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i-Series Cannabis Analyzer

- for Potency Testing -

CIPS
Shimadzu Europa GmbH

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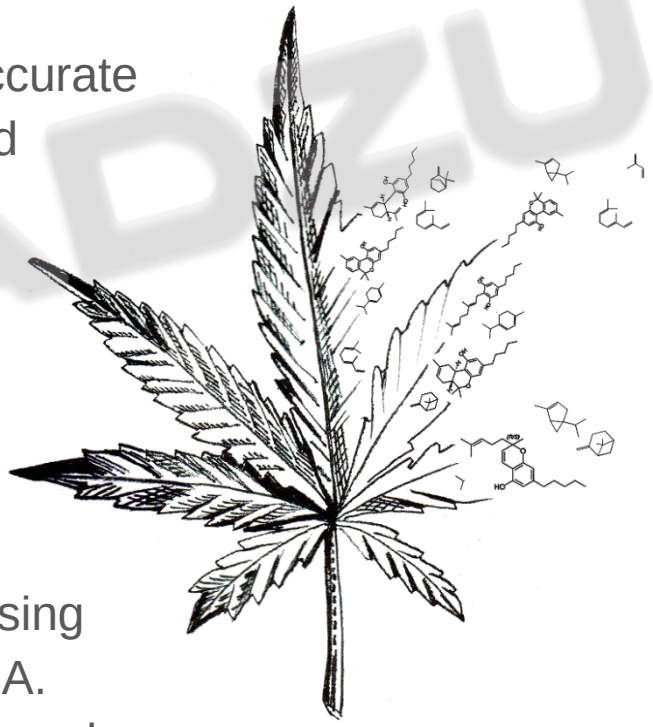
Outline

1. **Introduction**
   - Overview of cannabis chemistry and potency testing
2. **The Cannabis Analyzer for Potency Testing**
   - What is included in the package
   - Goal oriented analytical methods
   - Quantitative accuracy and analytical results
   - Sample preparation
   - Workflow in the dedicated software

Introduction

- **Overview of Cannabis Testing**
  - Cannabis contains more than 500 unique compounds, including over 80 alkaloids known as cannabinoids.
  - QC testing for cannabinoids is essential for the accurate labeling of cannabis products for both medical and recreational use.
  - Cannabis “potency” is normally reserved for the quantitation of the major cannabinoids, namely THCA, THC, CBD and CBN
  - HPLC has emerged as the gold standard for potency determinations because separation and detection of the cannabinoids is done without causing any decomposition of the naturally abundant THCA. This acid form undergoes decarboxylation to THC under the influence of heat and light.

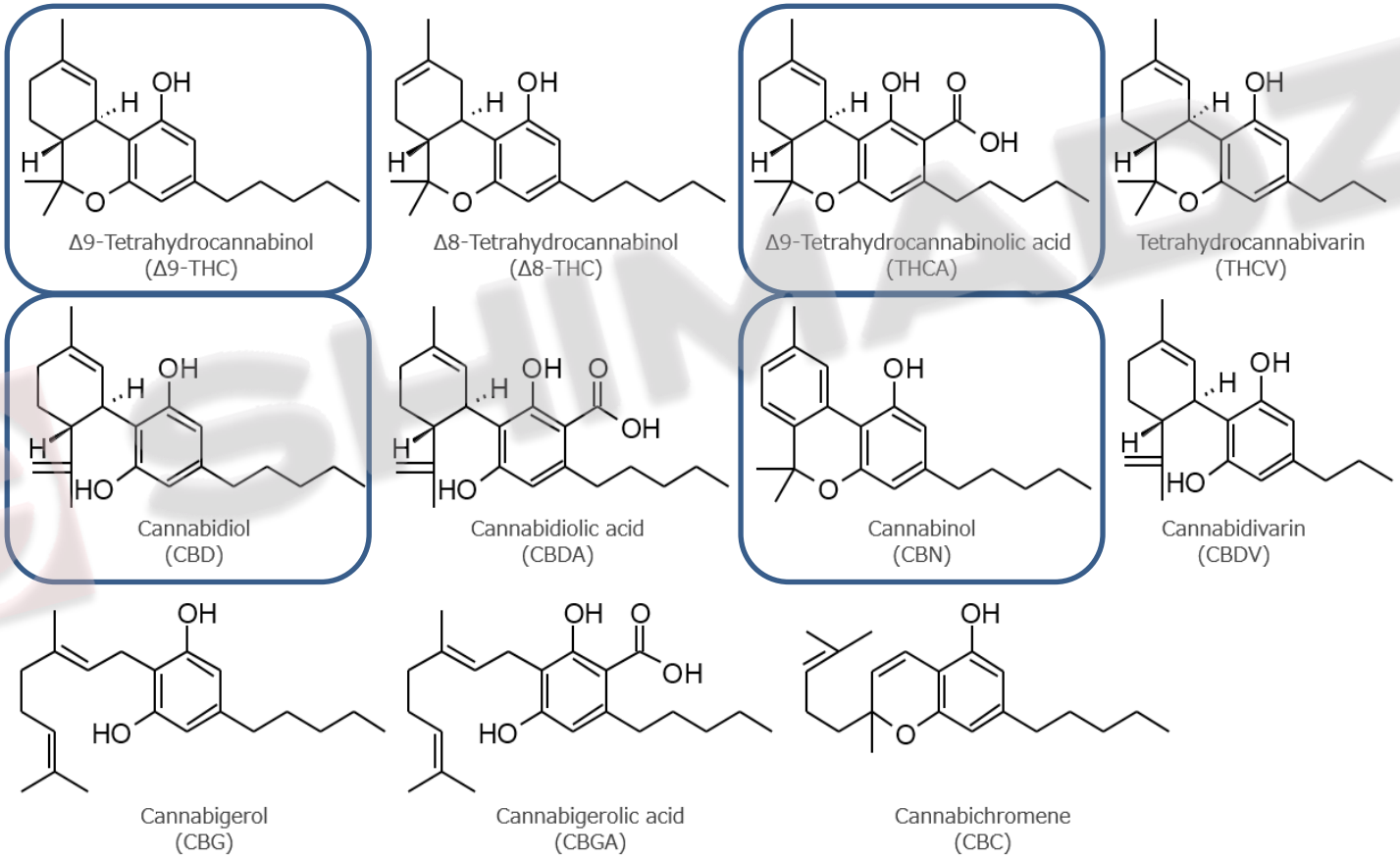


Importance of Cannabis

- THC does not occur naturally in cannabis plants. THC-A is the natural, non-psychoactive, carboxylic acid form of THC. It is rapidly converted to THC upon smoking or heating.
- The premium products sold in medicinal cannabis dispensaries are typically those with high THC concentrations, however, it's THC-A and the other non-psychoactive "CBx" cannabinoids, such as CBD that have been reported to reduce convulsions, inflammation, nausea and anxiety, and even eradicate tumors in some patients.
- CBN, only slightly psychoactive, is a degradation byproduct of THC and it is elevated when cannabis is poorly stored.

