

# RAMAN SPECTROSCOPY IN FORENSIC SCIENCE: DEVELOPMENTS AND FUTURE DIRECTIONS IN CANNABINOID ANALYSIS

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## ABSTRACT

**Purpose:** The purpose of this paper is to review how Raman spectroscopy - particularly portable Raman and SERS - supports cannabis-related forensic work across plant material characterisation and human biofluid detection, and to highlight methodological trends (chemometrics, computational modelling, AI, and advanced sampling) that are pushing the field toward reliable, field-deployable and legally robust workflows.

**Design/Methods/Approach:** Using narrative approach, critical synthesis of recent literature spanning: (i) plant-focused Raman/CARS microscopy and handheld classification for varietal and chemotype discrimination, stress diagnostics, and quantification; (ii) human-sample SERS using portable devices for minimally invasive detection in saliva, urine, plasma, and blood; and (iii) cross-cutting innovations - chemometrics (PCA/PLS/OPLS-DA), DFT-aided spectral assignment, AI/ML pipelines, and alternative excitation/sampling geometries (e.g., NIR, SORS).

**Findings:** Raman methods accurately differentiate hemp vs. drug-type cannabis, classify varieties/chemotypes in vivo, and detect early abiotic stress, while SERS enables sensitive detection of cannabinoids in complex biofluids with minimal preparation - preserving material for downstream DNA analysis. Emerging AI-assisted models and optimised excitation regimes improve classification, quantification, and interpretability, and handheld instruments demonstrate strong potential for on-site decision-making in forensic and regulatory settings.

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**Originality/Value:** This review integrates plant and human-matrix applications with the latest computational and instrumentation advances, framing Raman spectroscopy as a rapid, reagent-free, portable, and courtroom-defensible platform for cannabis forensics - bridging laboratory capability with operational field use.

**Keywords:** Raman spectroscopy; cannabis; cannabinoids; SERS; forensic toxicology; chemometrics; portable instrumentation.

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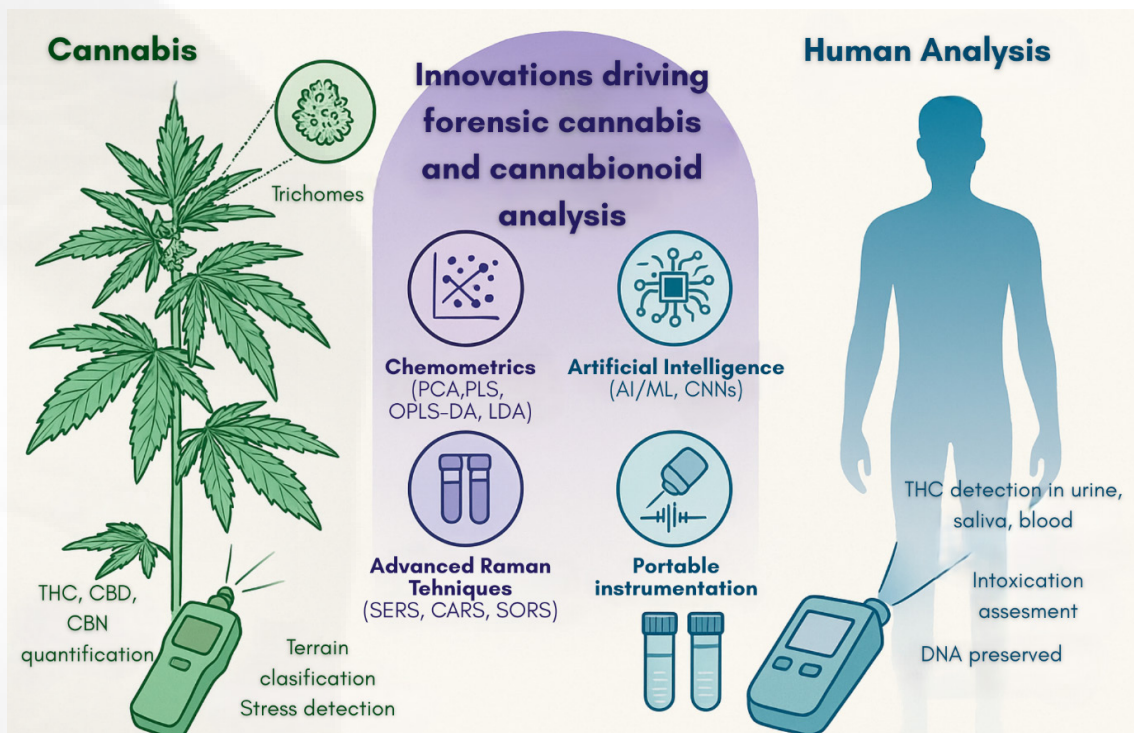
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## INTRODUCTION

