NEW PSYCHOACTIVE SUBSTANCES: ANALYTICAL CHALLENGES AND STRATEGIES

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What are New Psychoactive Substances (NPS)?

Novel Psychoactive Substances (NPS) are a diverse group of synthetic or naturally derived drugs not controlled by international drug conventions (the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances) designed to mimic the effects of controlled drugs (e.g., cannabis, cocaine, MDMA, LSD) while avoiding legal restrictions.

created by making minor alterations to the chemical structure of controlled drugs in order to bypass regulatory controls

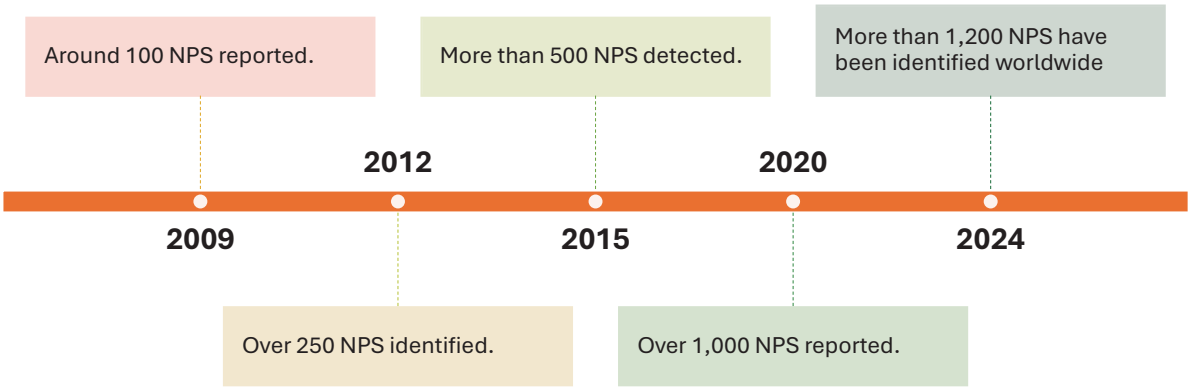
→

"Legal highs," "herbal highs," "research chemicals" and "bath salts" are also common names used to refer to NPS

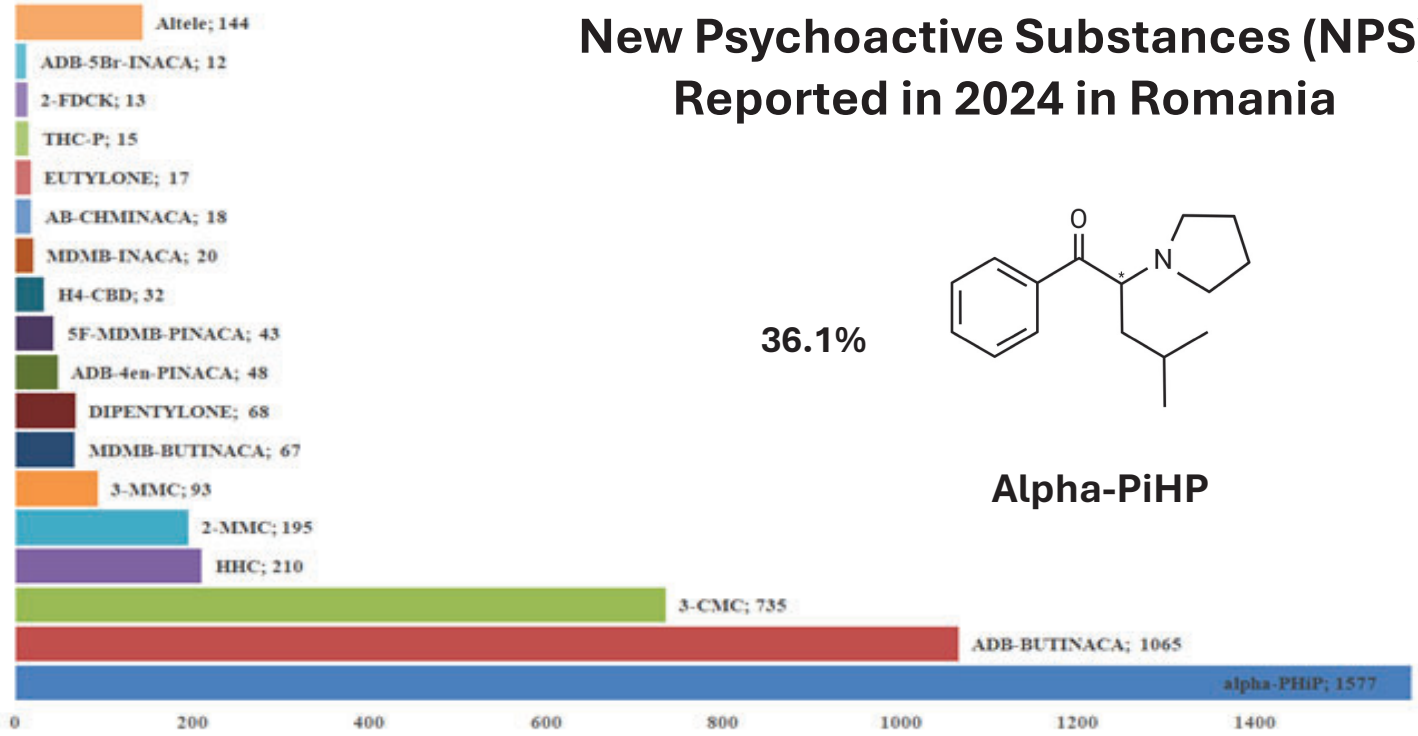
The idea behind NPS is to offer consumers a recreational and legal alternative

NPS are monitored by organizations like the **UNODC** (United Nations Office on Drugs and Crime) and **EMCDDA (European Union Drugs Agency)**, but their rapid evolution makes regulation challenging.

Global Trends (2009–2024) - Number of New Psychoactive Substances (NPS)



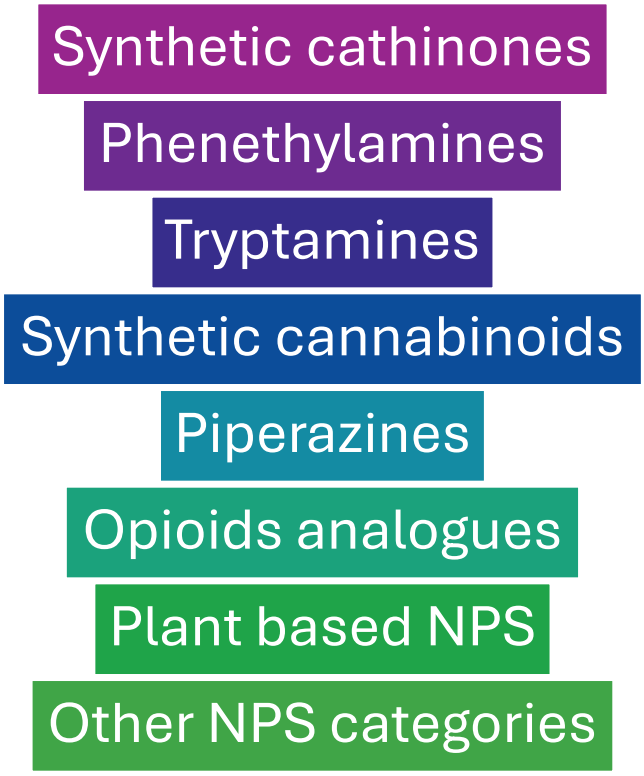
New Psychoactive Substances (NPS) Reported in 2024 in Romania