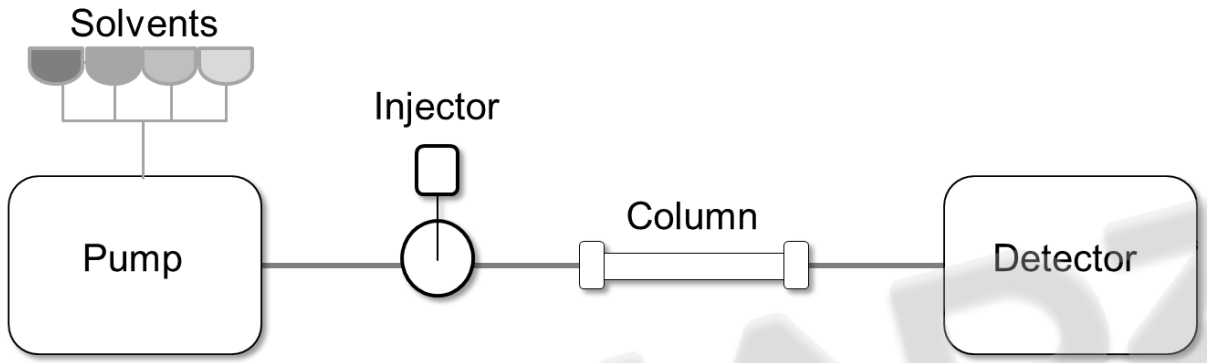
Structure Formula

- **Naturally occurring cannabinoids of primary interest for potency determination**

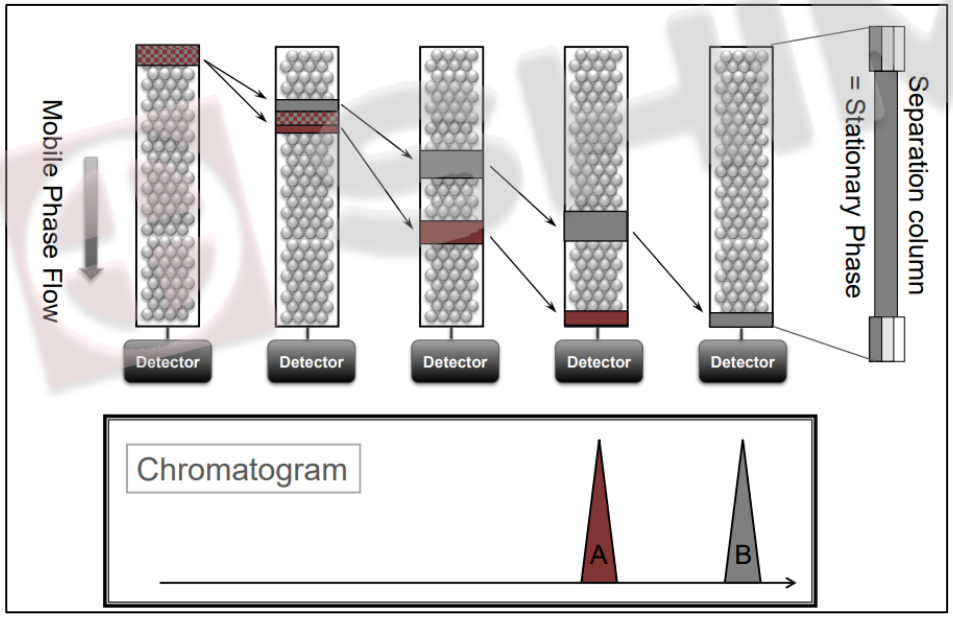


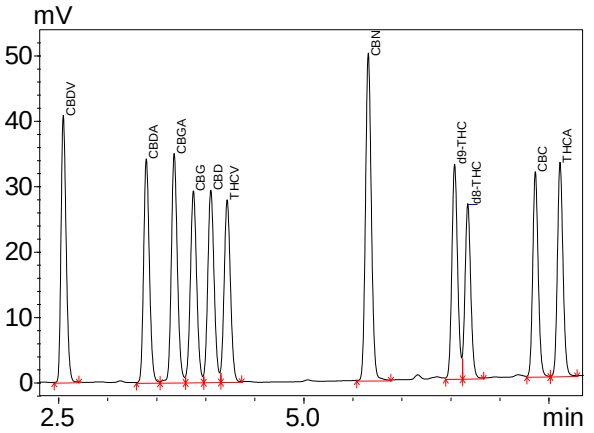
Principle of Liquid Chromatography

HPLC: High Performance Liquid Chromatography



Chromatogram





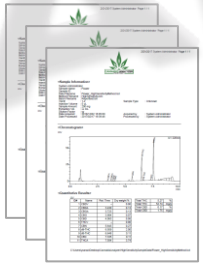
The i-Series Cannabis Analyzer

- **A complete solution for quantitative determination of cannabinoid content**
  - The analyzer is designed for potency determination by quantitation of the active constituents. It includes all required hardware, software, consumables and procedures, so the analyst is running samples in the shortest possible time.

Prominence-i compact HPLC instrument with integrated UV detector

Columns and guard columns

Software and Method Package



App notes, sample preparation procedures, report templates

  - Three proprietary instrument methods to meet your analytical objectives. No need to spend valuable lab time developing methods – just run samples.

Included Methods

- **High Throughput HPLC Method Package**
  - Designed for analysis of the 10 most commonly requested cannabinoids in under 8 minutes. (Does not include THCV)