Raman spectroscopy is an analytical method founded on the principle of inelastic light scattering, which arises when electromagnetic radiation interacts with molecular matter. As this interaction takes place, the vibrational energy levels of the molecules change. This results in the emission of photons whose wavelengths differ from those of the incident beam. This discrepancy, known as the Raman shift, underlies the identification of chemical structures (Penido et al., 2016).

This method is recognised as a valuable analytical tool in forensic science because of its non-destructive nature and its ability to identify (Doty et al., 2016). It has been shown to be highly effective in examining various types of forensic evidence, including fibres, bodily fluids, bones, hair and gunshot residue (Doty et al., 2016). The use of advanced data analysis techniques, particularly chemometrics improves the reliability and accuracy of Raman investigations, producing statistically sound results suitable for legal use (Doty et al., 2016). The operational simplicity and reagent-free nature of Raman spectroscopy make it highly suitable for preliminary screening in field laboratories and at crime scenes, where maintaining the chain of custody and minimising sample alteration are among the most important steps (Doty et al., 2016).

Traditional methods often require sample preparation, like staining for microscopy, which improves visibility but ruins the sample integrity (Hegde, 2024). They also tend to be slow, rely heavily on human effort, and struggle with dried or mixed fluids, which are complex and variable by nature (Harbison & Fleming, 2016). Overall, while these conventional approaches have been widely used, they are limited in sensitivity, specificity, and speed, showing the need for newer, more selective, and less destructive techniques (Virkler & Lednev, 2009; Hegde, 2024).

The identification of body fluids in the crime scene, for example, usually begins with simple screening tests - presumptive tests, and is followed by more precise confirmatory tests. Presumptive tests are helpful for quickly checking if evidence might be of relevance to the case, but they also often give



false-positive results and cannot be fully trusted just by themselves (Harbison & Fleming, 2016). If a presumptive test is negative, the sample is usually discarded, but if it is positive, confirmatory tests are done. A major problem is that chemical tests are destructive - they can damage or even destroy the evidence, sometimes through chemical reactions, colour changes, or exposure to strong light (Virkler & Lednev, 2009; Zapata et al., 2015). This is a serious issue because it can prevent further examinations, such as DNA analysis (Harbison & Fleming, 2016).

Another issue to consider is that body fluids are characterised by the presence of many shared components. For instance, amylase is present in both saliva and semen, while urea is found in urine, sweat, and semen, albeit in different quantities. Due to these overlaps, there is a possibility that tests may confuse one fluid for another, thus resulting in false positives (Virkler & Lednev, 2009). It is important to note that certain chemicals commonly utilised in presumptive testing, such as benzidine, have the potential to induce adverse reactions when exposed to certain substances, including plant enzymes. This interaction can result in the production of erroneous test results (Zapata et al., 2015).

Raman spectroscopy plays an important role in the specialised field of forensics, particularly in the detection of forgeries and the authentication of cultural heritage objects and financial fraud (Deneckere et al., 2012). As a vibrational spectroscopic technique, Raman spectroscopy can identify pigments and other organic and inorganic materials. This provides critical insights into the composition and provenance of artworks and banknotes found at crime scenes. Its ability to deliver detailed chemical information means that the authenticity of these items can be assessed without compromising their physical condition. In forensic fibre analysis, for example, Raman spectroscopy complements techniques such as microspectrophotometry by providing detailed molecular information about dyes, thereby enhancing the overall discriminatory power of the analysis (Lepot et al., 2008).