36.1%

Alpha-PiHP

New psychoactive substances (NPS)



Class	Examples	Mimics	Effects
Synthetic Cannabinoids ("Spice," "K2")	❖ JWH Series: JWH-018, JWH-073 ❖ Indazole/Indole Carboxamides: 5F-ADB, MDMB-4en-PINACA ❖ Cyclohexylphenols: CP 47,497	**Cannabis (THC)**	Potent CB1/CB2 receptor agonists (more intense than cannabis)
Synthetic Cathinones ("Bath Salts") β-keto-phenethylamine (similar to amphetamines but with a ketone group at the β-position)	❖ Primary Cathinones: Mephedrone (4-MMC), Methylone, Ethylone ❖ Pyrrolidinophenones: α-PVP (Flakka), MDPV ❖ N-Alkylated Cathinones: N-Ethylpentylone (Ephylone), Bupropion (medical use)	**Amphetamines/ MDMA**	Stimulant, entactogen, and hallucinogenic
Phenethylamines Phenethylamine backbone with various substitutions	❖ **2C Series (2,5-Dimethoxyphenethylamines)** 2C-B, 2C-I, 2C-E, 2C-T-7 ❖ **NBOMe Series (N-Benzyl Phenethylamines)** 25I-NBOMe, 25B-NBOMe, 25C-NBOMe ❖ **DOx Series (Dimethoxyamphetamines):** DOM, DOB, DOC	**LSD/Mescaline**	Psychedelic, hallucinogenic.
Tryptamines	5-MeO-DMT (powerful hallucinogen) 5-MeO-DiPT ("Foxy Methoxy") AMT (α-Methyltryptamine)	**Psilocybin** (magic mushrooms)/ **DMT** (ayahuasca)	Psychedelic, hallucinogenic (similar to serotonin)

Class	Examples	Mimics	Effects
Opioid Analogs	Fentanyl derivates (e.g., Carfentanil, Acetylfentanyl, Furanylfentanyl) Non-Fentanyl Opioids: U-47700, MT-45	**Heroin/ Morphine**	Extremely potent μ-opioid receptor agonists (high overdose risk
Piperazines	Benzylpiperazines (BZP): BZP, MDBP Phenylpiperazines: TFMPP, mCPP (also a metabolite of trazodone)	**MDMA**	Stimulant, entactogen
Benzodiazepine Analogs	Etizolam, Flualprazolam, Clonazolam	**Alprazolam, Diazepam**	Sedative-hypnotics
Plant based NPS	**Kratom** (Mitragynine, 7-Hydroxymitragynine) **Khat** (Cathinone, Cathine) **Salvia divinorum (**Salvinorin A) **Ayahuasca (**DMT)		pain relief, euphoria Stimulant Potent, short-acting hallucinogen Intense psychedelic experiences

Key challenges in NPS identification

Structural Diversity and Rapid Market Evolution

- NPS encompass a wide range of chemically diverse compounds
- New analogs constantly emerge to circumvent legal restrictions, making it difficult for laboratories to keep analytical methods updated

Low Concentrations in Samples

- Many NPS, especially hallucinogenic phenethylamines and tryptamines, are highly potent and consumed in microgram doses, leading to very low concentrations (pg/mL to ng/mL) in blood and urine.
- This demands highly sensitive analytical techniques (e.g., LC-MS/MS, HRMS) to detect and quantify these substances accurately.

Metabolic Complexity and Lack of Reference Standards

- Limited data exist on the metabolic pathways of many NPS, and reference standards for metabolites are often unavailable.
- For example, synthetic cathinones and phenethylamines may undergo extensive phase I and II metabolism, but laboratories often rely on parent drug analysis due to the lack of metabolite standards.

Key challenges in NPS identification

Regulatory and Legal Challenges

- Rapid legislative changes affect the availability of reference standards and the legality of new analogs.
- Cross-reactivity in immunoassays is often poor, limiting their utility for NPS screening.

Sample Preparation Challenges

- **Stability Issues**: Some NPS degrade under certain conditions (e.g., temperature, pH). For instance, NBOMes degrade at room temperature, and synthetic cannabinoids hydrolyze in blood.
- **Extraction Efficiency**: Basic NPS (e.g., phenethylamines, piperazines) require pH optimization during extraction (e.g., SPE with mixed-mode cartridges or LLE at alkaline pH).
- **Matrix Effects**: Complex biological matrices (e.g., blood, urine) can interfere with analysis, necessitating robust cleanup methods.

Analytical Techniques and Instrumentation

- Chromatographic Separation:
  - GC-MS may require derivatization for polar NPS (e.g., hydroxylated metabolites).
  - LC-MS is preferred for thermolabile and polar compounds but may struggle with isomeric separation (e.g., positional isomers of phenethylamines).
- Mass Spectrometry:
  - Low-resolution MS (e.g., triple quadrupole) is widely used for targeted analysis but may miss unknown analogs.
  - High-resolution MS (HRMS) is valuable for non-targeted screening but requires advanced data processing.

Gas chromatography-mass spectrometry (GC-MS)

Liquid chromatography (LC)-mass spectroscopy (LC-MS or LC-MS/MS)

Raman Spectroscopy & FTIR

High-Resolution Mass Spectrometry (HRMS)

Nuclear Magnetic Resonance (NMR)

Capillary Electrophoresis (CE-MS)

Gas chromatography-mass spectrometry (GC-MS)

Applications

- Volatile NPS (cathinones, some phenethylamines)
- Seized material analysis (powders, plant materials)
- Labs without LC-MS capabilities
- Confirmatory analysis when combined with LC-MS

Advantages

- High specificity, robust for volatile and thermally stable compounds.
- Well-established libraries for identification (e.g., NIST database).

Limitations

- Requires derivatization for polar/metabolized NPS (e.g., hydroxylated tryptamines).
- Not ideal for thermally labile compounds (e.g., LSD, some synthetic cannabinoids).