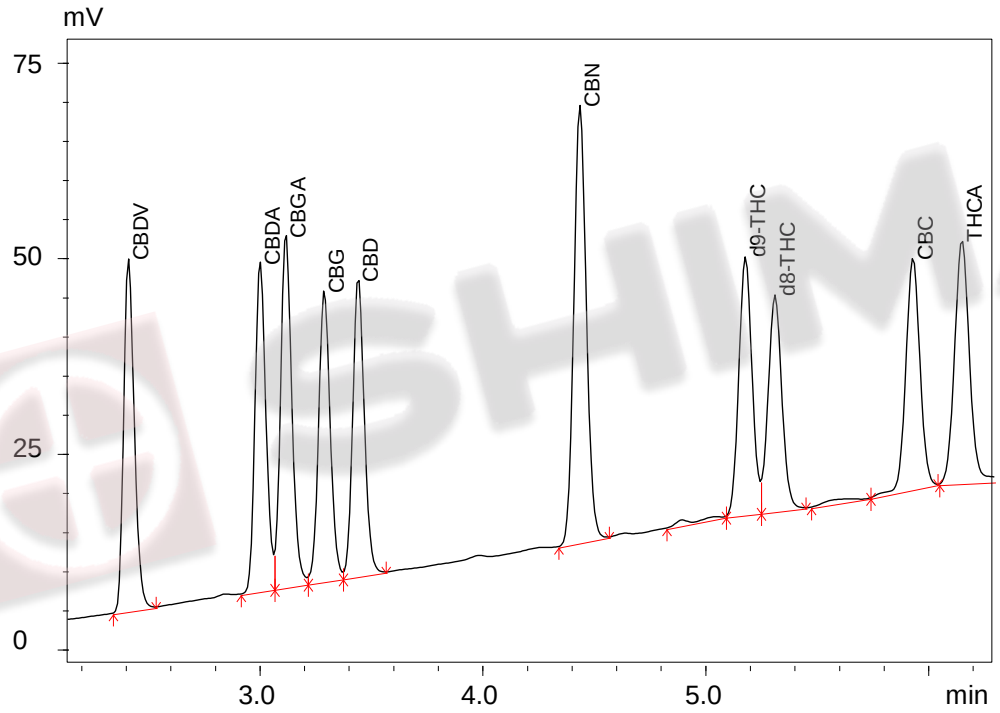
- **High Sensitivity HPLC Method Package**
  - Adds THCV to the target analyte list, with an instrument cycle time of under 10 minutes. The short analysis time produces the sharpest chromatographic peaks for the best overall sensitivity.

- **High Resolution HPLC Method Package**
  - Full baseline resolution for all 11 compounds and an analysis time under 30 minutes. This method is preferred for research purposes, or when additional compounds must be added to the analysis in response to new state regulatory requirements.

→ **All methods were exhaustively tested for ruggedness, repeatability and quantitative accuracy !**

High Throughput

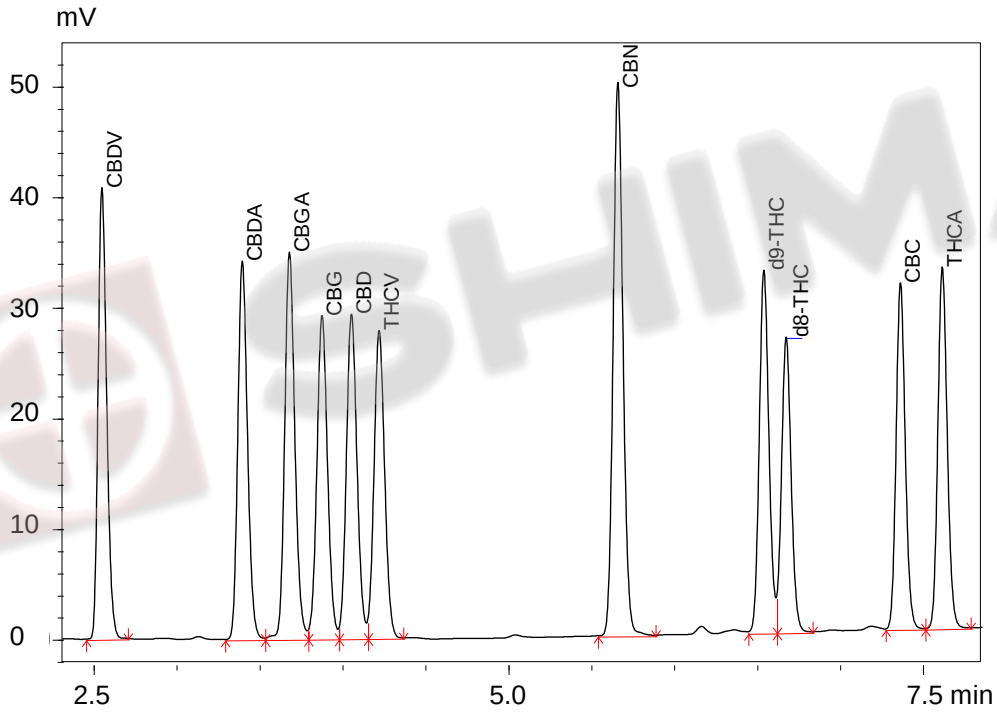
- **High Throughput HPLC Method Package**
  - Designed for analysis of the 10 most commonly requested cannabinoids in under 8 minutes. (Does not include THCV)



- When high sample throughput is paramount, 60 samples per 8 hr day
- Quantitative for 10 cannabinoids

High Sensitivity

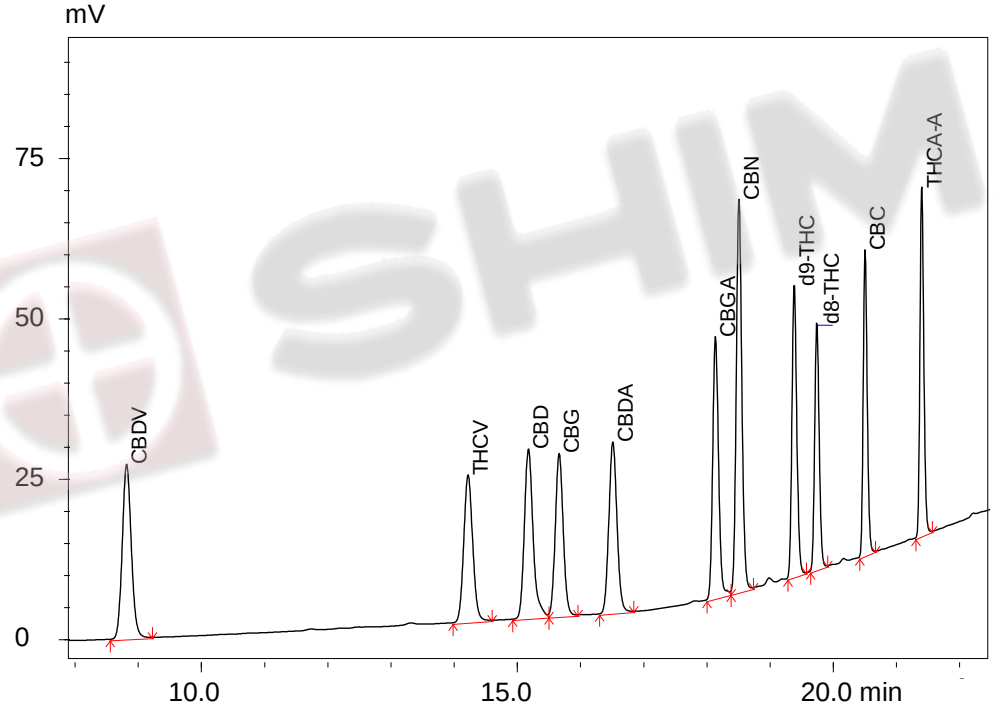
- **High Sensitivity HPLC Method Package**
  - Adds THCV to the target analyte list, with an instrument cycle time of under 10 minutes. The short analysis time produces the sharpest chromatographic peaks for the best overall sensitivity.