Beyond standard forensic applications, Raman spectroscopy has also found applications in homeland security and hazardous material detection (Izake, 2010). The invention of portable Raman instruments enables the non-contact detection of chemical and biological materials, which is essential for reducing the exposure risk to first responders in high-stakes environments (Izake, 2010). This approach allows the police and special forces to identify dangerous substances, including explosives and illicit drug mixtures, without any direct physical interaction. Furthermore, advanced technologies such as Spatially Offset Raman Spectroscopy (SORS) allow for the non-invasive chemical characterisation of substances concealed within opaque containers or packaging, which are typically present in illegal laboratories (Izake, 2010).

Advancements in the domain of portable Raman spectroscopy have led to an expansion in the applicability of this technique in forensic investigations conducted in the field. Commercially available handheld systems, Thermo Scientific TruNarc® and Chemring PGR-1064®, have been tested and gave very good results in presumptively identifying narcotics in the field. These systems demonstrate high reproducibility and analytical accuracy, particularly when enhanced with surface-enhanced Raman spectroscopy (SERS) substrates (Spicher, 2016). These instruments incorporate extensive onboard spectral libraries, enabling rapid, non-destructive analysis without the need for laboratory infrastructure (Spectroscopy Online, 2020). Portable Raman devices are not only useful for drug detection, but also for examining bodily fluids, fibres, counterfeit pharmaceuticals and explosive residues, thereby supporting a wide range of forensic scenarios (AZoM, 2019). By following core forensic principles, such as Locard's exchange principle, this technology enables swift, real-time decision-making without compromising the integrity of evidence. Together, these developments highlight the increasing importance of portable Raman spectroscopy as a reliable and adaptable tool in modern forensic science.

This review critically examines practices and emerging perspectives in Raman spectroscopy for forensic engineering, particularly its application to cannabinoid analysis. By providing an overview of the

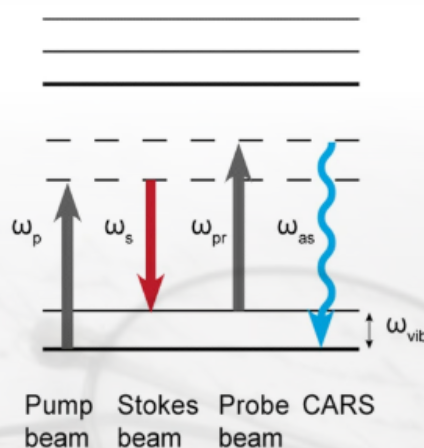


current literature and evaluating methodological advancements, the review aims to summarise the trends that are and might be followed in cannabis analysis using Raman spectroscopy.

## RAMAN SPECTROSCOPY FOR THE ANALYSIS OF CANNABIS PLANT SAMPLES

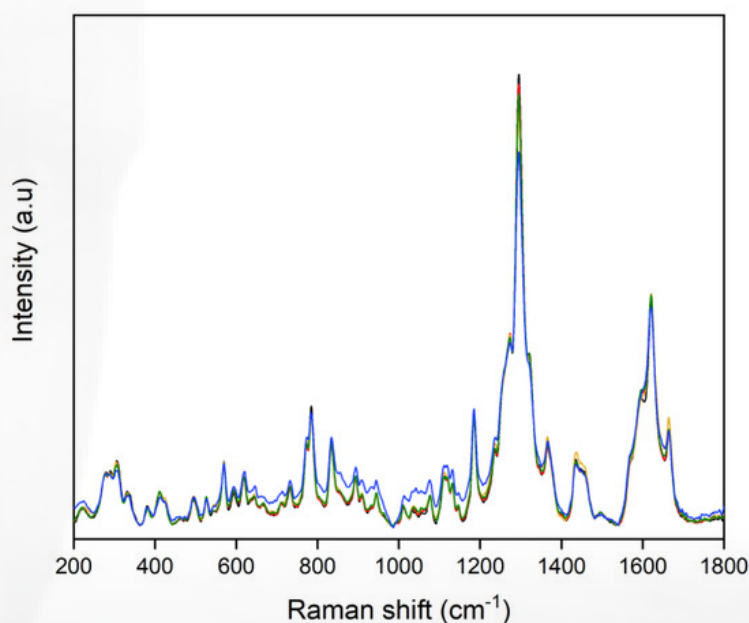
The application of Raman spectroscopy to cannabis plants has advanced rapidly, reflecting its adaptability from fundamental microscopy to practical tools for cultivation and regulation. Research in this field has undergone a shift in focus from the cellular-level visualisation of cannabinoids to the use of portable spectrometers for field classification, crop monitoring and stress diagnostics. This comprehensive collection of works underscores the evolution of Raman from a mere laboratory technique to a multifaceted platform for both scientific and agricultural applications.