Challenge	Impact	Workarounds
Thermal degradation e.g., synthetic cannabinoids, LSD analogs, and some cathinones) are thermally labile and degrade in the GC inlet or column	False negatives for labile NPS	Use LC-MS
Derivatization needed Polar NPS (e.g., hydroxylated metabolites, glucuronides) require chemical derivatization (e.g., silylation, acylation) to improve volatility. e.g. Psilocin must be derivatized for GC-MS analysis.	Extra prep time, risk of incomplete derivatization or side reactions	Optimize derivatization
Isomer separation issues 2-MMC vs. 3-MMC vs. 4-MMC (positional isomers) struggles with stereoisomers (R/S enantiomers of amphetamine-type NPS)	Misidentification of similar NPS	Comparation with analytical standard HRMS or LC-MS/MS FTIR/Raman/GC-MS-FTIR
Low sensitivity (e.g., fentanyl analogs) may be present at sub-ng/mL levels, below GC-MS detection limits	Misses ultra-potent NPS	LC-MS/MS

Challenge	Impact	Workarounds
Matrix interference Complex matrix may contain co-eluting compounds that can mask NPS signals (e.g.Phospholipids and fatty acids can interfere with synthetic cannabinoid detection)	Reduced accuracy in complex samples Extra prep time	Better sample cleanup Selective ion monitoring (SIM) to reduce noise
Inability to Detect Non-Volatile or High-MW NPS Large or polar molecules (e.g., synthetic cannabinoid glucuronides, peptide-based NPS) do not vaporize well in GC.	Cannot detect large/polar compounds	LC-MS
Limited EI Fragmentation Libraries for NPS While GC-MS has robust electron ionization (EI) libraries (e.g., NIST), many new NPS lack reference spectra.	Hard to identify novel NPS	Custom libraries (time-consuming), HRMS
Long run times GC-MS methods often require longer chromatographic runs (~15–30 min)	Slower throughput in high-volume labs.	Fast GC or LC-MS

Liquid chromatography (LC)-mass spectroscopy (LC-MS or LC-MS/MS)

Applications

- Gold standard for NPS confirmation in blood, urine, and oral fluid.
- Detect NPS at very low concentrations (ng/mL or pg/mL)

Advantages

- No derivatization needed; works for polar, non-volatile, and thermally labile NPS.
- High sensitivity and selectivity (MRM mode for quantification).

Limitations

- Requires authentic standards

Challenge	Impact	Workarounds
Isomer Differentiation Even with advanced columns, some isomers co-elute (e.g., 3-MMC vs. 4-MMC)	False IDs of positional/stereoisomers	Complementary spectrometric techniques for confirmation Ultra-high-performance LC columns
Matrix Effects	Ion suppression (up to 90% signal loss) False negatives in complex matrices	Extensive sample cleanup (SPE > LLE) Stable isotope-labeled internal standards
Reference Standard Gaps Requires authentic standards for: Retention time matching Optimal MRM transitions Quantification	Cannot confirm novel NPS (30-40% lack standards)	HRMS with in-silico fragmentation prediction Class-based identification Collaborative standard sharing programs
Metabolite Detection	Misses conjugated metabolites (glucuronides/sulfates); unknown pathways	Enzymatic hydrolysis Predictive metabolite software (e.g., Meteor) HRMS/MS^n fragmentation trees

Challenge	Impact	Workarounds
Dynamic Range Limits Ultra-potent NPS (e.g., fentanyl analogs) require pg/mL sensitivity High-dose NPS (e.g., phenethylamines) may saturate detectors	Misses ultra-potent NPS (pg/mL) or saturates with high-dose compounds	Dynamic exclusion in DDA Dual MRM ranges (high/low sensitivity) Sample dilution protocols
System Maintenance	Frequent source cleaning (every 100-200 runs); short column lifetimes	Online SPE cleanup Scheduled maintenance protocols Dual LC systems for high throughput
Operational demand Expertise Needed: Method development requires specialized knowledge		Data interpretation needs experienced analysts

Raman Spectroscopy & FTIR

Applications

- Used for **preliminary identification of bulk drugs** (powders, tablets, liquids).

Advantages

- Non-destructive
- Fast and simple sample prep
- Useful for distinguishing between structurally similar compounds (e.g., synthetic cannabinoids, cathinones, phenethylamines)
- determines the form of the NPS (free base, hydrochloride salt, etc.)

Limitations

- Difficult to interpret spectra of mixtures or novel analogs without references
- May require experienced analysts for complex spectra

Nuclear Magnetic Resonance (NMR)

Structural elucidation of unknown compounds (e.g., seized powders)

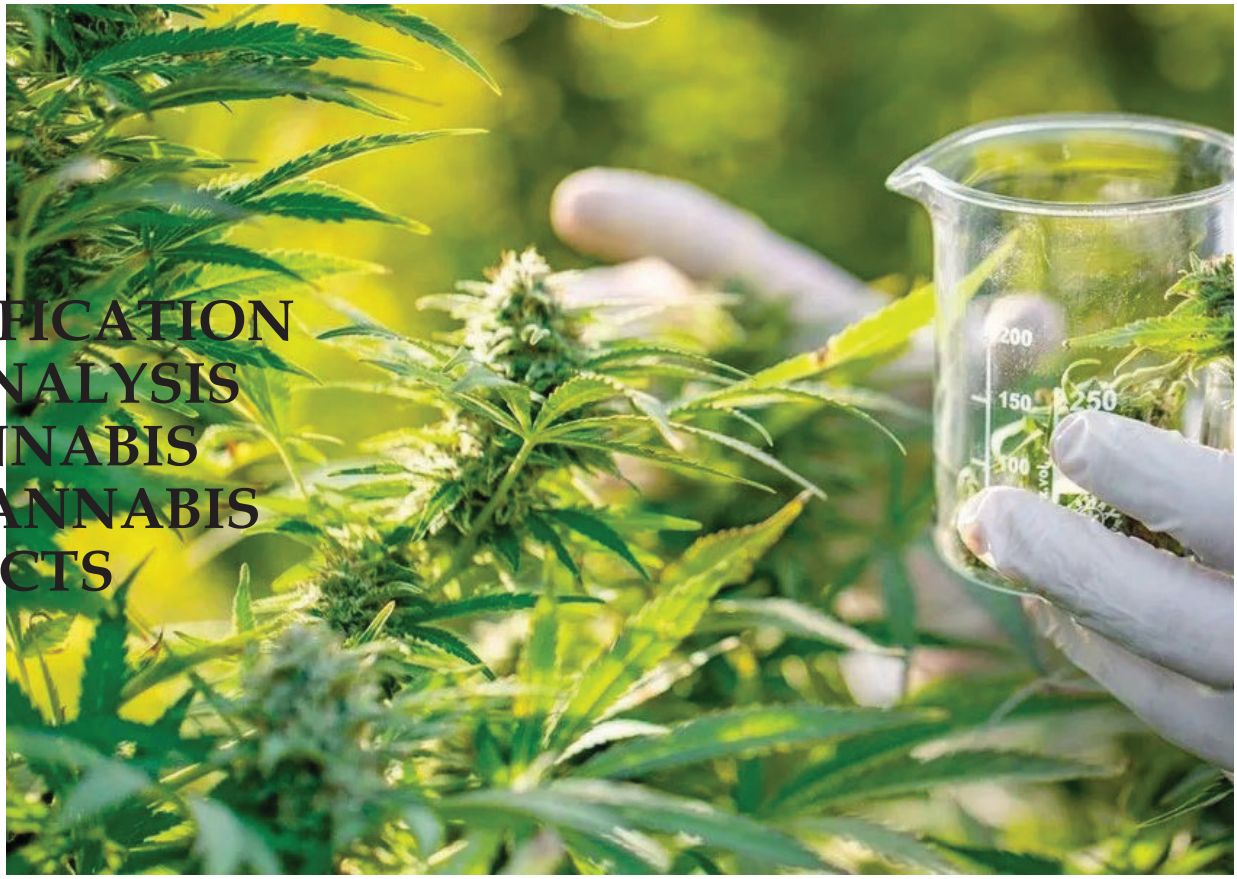
high cost and low sensitivity

High-Resolution Mass Spectrometry (HRMS)