- When THCV is important, yet with good sample throughput,
  48 samples per 8 hr day
- Quantitative for 11 cannabinoids

High Resolution

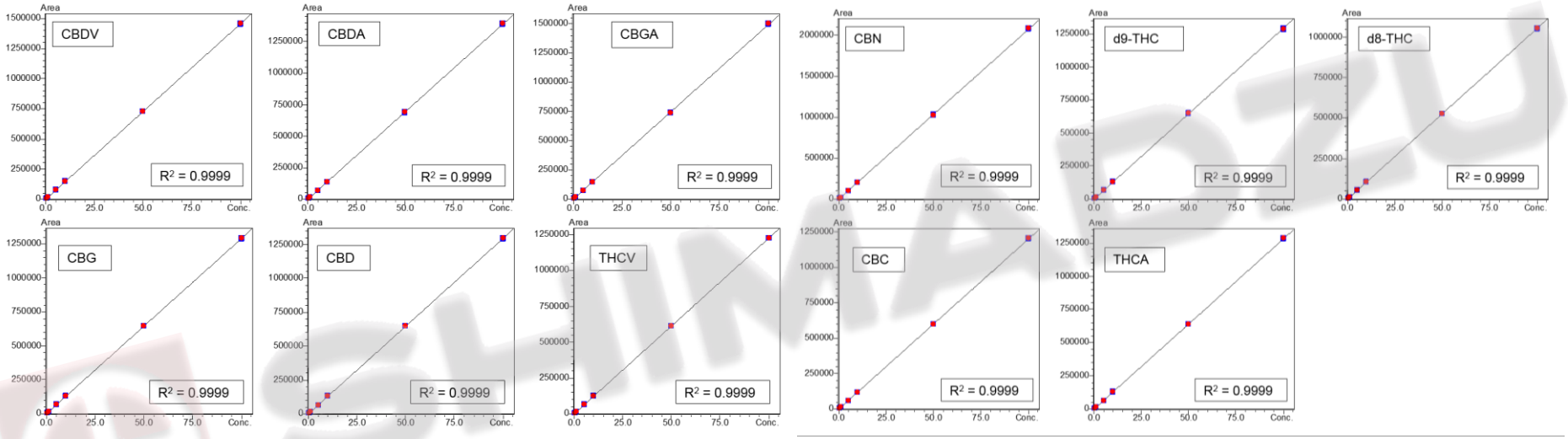
- **High Resolution HPLC Method Package**
  - Full baseline resolution for all 11 compounds and an analysis time under 30 minutes. Preferred method for research purposes, or when additional compounds must be added in response to new regulatory requirements.



- For baseline separation of 11+ components, including THCV.
  Minimum Resolution = 2.0
- 16 samples per 8 hr day
- Add cannabinoid targets as regulations change

Quantitative Accuracy

- **Standard Calibration Curves (High Sensitivity Method)**
  - R^2 > 0.999 for all targets



Level (ppm)	Accuracy % (Bias)	Range
Low 2	109.7 – 111.1	± 1.4
Med 20	101.2 – 103.4	± 2.2
High 70	99.2 – 100.2	± 1.0

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Limits of Detection and Quantitation

- **Determined LOQs and LODs (High Sensitivity Method)**
  - LOQ ≤ 0.64 mg/L for all targets
  - LOD ≤ 0.21 mg/L for all targets

Target Compound List	Abbreviation	LOQ (mg/L)	LOD (mg/L)
Cannabichromene	CBC	0.60	0.20
Cannabidiol	CBD	0.59	0.20
Cannabidiolic acid	CBDA	0.55	0.18
Cannabidivarin	CBDV	0.43	0.14
Cannabigerol	CBG	0.59	0.20
Cannabigerolic acid	CBGA	0.53	0.18
Cannabinol	CBN	0.37	0.12
d8-Tetrahydrocannabinoid	d8-THC	0.64	0.21
d9-Tetrahydrocannabinoid	d9-THC	0.52	0.17
Tetrahydrocannabivarin	THCV	0.62	0.20