Ebersbach et al. (2018) provided an early demonstration of coherent anti-Stokes Raman scattering (CARS) microscopy to investigate individual glandular trichomes of *Cannabis sativa*. This non-labelling technique facilitated direct observation of cannabinoid distribution, notably delta-9-tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), without the necessity for destructive preparation. By producing chemical “fingerprints” at the cellular level, the study advanced the understanding of cannabinoid biosynthesis and storage, establishing Raman microscopy as a high-resolution, non-invasive alternative to conventional methods for plant tissue analysis. In this study, the authors used CARS microscopy because it produces a much stronger signal than conventional Stokes Raman scattering. Traditional Stokes Raman yields useful vibrational fingerprints but its weak signal requires long acquisition times, which can make imaging fragile plant tissues challenging. By contrast, CARS relies on a nonlinear interaction of laser beams to generate a bright anti-Stokes signal that is shifted to a higher frequency than the incident light. This coherent signal allows imaging to be performed more quickly and with higher spatial resolution, while lowering the risk of damaging the sample. These advantages make CARS particularly suitable for studying individual glandular trichomes of *Cannabis sativa*, where both sensitivity and tissue preservation are essential (Figure 1).



**Figure 1:** Illustration of the CARS process. A pump photon ( $\omega_p$ ) excites a molecule to a virtual state, which is influenced by a Stokes photon ( $\omega_s$ ). A probe photon ( $\omega_{pr}$ ), supplied from the same source as the pump in this setup, raises the molecule to a higher virtual level. The anti-Stokes signal ( $\omega_{as}$ ) that results is shifted to a higher frequency than the incoming light and, under the right conditions, becomes coherently amplified (adapted from Ebersbach et al., 2018).

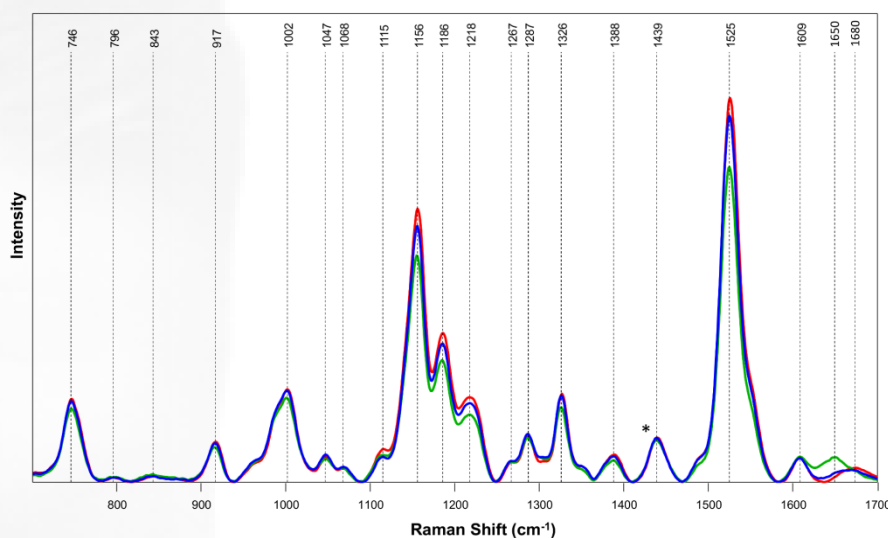
The transition towards portable instrumentation for operational contexts was illustrated by Sánchez et al. (2020), who reported the use of an 831 nm portable Raman spectrometer to discriminate hemp from cannabis. Hemp and cannabis are distinguished by their THCA content, with cannabis containing much higher levels than hemp. The study showed that Raman spectroscopy can differentiate between the two with 100% accuracy and even quantify THCA, offering a non-invasive and reliable tool for regulation and breeding. This finding emphasised the confirmatory power of Raman in distinguishing legal hemp from illegal cannabis, a critical task in forensic and regulatory settings. Furthermore, extending the scope of classification, Ramos-Guerrero et al. (2022) demonstrated the use of Raman microscopy to analyse the trichome heads of cannabis plants, where cannabinoids are concentrated. The researchers carefully adjusted the laser settings to avoid damaging the samples and collected spectra from five marijuana varieties - four Sativa and one Indica. To interpret these data, they applied Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) - chemometric analysis, and compared the spectra with pure cannabinoid standards delta-9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), as well as quantum-chemical simulations of acidic and neutral forms. This combined approach enabled them to clearly distinguish Indica from Sativa and even differentiate between individual Sativa strains, achieving 100% classification accuracy. Raman spectra of their results are shown in figure 2.



