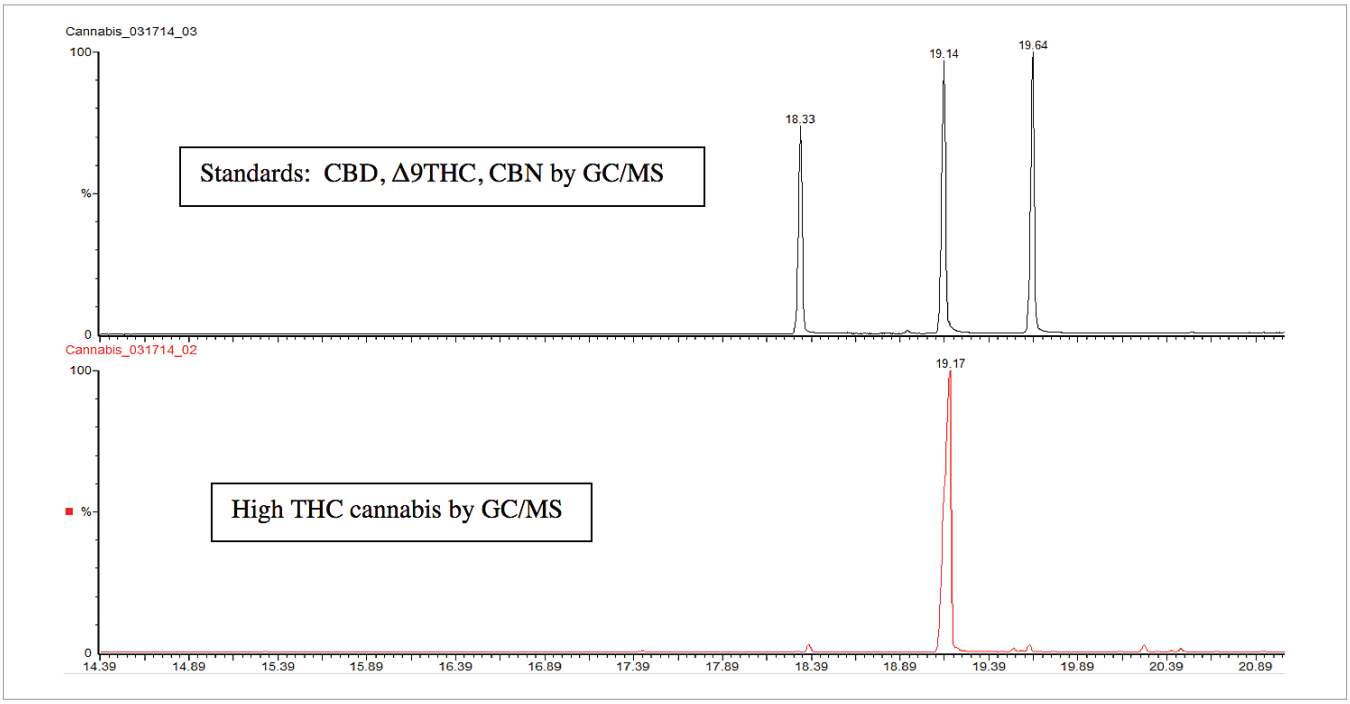

Figure 2. Cannabinoids by GC/MS.