All values correspond to Dry WT % of less than 0.1%

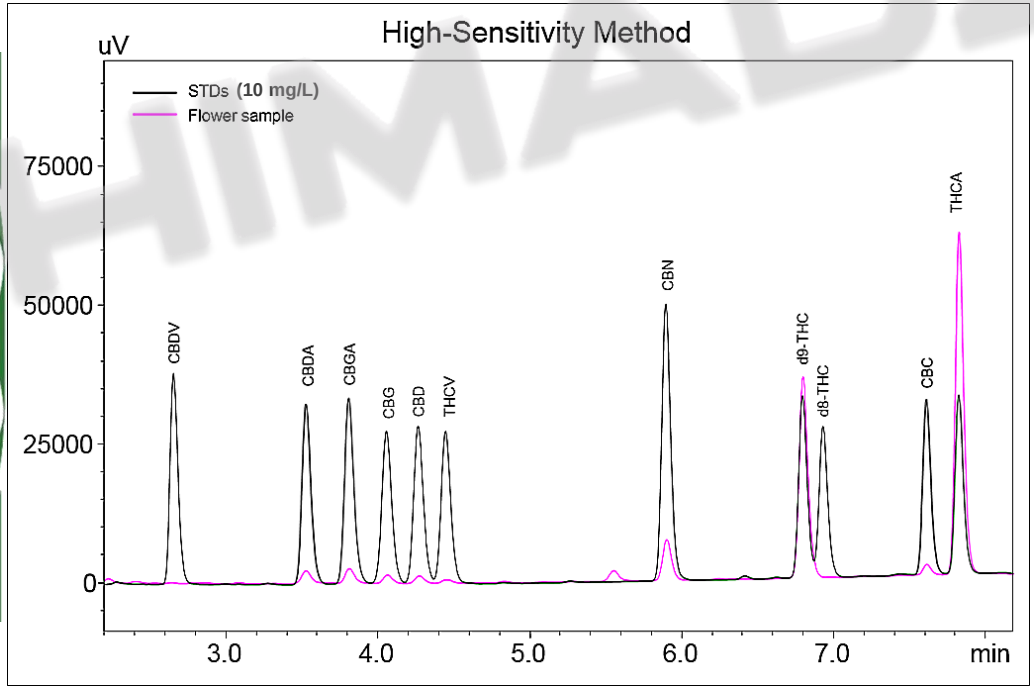
Analytical Results

- **Example of quantification from 200 mg cannabis flower**

→ DRY WT% = [Target] x (Dilution) x (Extraction Vol./Dry wt mg) x 100

THCA WT% = 3.8 % = [18.9 mg/L] x (20/1) x (0.02 L/200 mg) x 100

d9-THC WT% = 2.0 % = [10.2 mg/L] x (20/1) x (0.02 L/200 mg) x 100



Sample Preparation of Flowers

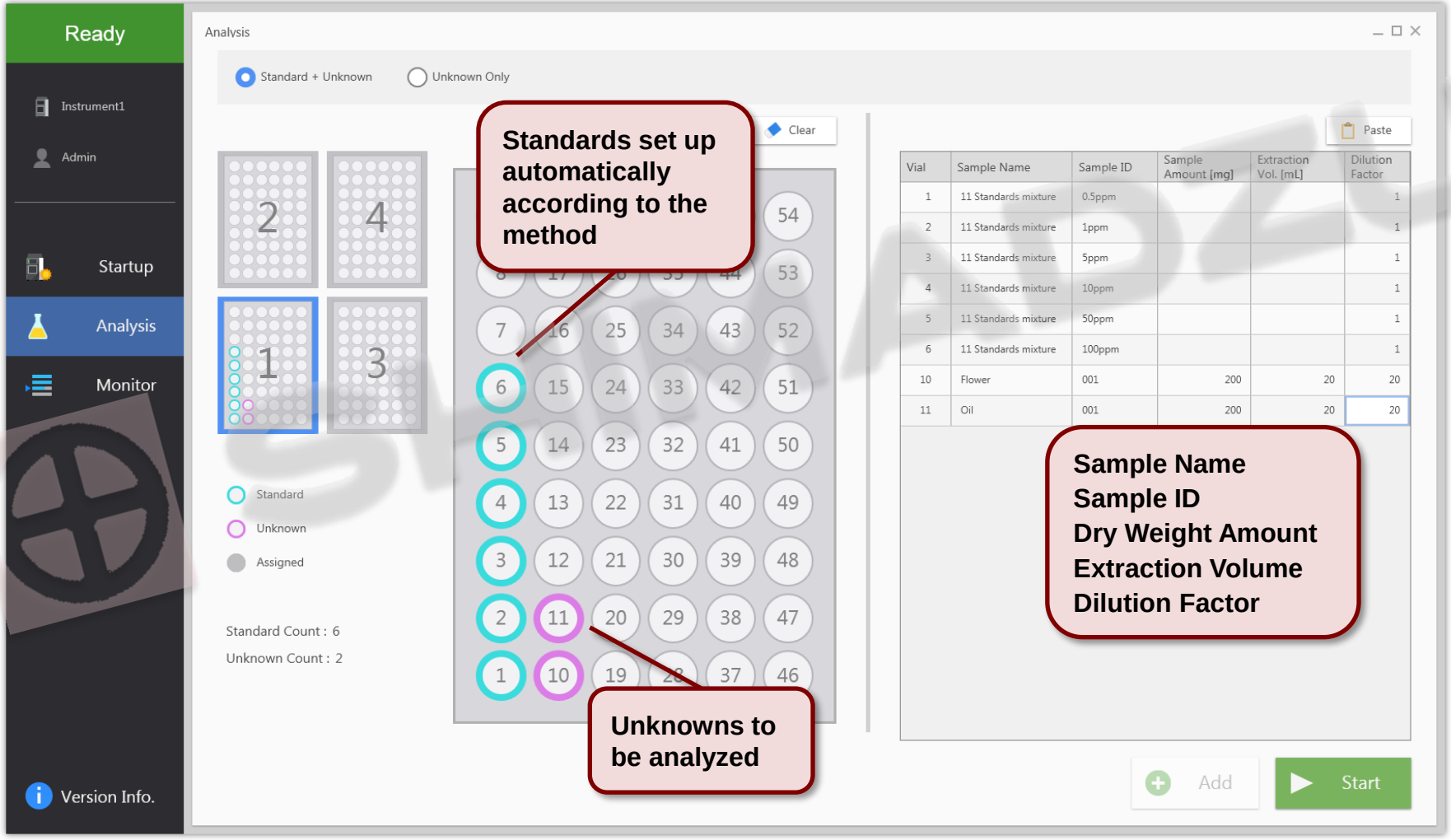
- **Prepare extracts from flowers, leaves or stems**
  - Weigh 200 mg of flower or leaf cuttings into 50 mL centrifuge tube
  - Add two 9.5 mm steel balls into the tube
  - Shake at 1000 rpm for 1 min with a Grinder or similar
  - Add 20 mL of methanol to the tube
  - Shake at 1000 rpm for 1 min
  - Wait for 15 min
  - Mix using a vortex mixer for 1 min.
  - Transfer 1 mL of the mixture into a 1.5 mL micro-tube and centrifuge at 3000 rpm for 5 min
  - Transfer 100 µL of supernatant to a new 1.5 mL micro-tube
  - Add 900 µL of methanol
  - Filter the mixture through a 0.45 µm syringe filter and transfer to a 1.5 mL sample vial

Sample Preparation of Oils

- **Prepare extracts from flowers, leaves or stems**
  - Add 400 µL isopropanol to a 2 mL glass vial
  - Add 10 µL hemp oil sample and completely dissolve
  - Agitate for 30 seconds
  - Add 400 µL methanol to the mixture
  - Agitate for 30 seconds
  - Filter the mixture through a 0.2 µm PTFE syringe filter into an HPLC vial
  - (Note: Total dilution factor x 81)