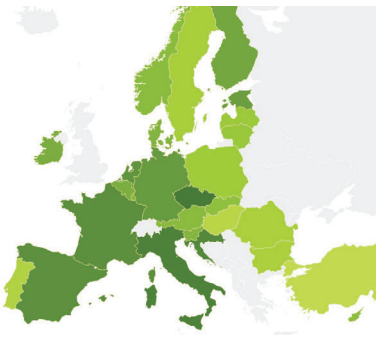
invaluable for non-targeted screening, metabolite identification, and novel NPS detection

limitations in sensitivity, isomer differentiation, cost, and data complexity

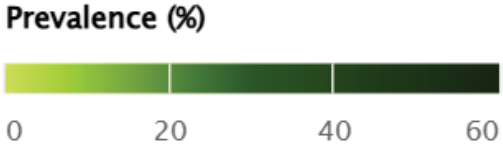
IDENTIFICATION AND ANALYSIS OF CANNABIS AND CANNABIS PRODUCTS



Prevalence of cannabis use in Europe (%)



- **Cannabis** is produced in almost all countries worldwide and cannabis products are the most widely trafficked drugs.
- An estimated 8 % of European adults (22.8 million aged 15 to 64) have used cannabis in the last year.

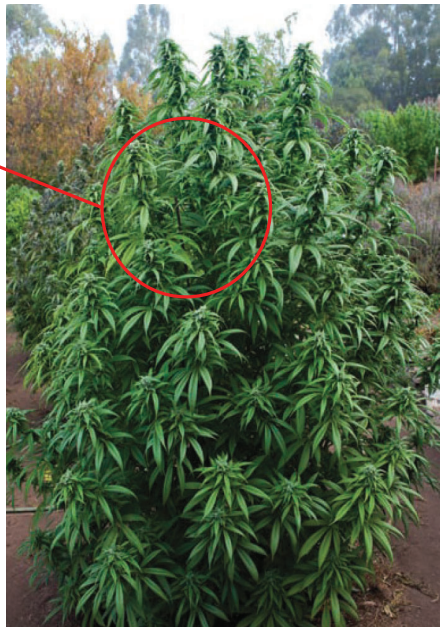


Prelevance in Romania – 47% of the samples is Cannabis/Cannabis Products

Definitions

"Cannabis" - flowering or fruiting tops of the cannabis plant (excluding the seeds and leaves when not accompanied by the tops) from which the resin has not been extracted

"Cannabis plant" - any plant of the genus Cannabis.



"Cannabis resin" the separated resin, crude or purified, obtained from the cannabis plant.

Street names: marihuana, pot, ganja, grass, chanvre

Cannabis products

- Herbal cannabis
- Cannabis resin (hashish)
- Cannabis oil (hashish oil)
- Cannabis edibles (Gummies and Chocolates, Baked Goods, Beverages)
- Topicals (creams, balms, lotions, and oils)
- Vapes

Developments in legislation resulted in changes in the dynamics of cultivation, production and markets of cannabis and cannabis products.

Availability in illicit markets of a wide range of cannabis with increasing levels of **tetrahydrocannabinol (THC)**, which refers to the **psychoactive components of cannabis** and comprises a number of isomers and stereochemical variants that are included in the international drug control conventions.

Increase in the variety of products containing THC (edibles, vapes and dabs) as well as an increased availability of cannabis products containing mainly **cannabidiol (CBD)**, but which could also contain low levels of THC.

As a result, the analysis of cannabis and cannabis products has become more complex for forensic drug testing laboratories, with a need to identify and often quantitate low levels of THC, differentiate its isomers, in particular *delta*-9-THC (Δ9-THC) and *delta*-8-THC (Δ8-THC), and identify other cannabinoids that are present.

This poses a variety of analytical challenges requiring reliable, reproducible, and sensitive analytical methods and techniques.

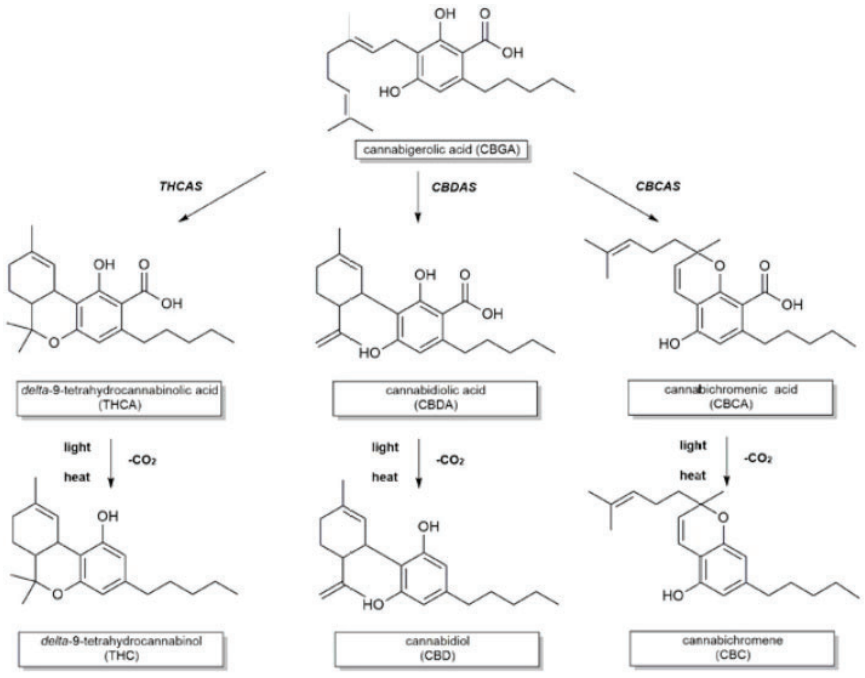
Chemistry of cannabis

The main phytocannabinoid constituents of cannabis are delta-9-THC (delta-9-tetrahydro

Cannabinol), CBD (cannabidiol) , and CBC (cannabichromene).

Tetrahydrocannabinol (THC) refers to the psychoactive components of cannabis and includes a number of isomers and stereochemical variants that are included in the international drug control conventions

(-)-trans-.9-THC is the only of these stereochemical variants that occurs naturally in the cannabis plant and is considered the primary psychoactive component of cannabis.