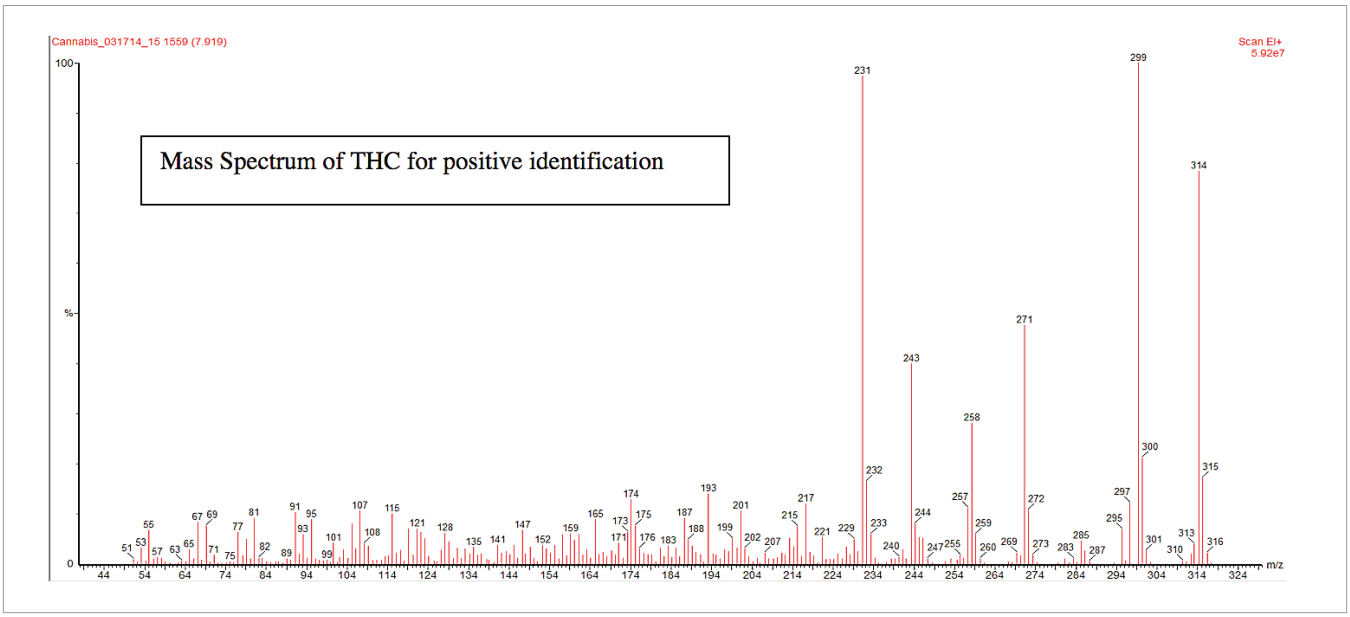

Figure 3. EI-Mass Spectrum of THC.

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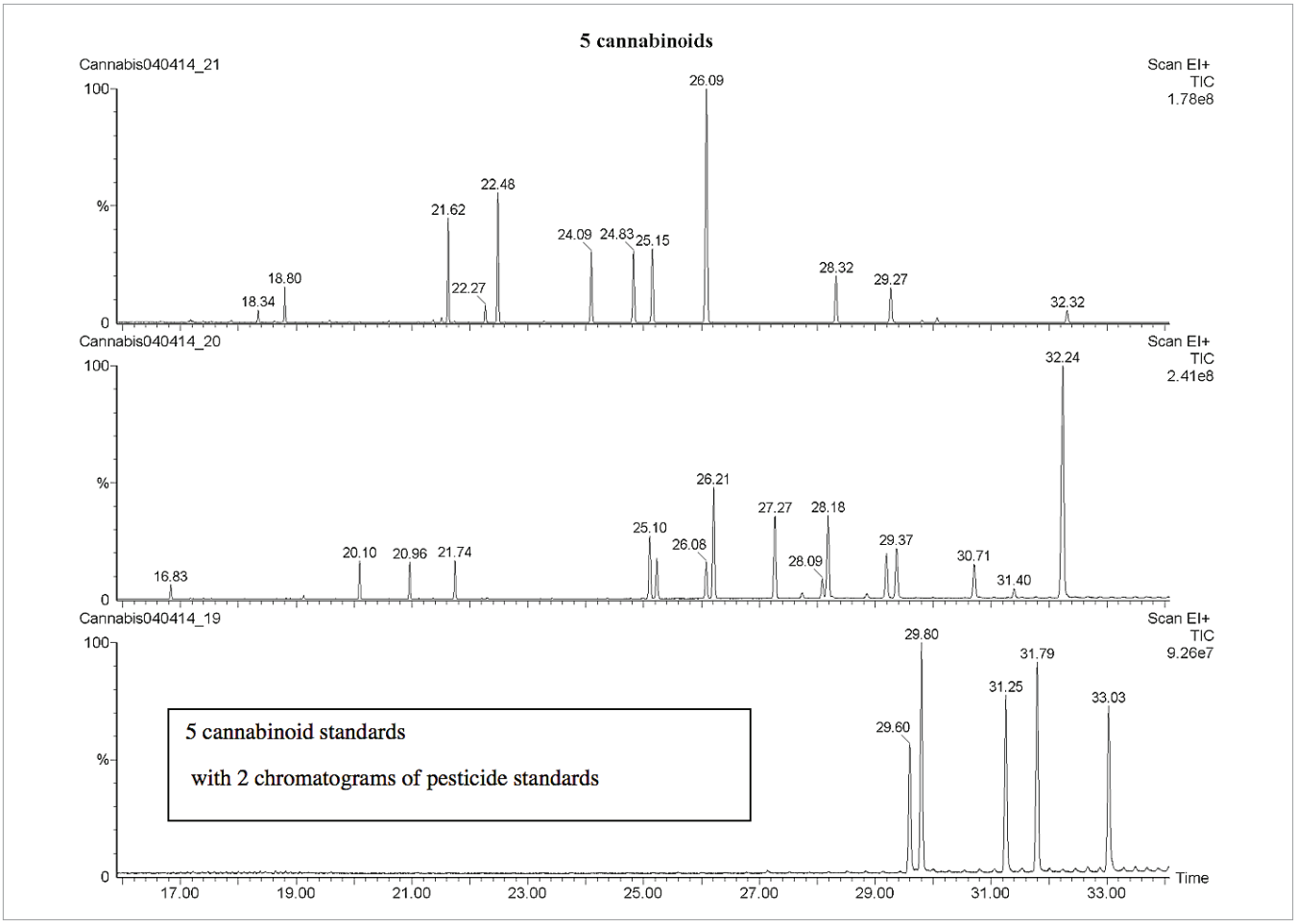

Figure 4. Pesticide elution times relative to cannabinoids.

Conclusion

GC/FID can be used for rapid determination of THC and CBD concentrations in cannabis to characterize and grade plant material for recreational and medical marijuana applications. GCMS can be used for rapid positive identification of cannabis samples. GC/MS can also be used for additional safety testing of cannabis.

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