**Figure 2:** Comparison of average Raman spectra for Indica (Black Domina, black) and Sativa varieties (Amnesia Haze, red; Amnesia Hy-Pro, gold; Original Amnesia, blue; Y Griega, green) within the 200–1800  $\text{cm}^{-1}$  range. The spectra appear broadly similar, with small differences in peak intensity and features (adapted from Ramos-Guerrero et al., 2022).

Applications also moved into the domain of cultivation and breeding. Goff et al. (2022) utilised a hand-held Raman spectrometer to differentiate hermaphrodite cannabis plants from male and female counterparts in vivo. Differentiating the sex of *Cannabis sativa* plants is important because only female plants produce trichomes that are rich in cannabinoids, while male plants do not. Hermaphroditic plants present an additional problem since they develop both female flowers and male anthers, which can fertilise females. This fertilisation reduces crop quality by leading to seed production and lowering cannabinoid content. Reliable sex identification therefore plays a key role in maintaining yield and ensuring the desired chemical composition of cannabis crops. The flowers of male, female, and hermaph-

roditic *Cannabis sativa* plants were examined with a 532 nm Raman laser, and spectra were collected from the calyx and trichome regions where cannabinoids are typically concentrated. Chemometric analysis, specifically OPLS-DA, was then applied to the spectral data to identify subtle biochemical differences linked to plant sex. This approach successfully classified all male and female plants with 100% accuracy and hermaphroditic plants with 98.7% accuracy; spectra are shown in Figure 3.



**Figure 3:** Averaged Raman spectra of *Cannabis sativa* leaves from male (blue), female (red), and hermaphroditic (green) plants, showing distinct spectral profiles used for sex classification (adapted from Goff et al., 2022).

In addition to its applications in genetic and morphological classification, Raman spectroscopy has also demonstrated its value in the assessment of plant responses to environmental stress. In a recent study, Steczkowski et al. (2023) utilised Raman spectroscopy to assess the effects of drought, heat, and light on various hemp varieties, identifying spectral alterations associated with carotenoids, lignin, and proteins. The outcomes of the study demonstrated the presence of both common and variety-specific stress responses, thereby underscoring Raman's capacity for early, non-destructive detection of abiotic stress and reinforcing its role in crop monitoring and precision agriculture. Cannabis plants exhibit distinct biochemical changes when exposed to environmental stressors such as drought, heat, or high light. These alterations can influence the production and distribution of key cannabinoids like THC and CBD, as well as their spectral signatures during analysis. In forensic contexts - whether in police laboratories, at border inspections, or in legal proceedings - accurate discrimination between hemp and cannabis is essential. Stress-induced chemical variation has the potential to complicate this distinction, underscoring the need for analytical methods capable of recognising and accounting for such effects.

In 2023, Dilena et al. examined how nitrogen fertilisation and pruning affect biomass production and cannabinoid yield in a CBD-type *Cannabis sativa* cultivar. Plants were cultivated in a glasshouse with coarse sand and, after a vegetative phase, were subjected to four nitrogen levels (30, 60, 210, and 500 mg/L) for twelve weeks under flowering conditions. A double stem pruning treatment was also applied, and at harvest, biomass, tissue nitrogen concentration, and cannabinoid levels in both leaves and inflorescences were quantified. The results showed that higher nitrogen levels increased vegetative growth, particularly leaf biomass, but caused a reduction in cannabinoid concentration and overall cannabinoid yield, while pruning had no positive effect. An additional observation revealed that plants grown on the edges of the greenhouse, exposed to greater sunlight, produced more biomass and cannabinoids, further highlighting environmental influences. These findings are forensically signifi-

cant because they demonstrate how cultivation practices and nutrient regimes can alter the chemical profile of cannabis. Stress or excessive fertilisation may reduce cannabinoid levels below legal thresholds, potentially complicating the distinction between hemp and drug-type cannabis. Recognising how environmental and agronomic factors shape cannabinoid content is therefore essential for ensuring accurate forensic classification and interpretation.