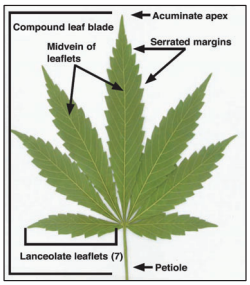


Biosynthesis of main phytocannabinoids

Qualitative and quantitative analysis of cannabis products

Physical examination

Morphological characteristics

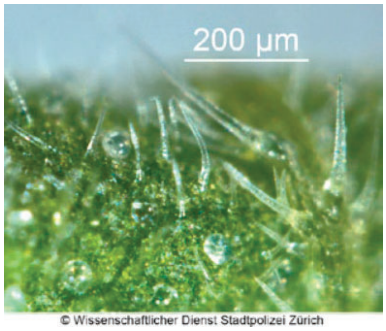


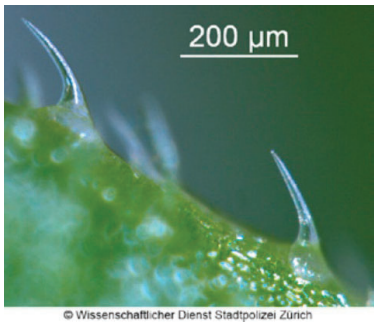
Adaxial (upper) and abaxial (lower) surfaces of Cannabis sativa L. leaves

Clusters of male (left) and female flowers (right)

Close up of a dried pistillate inflorescence

Microscopic characteristics





trichomes (i.e. hair-like projections from a plant epidermal cell)

Colour tests

Thin layer chromatography (TLC)

Gas chromatography-flame ionization detector (GC-FID)

Gas chromatography-mass spectrometry (GC-MS)

Liquid chromatography (LC) or liquid chromatography-mass spectroscopy (LC-MS or LC-MS/MS)

Gas chromatography-mass spectrometry (GC-MS)

Applications

1. **Potency Testing:** Quantifies THC, CBD, minor cannabinoids.
2. **Terpene Profiling**: Identifies myrcene, limonene, β-caryophyllene, etc.
3. **Contaminant Screening**: Pesticides, solvents (e.g., butane), mycotoxins.

Advantages

- **High Sensitivity**: Detects trace cannabinoids (e.g., THCV, CBC) at ppm levels.
- **Decarboxylation**: Converts acidic cannabinoids (THCA, CBDA) to neutral forms (THC, CBD) during heating.
- **Compound Identification**: Mass spectra library matching (e.g., NIST) confirms cannabinoids/terpenes.

Limitations

- **Decarboxylation Artifact**: Overestimates THC (since THCA → THC during analysis).
- **Thermal Degradation**: High temps may degrade terpenes or generate artifacts.
- **Non-Volatile Compounds**: Poor for flavonoids or large molecules (HPLC better).

Sample preparation

Drying & Grinding:

- ***Herbal cannabis*** is dried (~8–13% moisture) and finely ground. If necessary seeds/stems (>3 mm) are removed to avoid dilution effects.
- ***Edibles*** are ground

Extraction

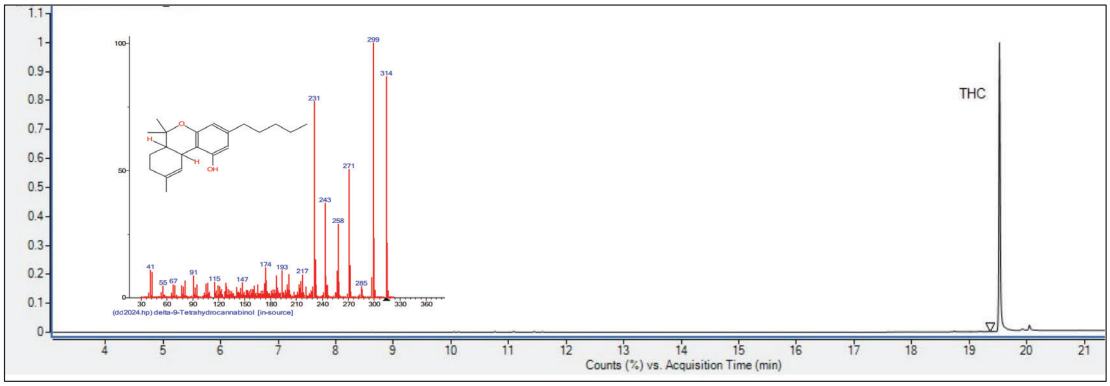
Solvent: Methanol, ethanol, or chloroform (e.g., 1:9 chloroform/methanol), hexane.

Herbal cannabis: Sonnication, maceration

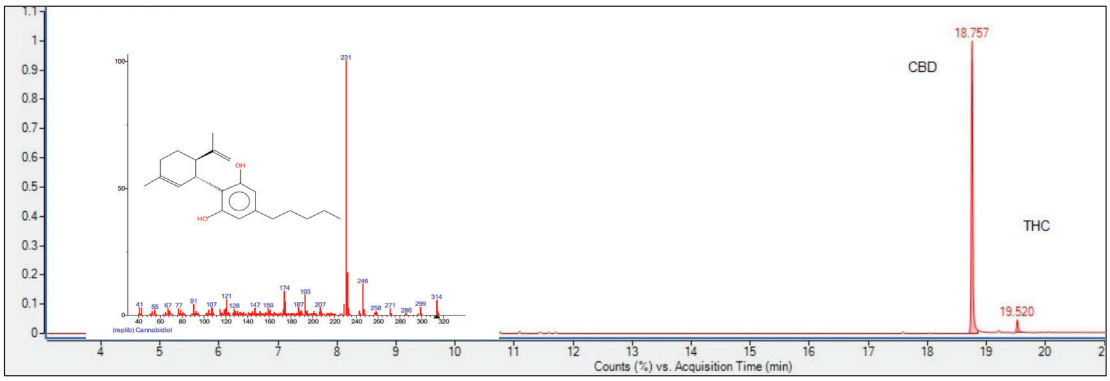
***Edibles*:** matrix-specific extraction (matrix solid-phase dispersion (MSPD) or microwaved- assisted extraction (MAE)

GC-MS Instrumentation & Recommended Parameters

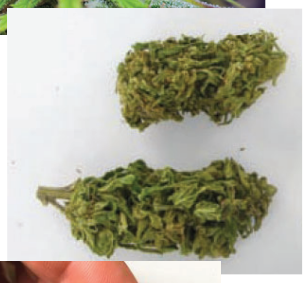
Parameter	Setting
Column	DB-5ms, 30 m × 0.25 mm × 0.25 µm (or equivalent)
Carrier Gas	Helium (1.0 mL/min)
Injection	Split mode (50:1), 1 µL volume
Injector Temp	240-280°C
Oven Program	50°C (1 min) → 300°C at 10°C/min (hold 5 min)
MS Interface	280°C
Ion Source	230°C
Detection	Electron Ionization (EI, 70 eV), scan mode (m/z 40–500)