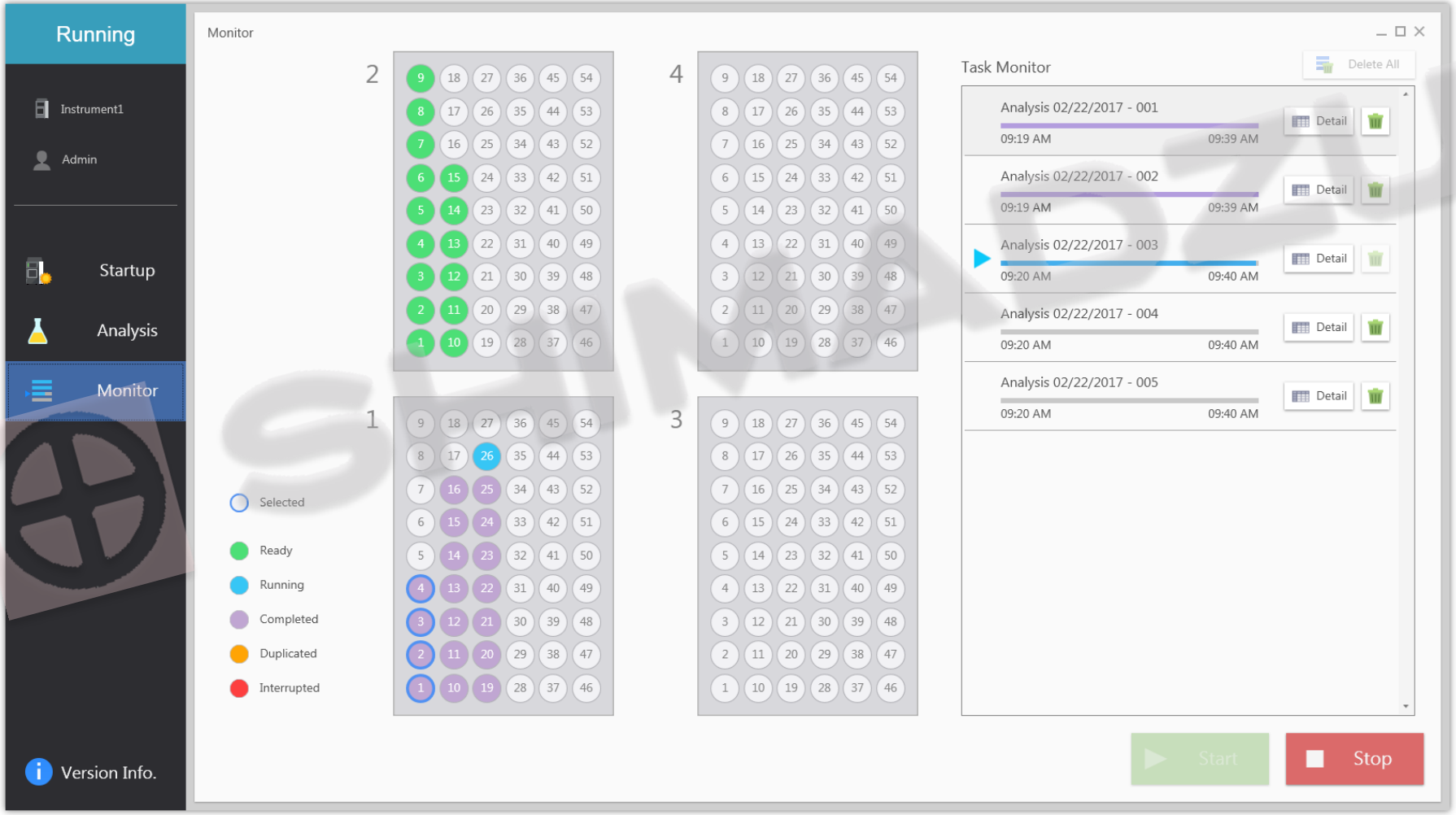
Workflow in the Software (1)

1. Setting samples in the Analysis Screen



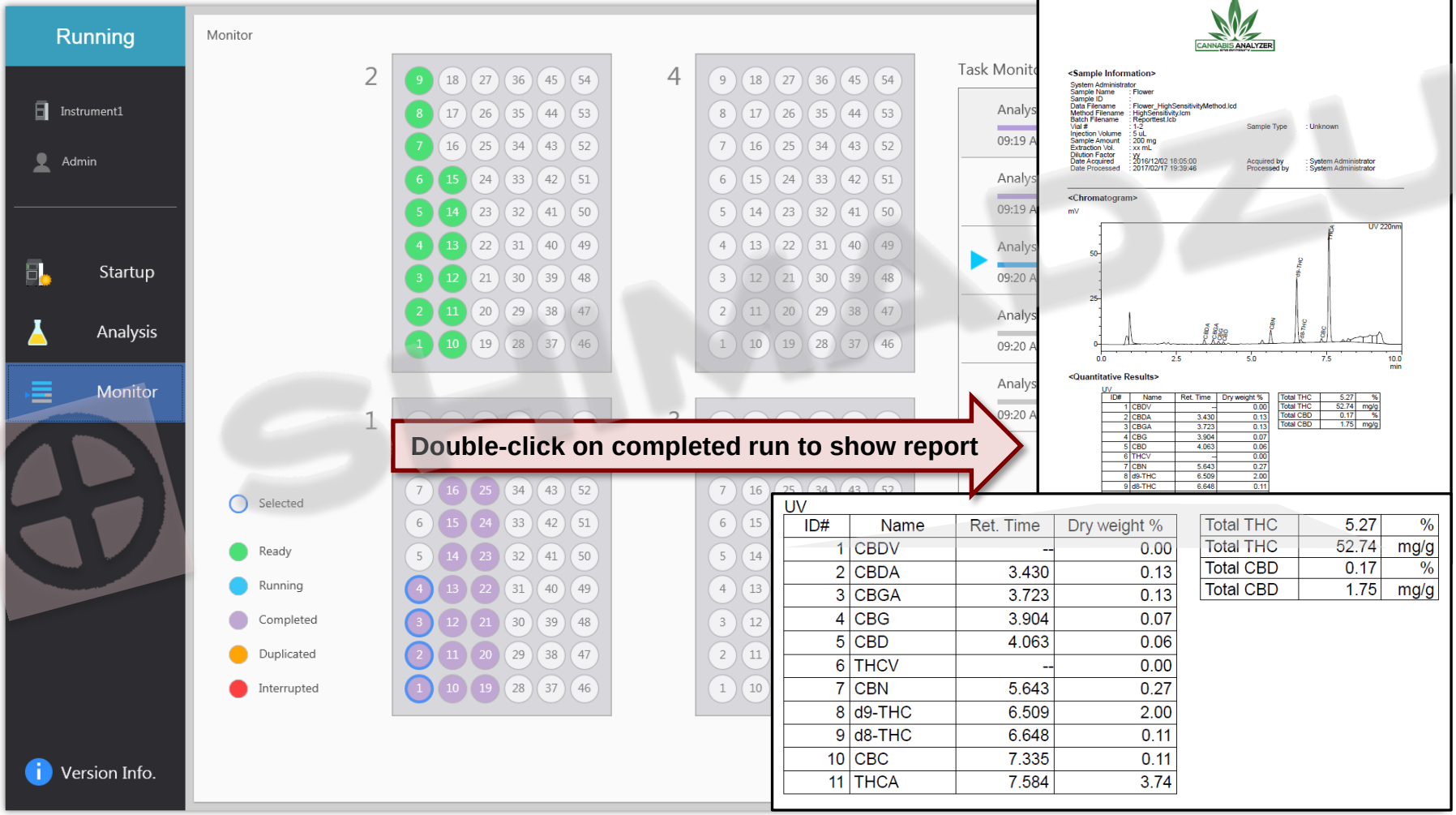
Workflow in the Software (2)