**Table 1:** Summary Raman Spectroscopy for the Analysis of Cannabis Plant Samples

| Study                        | Sample                                                  | Method                                              | Excitation (nm) | Chemometrics                               | Accuracy                                        | Key Findings                                                                                                                | Forensic Relevance                                                                       |
|------------------------------|---------------------------------------------------------|-----------------------------------------------------|-----------------|--------------------------------------------|-------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Ebersbach et al., 2018       | Cannabis sativa glandular trichomes (single-cell)       | CARS microscopy (nonlinear Raman)                   | NR              | Qualitative spectral mapping               | Fast imaging; qualitative THCA/CBDA mapping     | Label-free visualisation of THCA/CBDA distribution; strong anti-Stokes signal enables faster imaging vs. Stokes Raman       | Non-destructive localisation of cannabinoids; supports evidentiary integrity             |
| Sánchez et al., 2020         | Dried plant material (hemp vs. cannabis)                | Handheld Raman                                      | ≈ 831           | Classification (thresholding/chemometrics) | 100% differentiation (hemp vs. cannabis)        | 100% differentiation hemp vs. cannabis; THCA quantification feasible                                                        | Rapid, non-destructive legal status screening in field/regulatory settings               |
| Ramos-Guerreiro et al., 2022 | Trichome heads; multiple varieties                      | Raman microscopy                                    | NR              | OPLS-DA + quantum-chemical simulations     | 100% Indica vs. Sativa; strain-level separation | 100% classification of Indica vs. Sativa; differentiation among Sativa strains; spectral assignment improved by simulations | Varietal/chemotype discrimination; supports provenance and regulatory assessment         |
| Goff et al., 2022            | Leaves/flowers of male, female, hermaphrodite (in vivo) | Handheld Raman (532 nm)                             | 532             | OPLS-DA                                    | 100% male/female; 98.7% hermaphrodites          | 100% accuracy for male/female; 98.7% for hermaphrodites                                                                     | Crop quality control; helps interpret seized plant material characteristics              |
| Steczowski et al., 2023      | Hemp varieties under drought/heat/light stress          | Raman spectroscopy (bench/portable)                 | NR              | Spectral feature analysis                  | Stress signatures detected (qualitative)        | Detected common and variety-specific stress signatures (carotenoids, lignin, proteins)                                      | Explains stress-induced chemical variability affecting legal thresholds                  |
| Dilena et al., 2023          | CBD-type cultivar (N fertilisation + pruning)           | Chemical quantification (paired with Raman context) | —               | Quant / correlation analyses               | Agronomic effect; not a Raman accuracy metric   | Higher N increased biomass but reduced cannabinoid concentration/yield; pruning had no positive effect                      | Agronomy affects cannabinoid content; implications for hemp vs. drug-type determinations |
| Porcu et al., 2022           | Cannabis material (THC/CBD)                             | 1064 nm Raman (NIR to suppress fluorescence)        | 1064            | Peak-based quant/ID                        | Qualitative ID (reduced fluorescence)           | Rapid in-situ THC/CBD detection with reduced fluorescence interference                                                      | Improved on-site screening when fluorescence is problematic                              |
| Komisar et al., 2025         | Living plants (leaf surfaces); standards/distillates    | Handheld Raman                                      | 785, 830        | PLS-DA, PCA                                | ≈90% (chemotype classification, 830 nm)         | ~90% accuracy (830 nm) for THC-dominant vs. CBD-dominant chemotypes; phytocannabinoid-associated peaks identified           | Field chemotype verification to support rapid decision-making                            |

Most recently, Komisar et al. (2025) demonstrated the application of portable Raman spectroscopy for the classification of cannabis chemotypes. Using handheld devices equipped with 785 nm and 830 nm excitation sources, they collected spectra directly from the leaves of living plants, supplemented with



measurements of THC and CBD standards and distillates. The spectral data were analysed with Partial Least Squares Discriminant Analysis (PLS-DA), and the 830 nm spectrometer achieved approximately 90% accuracy in distinguishing THC-dominant from CBD-dominant chemotypes. Principal Component Analysis (PCA) further revealed phytocannabinoid-associated Raman peaks, indicating the potential for future quantitative profiling. These findings are important for forensic science, because they demonstrate that portable Raman instruments can provide rapid and reliable chemotype verification directly in the field, supporting timely decision-making in cultivation monitoring, border inspections, and law enforcement contexts.