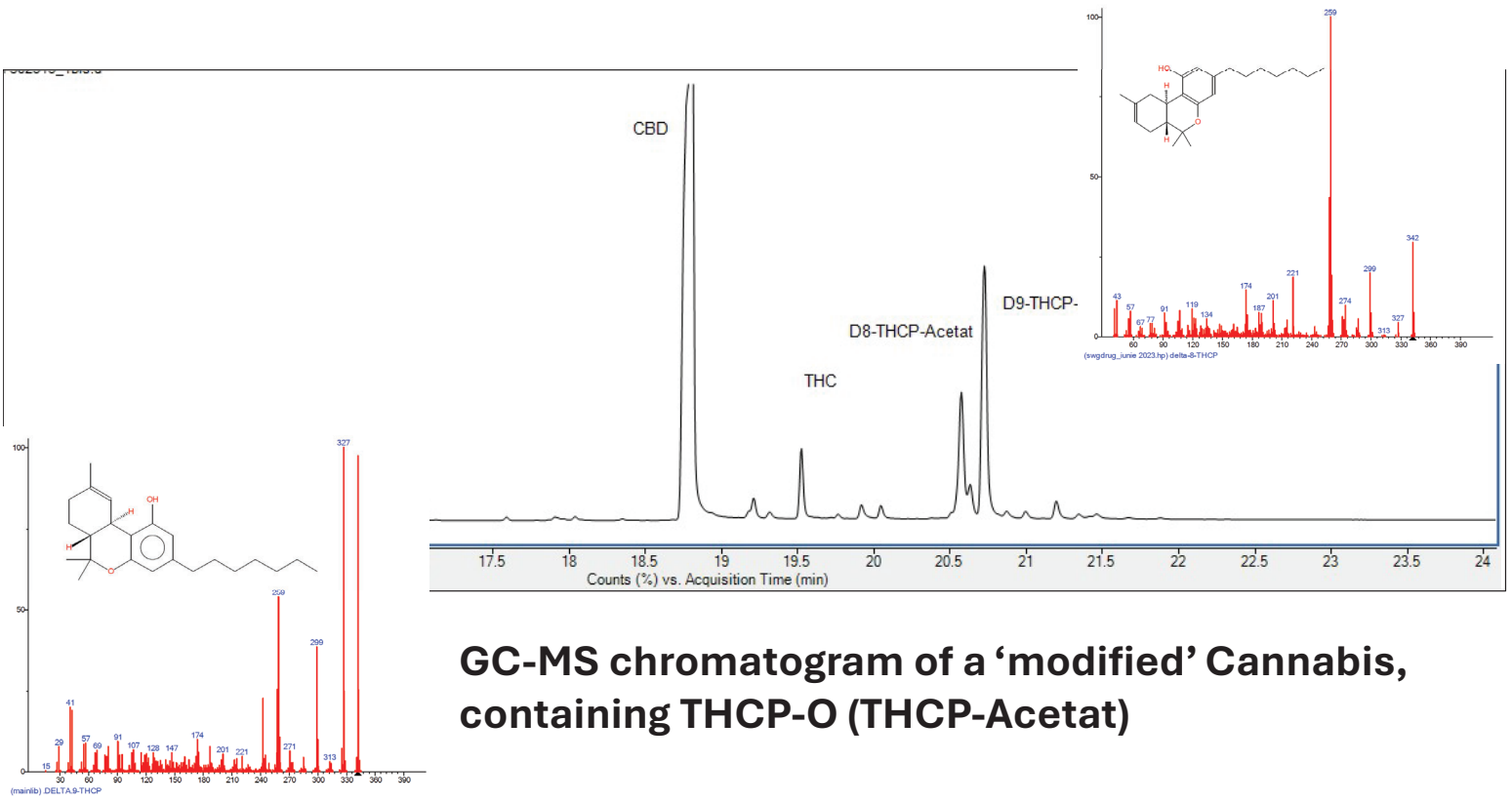


GC-MS chromatogram of a high THC inflorescence

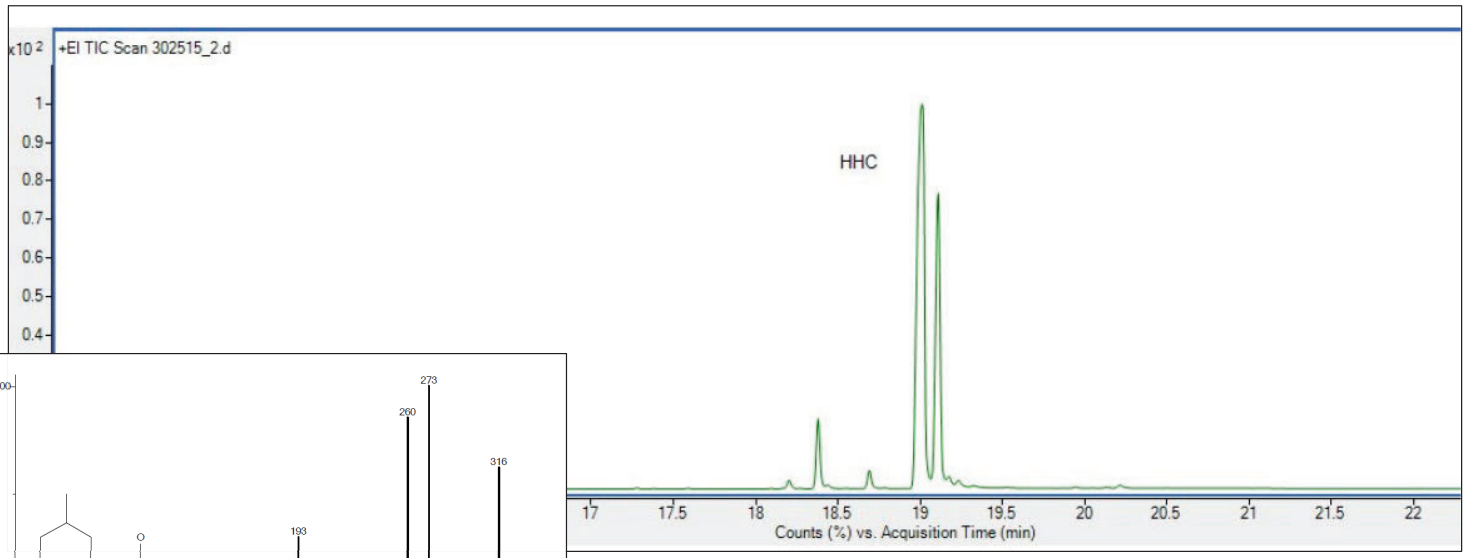


GC-MS chromatogram of a high CBD inflorescence

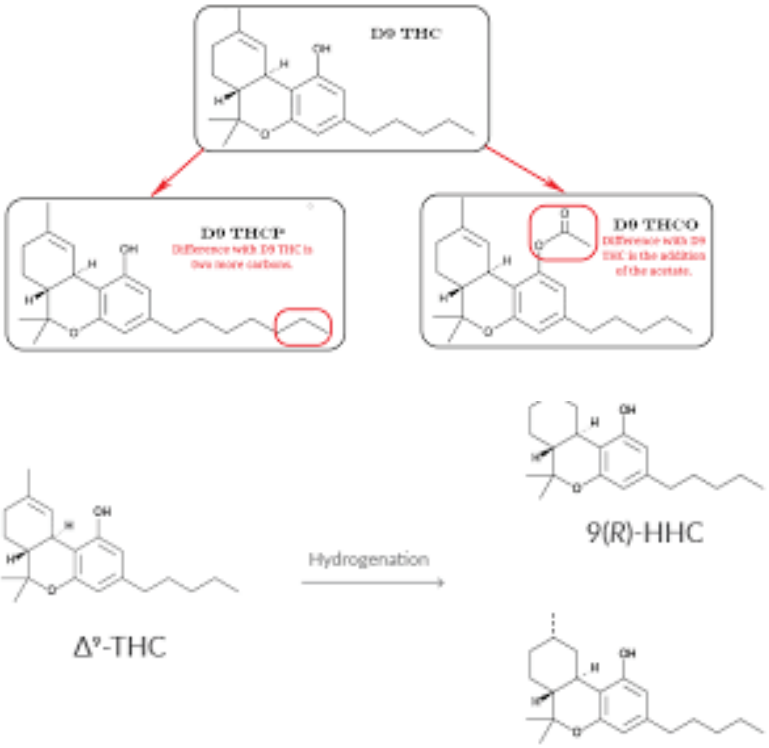




GC-MS chromatogram of a ‘modified’ Cannabis, containing THCP-O (THCP-Acetat)



GC-MS chromatogram of a vape containing HHC



Gas chromatography-flame ionization detector (GC-FID)

Advantages

- **High Precision**: Excellent for quantifying major cannabinoids (THC, CBD, CBN).
- **Decarboxylation**: Converts THCA → THC during injection (reports "total THC").
- **Cost-Effective**: Simpler and cheaper than GC-MS.

Limitations

- **No Compound ID**: Cannot distinguish co-eluting peaks (unlike GC-MS).
- **Thermal Degradation**: May degrade heat-sensitive compounds (terpenes, acidic cannabinoids).
- **Requires Standards**: Must run pure cannabinoids for calibration.

Applications

1. **Potency Testing**: Measures THC, CBD, CBN for compliance.
2. **Strain Profiling**: Compares cannabinoid ratios (e.g., THC:CBD).
3. **Quality Control**: Detects degradation (e.g., THC → CBN).

Key Steps

Drying & Grinding:
- Cannabis inflorescence is dried (~8–13% moisture) and finely ground.
- If necessary seeds/stems (>3 mm) are removed to avoid dilution effects.

Extraction
- Extract **200 mg** of dried, homogenized herbal cannabis (or **100 mg** of cannabis resin, **50 mg** of cannabis oil) with **20 mL** of internal standard (ISTD) solution (e.g. tribenzylamine in ethanol, 0.5 mg/mL) for **15 minutes** in an ultrasonic bath.

Decarboxylation (to convert THCA to THC)
- Transfer **500 µL** of the extract to a GC vial, evaporate solvent, and heat at **150°C for 12 minutes**.
- Reconstitute the residue in **1.5 mL ethanol** for analysis.

Derivatization (optional)
- If THCA must be analyzed separately (without decarboxylation), derivatize with **MSTFA** (N-methyl-N-trimethylsilyltrifluoroacetamide) or **BSTFA/TMCS** after solvent removal.

Calibration:
- Use **CBN (cannabinol)** as the calibration standard (due to THC degradation risks).
- Prepare a series of standard solutions (e.g., 0.1% to 30% THC equivalent) with ISTD.

Notes

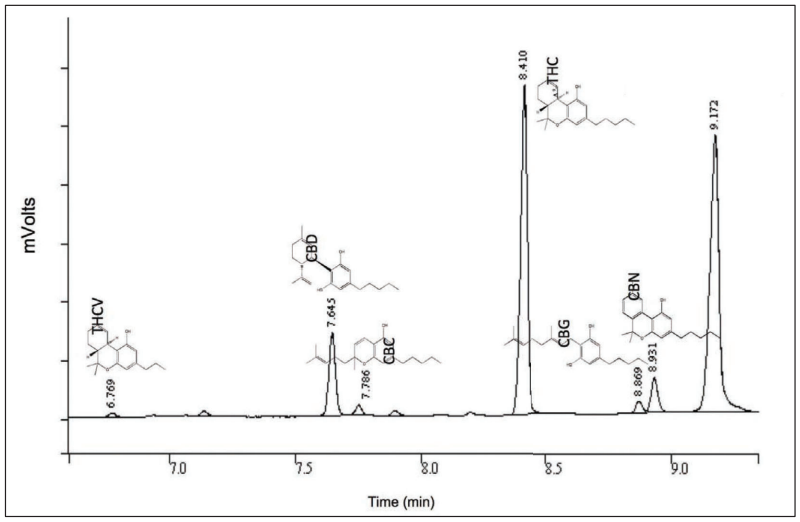
- Decarboxylation is critical for accurate total THC measurement, as THCA converts to THC under heat.
- Avoid acidic derivatizing agents (e.g., TFAA-HFIP) to prevent THC isomerization.
- Store standards in cool, dark conditions (up to 4 months stability).

GC-FID Instrumentation & Recommended Parameters

Parameter	Setting
Column	5% Diphenyl – 95% Dimethylpolysiloxane (15 m × 0.25 mm × 0.25 µm)
Carrier Gas	Nitrogen (1.0 mL/min)
Injection	Split (20:1), 1 µL volume
Injector Temp	280°C
Oven Program	2 min at 200°C, 10°C/min 200-240°C, 2 min at 240°C
Detector (FID)	300°C
H_2/Air Flow	35/350 mL/min

Results

- Elution Order: CBD, THC, CBN.



Liquid chromatography (LC) or liquid chromatography-mass spectroscopy (LC-MS or LC-MS/MS)

Applications

1. **Regulatory Compliance**: Measures THCA/THC for legal limits (e.g., <0.3% THC in hemp).
2. **Strain Characterization**: Profiles CBD:THC ratios for medical cannabis.
3. **Stability Testing:** Tracks degradation (e.g., THC → CBN).
4. **Increased selectivity and reduced interference** (low concentrations of cannabinoids in complex herbal mixtures and matrices such as cannabis edibles) – LC-MS/MS

Advantages

- **Preserves Acidic Cannabinoids**: Directly quantifies THCA, CBDA (no decarboxylation)
- **High Accuracy**: Resolves THC/CBD from interferents (e.g., CBN, CBG)
- **Flexible Detection**: UV (low-cost), PDA (spectral confirmation), or LC-MS (high sensitivity)

Limitations

- **Slower than GC**: Run times typically longer (15–30 min vs. 10–15 min for GC)
- **No Terpene Analysis**: Poor for volatile compounds (GC-MS preferred)
- **Cost**: More expensive columns/solvents vs. GC

Key Steps

Drying & Grinding:
- Cannabis inflorescence is dried (~8–13% moisture) and finely ground.
- If necessary seeds/stems (>3 mm) are removed to avoid dilution effects.