2. Monitoring of progress on the Analysis Screen



Workflow in the Software (3)

3. Quick access to quantitative reports



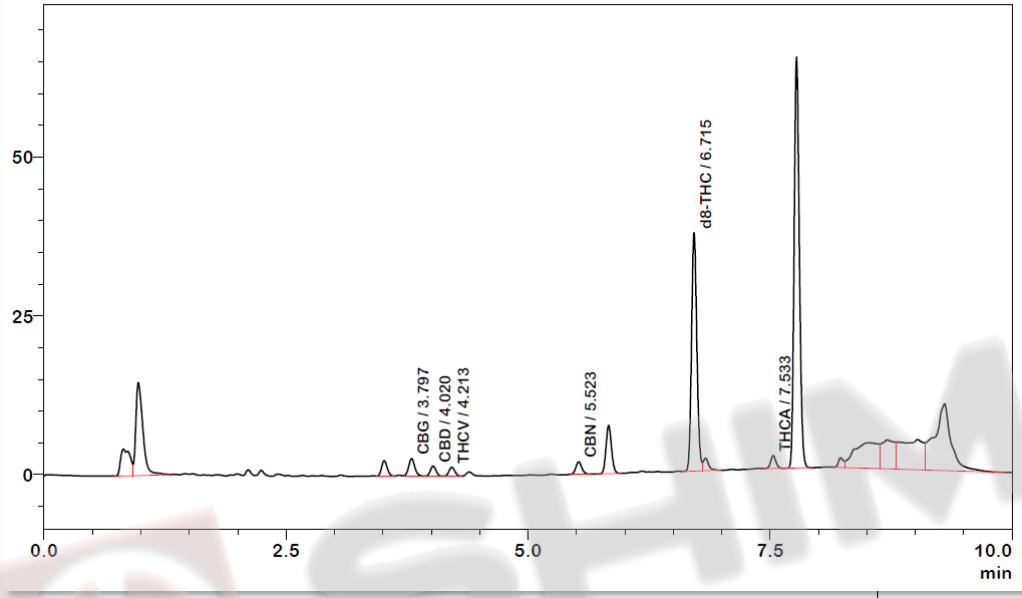
UV

ID#	Name	Ret. Time	Dry weight %
1	CBDV	--	0.00
2	CBDA	3.430	0.13
3	CBGA	3.723	0.13
4	CBG	3.904	0.07
5	CBD	4.063	0.06
6	THCV	--	0.00
7	CBN	5.643	0.27
8	d9-THC	6.509	2.00
9	d8-THC	6.648	0.11
10	CBC	7.335	0.11
11	THCA	7.584	3.74

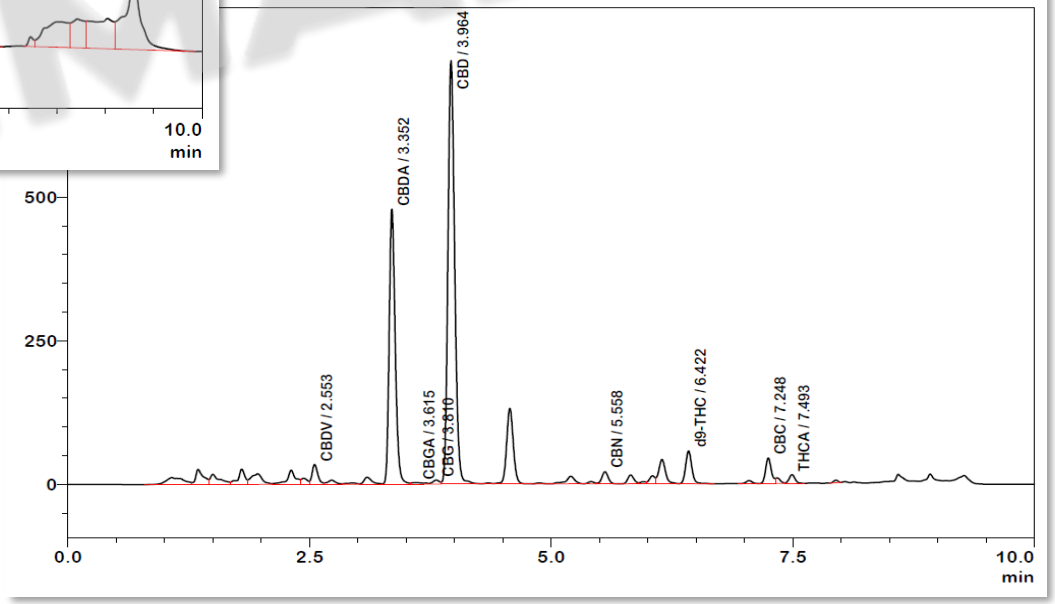
Total THC	5.27	%
Total THC	52.74	mg/g
Total CBD	0.17	%
Total CBD	1.75	mg/g

Examples of Real Samples

Flower Sample



Hemp Oil Sample



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Supporting Documentation

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Application News

High Performance Liquid Chromatography

The Determination of CBD and General Cannabinoid Content in Hemp Oils Using HPLC with UV Detection

No. HPLC-018

■ Introduction

Medical marijuana generally possesses high levels of the therapeutic cannabidiol, CBD, and lower levels (generally less than 0.3%) of the psychotropic tetrahydrocannabinol, d9-THC. Pain mitigation and reduced severity of nausea and seizures are just a few of the therapeutic benefits reported by medical cannabis patients. Little has been done to better understand the chemistry of benefits from CBD. To complicate matters, there is evidence that a combination of CBD, a host of other minor cannabinoids and a complex array of terpenoids may be the most beneficial – called the "entourage effect." CBD-rich oil has become increasingly popular and is administered via sublingual drops, gel capsules or as a topical ointment.

The main source of CBD-rich oil is industrial hemp. Hemp is considered a rustic plant as it is frost resistant, adapts to poor soil, reproduces easily, and does not require chemical fertilizers/pesticides/herbicides/fungicides to thrive. A hemp crop tends to resists mildew and requires less water than cotton. Hemp textiles are considered softer than cotton.

CBD oil is derived as concentrate from CO2 or butane extraction of hemp, sometimes followed steam distillation or ethanol distillation for purification. The Farm Bill of 2014 distinguishes hemp from marijuana, yet interpreting the law i difficult in that "CBD oil" may be classified as marijuana.

The FDA has issued warning letters to firms that market unapproved new drugs allegedly contai CBD. As part of these actions, the FDA has determined the cannabinoid content of some h products and many were found to contain level CBD that are very different from the label claim important to note that such products are not approved by the FDA for the diagnosis, cure, mitigation, treatment, or prevention of any dise

Like cannabis, hemp oil may be analyzed easily effectively for its cannabinoid content. This application note highlights the use of a High Sensitivity HPLC method used with Shimadzu's "Cannabis Analyzer for Potency" to determine important cannabinoids, including CBD (Figure hemp oil.