Extraction
- Extract 500 mg of dried, homogenized cannabis with 5 mL methanol:chloroform (90:10 v/v).
- Vortex, sonicate (15 min), and centrifuge

Decarboxylation (to convert THCA to THC)
- Evaporate 200 µL of extract, heat at 210°C for 15 min to convert THCA → THC.
- Reconstitute in 200 µL methanol:chloroform (90:10) and dilute (10x or 100x).

Calibration:
- THC standard solutions (0.001–0.1 mg/mL).
- Total THC = THC + (THCA × 0.877 [MW correction])

Notes

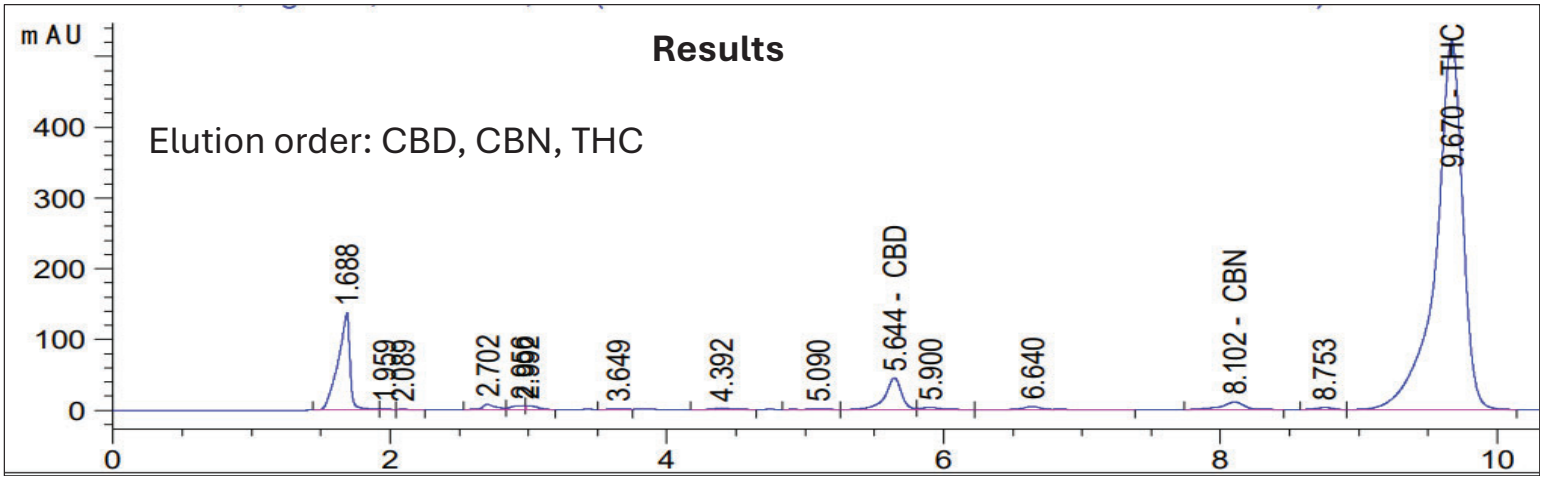
- No decarboxylation → preserves acidic cannabinoids (THCA, CBDA).
- Suitable for Δ^8-THC vs. Δ^9-THC differentiation.

HPLC Instrumentation & Recommended Parameters

Column type	Hypersil ODS 4.6x250 mmx5µm (or equivalent)
Column temperature	30°
Mobile phase	ACN (80%)-H2O (20%)
Flow rate	1ml/min
Detection	UV Lamp, Ref 360 nm

Results

Elution order: CBD, CBN, THC



Challenges in Cannabis Chemical Identification and Profiling

Analytical Methodologies

- **Gas Chromatography (GC):**
  - Commonly used but can decarboxylate acidic cannabinoids during analysis, leading to overestimation of THC and underestimation of THCA.
  - Requires validation to ensure complete decarboxylation without THC degradation.
- **High-Performance Liquid Chromatography (HPLC):**
  - Preferred for quantifying acidic and neutral cannabinoids separately but is less commonly available in some labs.
- **Thin-Layer Chromatography (TLC):**
  - Useful for qualitative identification but lacks the precision for quantitative analysis compared to GC or HPLC.

Instrumentation and Expertise

- Advanced techniques like LC-MS/MS or GC-MS are needed for comprehensive profiling but may not be accessible to all testing facilities.
- Interpretation of results requires specialized knowledge to account for matrix effects, interferences, and methodological biases

Sampling Challenges

- Cannabinoid concentrations vary within a single plant (e.g., higher in flowers than leaves) and between plants of the same strain. Representative sampling is critical but often inadequately addressed, leading to inconsistent potency claims.

Taxonomic and Chemotypic Variability

- Cannabis exhibits significant genetic diversity, leading to variations in cannabinoid and terpenoid profiles. The plant can be classified into different chemotypes (e.g., THC-dominant, CBD-dominant, or mixed), which complicates standardization.
- Hybridization and selective breeding have blurred traditional distinctions between *Cannabis sativa*, *C. indica*, and *C. ruderalis*, making it difficult to correlate chemical profiles with botanical classifications.

Cannabinoid Complexity

- Over 100 cannabinoids have been identified, with Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) being the most studied. These compounds exist in acidic (e.g., THCA, CBDA) and neutral forms (e.g., THC, CBD), which interconvert through decarboxylation when exposed to heat or light.
- Quantifying cannabinoids accurately requires distinguishing between acidic and neutral forms, as their pharmacological effects differ. For example, THCA is non-psychoactive, while THC is psychoactive.

Terpenoid and Flavonoid Variability

- Cannabis contains over 200 terpenoids and numerous flavonoids, which contribute to its aroma and may modulate cannabinoid effects. These compounds are highly volatile and degrade during storage or processing, complicating their quantification.
- Terpenoid profiles vary by strain, growing conditions, and post-harvest handling, making it difficult to establish consistent chemical fingerprints.

Degradation and Stability

- Cannabinoids degrade over time, with THC converting to cannabinol (CBN) under oxidative conditions. This degradation affects potency and therapeutic efficacy.
- Terpenoids are particularly labile, with significant losses occurring during drying, storage, or exposure to light and heat.

Contaminants and Adulterants

- Illicit cannabis may contain pesticides, heavy metals, microbial contaminants (e.g., *Aspergillus*), or synthetic additives (e.g., glass beads to mimic trichomes). Detecting these requires additional analytical steps beyond cannabinoid profiling.
- Legal cannabis in regulated markets must meet safety standards for contaminants, but testing protocols vary by jurisdiction.

Legal and Regulatory Variability

- Discrepancies in legal definitions (e.g., "total THC" vs. "THC content") and testing requirements across regions complicate standardization. For example, some jurisdictions require reporting of total THC (THCA + THC), while others focus only on THC.
- Lack of harmonized international standards for cannabis analysis hinders comparability of data.

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Thank you !