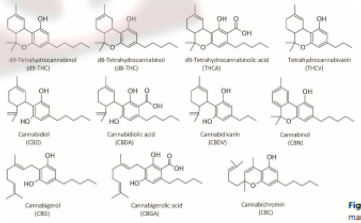

Figure 1: Cannabinoids found in hemp and marijuana

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Application News

High Performance Liquid Chromatography

The Potency Determination of 16 Cannabinoids by UHPLC with Diode-Array Detection

No. HPLC-020

■ Introduction

Since the legalization of cannabis in several US states and, recently, Canada, the quantitative determination of cannabinoids in cannabis products has been of great interest. There are more than 100 cannabinoids that can be found in the plant or extracts[1]. Tetrahydrocannabinol (THC) and cannabidiol (CBD) are two of the highest priority in potency testing along with their acidic forms. The acidic forms, Tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), are primarily found in the plant, subsequently converting to THC and CBD through decarboxylation from exposure to heat and light[2].

Traditional HPLC is the gold standard for cannabinoids analysis, including the acidic forms, providing nearly complete separation of the cannabinoids, and robust quantitation. Several methods have been developed for optimal results of resolution, sensitivity, and throughput. To assist in optimizing for high throughput while maintaining sensitivity and resolution, this application note proves a 4.5-minute isocratic method using a UHPLC system.

■ Experimental

Potency analysis was performed using a Shimadzu Nexera-i (LC-2040C 3D) UHPLC with a photodiode array detector. The method conditions are shown in Table 1. Historically, 276 nm is ideal for acidic cannabinoids, but non-acidic cannabinoids give weak responses. Consistent with previous literature, a wavelength of 228 nm wash chosen as an acceptable compromise[3]. Experimentation with the PDA supported this finding (Figure 1).

Table 1: Instrument Method Parameters

Liquid Chromatography	Nexera-i (LC-2040C 3D)
Mobile Phase A	Water, 5 mM Ammonium Formate, 0.1% Formic Acid
Mobile Phase B	Acetonitrile, 0.1% Formic Acid
MP Composition	Isocratic, 25/75
Column	Shimadzu NexLeaf CBX II, 1.8 µm, 3.0 x 100mm (220-91525-75) Shimadzu NexLeaf CBX II Guard, 1.8 µm (220-91525-76)
Oven temperature	30°C
Flow rate	1.0 mL/min
Wavelength Monitored	228 nm

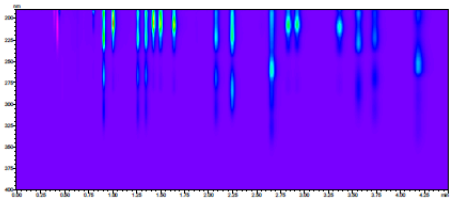

Figure 1: PDA contour plot showing wavelengths 190 to 400 nm

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Application News

High Performance Liquid Chromatography, i-series, Cannabinoids

The Determination of Cannabinoids Content within Gummy Based Confectionary

Angela Jein [1]
1 Shimadzu UK Ltd, United Kingdom

User Benefits

- Extraction of Cannabinoids from Confectionary Products
- Accurate analysis of Cannabinoids
- Extraction of spiked samples and Commercially available products

■ Introduction

The growth of the Cannabinoid industry has led to a wide variety of nutraceutical products being commercially available. The leading products within this range are confectionary in nature, typically gummy based sweets. This application looks at the extraction method from the confectionary utilizing Shimadzu's High Sensitivity Cannabinoid method for the analytical analysis.

The extraction method is tested using standard spiking addition techniques using non cannabinoid containing gummy confectionary, as well as using commercially available CBD gummy products. This combination of testing procedures ensured a robust and accurate extraction method was developed for a variety of gummy based confectionary products. The standard spiking tested out the methods precision, accuracy across differing spiking levels and specificity.

Investigations were also carried out to ensure the method is suitable for gummy based confectionary that is suitable for vegans, which does not contain gelatin.

■ Analytical Conditions

Analysis of all samples was carried out using Shimadzu's High Sensitivity method (HPLC-018), using a Nexera i-series (2060) with photodiode array detector. Reference standards were prepared from individual cannabinoid standards across a concentration range of 0.5 to 90.9 ppm for 11 specified cannabinoids.

■ Spiking Solutions

Spiking solution (A) for CBD only was prepared at approximately 100 mg/L using an isolate of CBD with known purity in Methanol.

Spiking solution (B) for all cannabinoids was prepared using a known volume of each cannabinoid directly into the sample.

■ Extraction Sample Preparation

Testing of the extraction method used a control gummy confectionary, commercially available without cannabinoids present. These gummies were cut into smaller pieces and thoroughly mixed to form a representative sample.

Spiking with specific quantities of spiking solution A or B was carried out and allowed to absorb into the gummy before proceeding with the extraction path shown in Figure 1.

Figure 1 Extraction Flow Path

Gummy confectionary 2.0 g ± 0.2 g

- Spike if required and leave to absorb
- Add 20 mL water
- Warm in water bath set at 50° C with occasional agitation
- Ensure all Gummy is dissolved

- Add 20 mL acetonitrile
- Agitate for 5 minutes (manually or mechanically)

- Add 8.0 g ± 0.25 g Magnesium Sulfate [2]
- Agitate for 5 minutes (manually or mechanically)
- Allow sample to settle into distinct layers

- Filter an aliquot (top layer) using 0.2 µm PTFE syringe filter (A)
- Sample A used for minor cannabinoid content
- Dilute by a factor of 4 with methanol for CBD content

HPLC Analysis

2 – Magnesium Sulfate (MgSO4) Extra Pure Dried

■ Extraction Testing

Precision samples were prepared by spiking with spiking solution A for a final concentration of 20 ppm. Six replicates were prepared.

Additional precision samples were prepared using spiking solution (B), these were carried out with no final dilution, duplicate samples were prepared.

Commercially available products are sold in a wide range of concentrations and gummy sizes, therefore Accuracy testing was carried out at 5ppm, 20 ppm and 40 ppm, each was carried out in duplicate.

Specification testing was carried out to test for peaks present within the gummy/solvent itself that may interfere with cannabinoid determination. Therefore, the following specification criteria were tested:

1. Clear only gummy / no spike
2. Red only gummy / no spike
3. Orange only gummy / no spike
4. Yellow only gummy / no spike
5. Green only gummy / no spike
6. All coloured gummy / no spike
7. Random coloured gummy / no spike
8. No gummy present / spike
9. No gummy present

Disclaimer

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