In forensic contexts, Raman spectroscopy offers an invaluable means of distinguishing between different types of cannabis and assessing plant material without the need for destructive preparation. The instrument capacity for rapid chemotype classification, varietal identification, and the detection of plant stress markers provides tools that are directly applicable to law enforcement, regulatory inspection, and quality assurance of seized samples. The combination of portability with chemical specificity in Raman techniques has been demonstrated to enhance the reliability of plant-based forensic investigations and support legally defensible outcomes. Based on the studies above, Table 1 summarises the key findings and forensic relevance of utilising Raman spectroscopy for the analysis and discrimination of cannabis plant samples.

## RAMAN SPECTROSCOPY FOR THE DETECTION OF CANNABINOIDS IN HUMAN SAMPLES

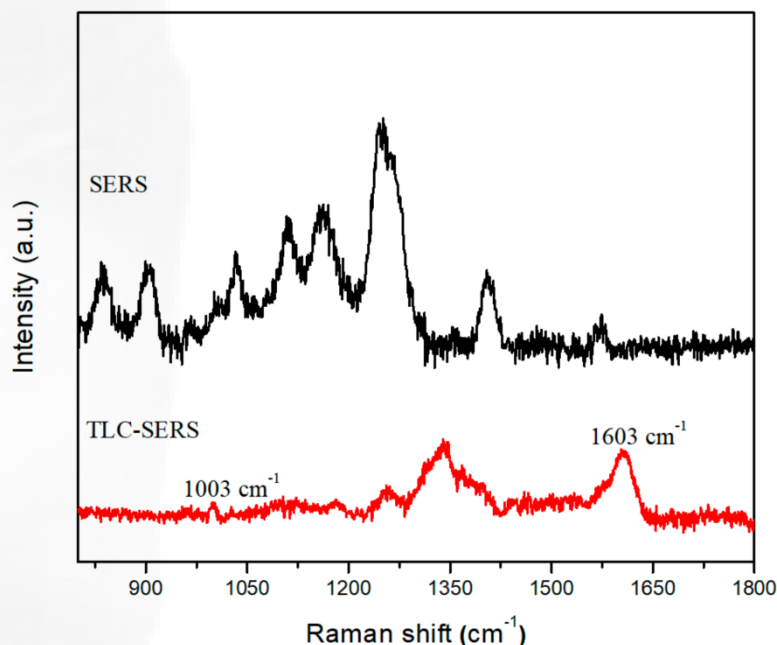
The application of SERS to the detection of cannabinoids in human samples has developed steadily, progressing from proof-of-concept demonstrations toward clinically relevant and forensic-ready workflows. Farquharson et al. (2017) conducted one of the earliest evaluations of a portable, point-of-care SERS saliva analyser. By directly comparing the results of the device with those of urinalysis, the study demonstrated that the device correctly identified the presence or absence of THC in 13 out of 14 patient samples. The nanoparticle substrates generated clear Raman signatures at concentrations relevant to human physiology, and the handheld design made it possible to conduct fast, on-site testing. This work established the practical potential of Raman-based devices as non-invasive alternatives for roadside, workplace, or emergency drug testing.

Expanding the scope of biofluid testing, Sivashanmugan et al. (2019) developed a portable SERS-based approach for detecting THC in biological fluids, specifically saliva and urine. The method employed diatomaceous earth substrates integrated with gold nanoparticles as SERS-active surfaces, enabling strong signal enhancement. Saliva samples were analysed directly, while urine required a thin-layer chromatography (TLC) separation step on diatomaceous channels to resolve THC from interfering compounds such as urea. With a portable 785 nm Raman device, the authors detected distinct THC vibrational bands at 1601 and 1681  $\text{cm}^{-1}$  in saliva, and showed that TLC pretreatment allowed unambiguous THC identification in urine. Validation through PCA confirmed clear separation between THC-containing and control spectra. This article highlights the feasibility of Raman spectroscopy combined with SERS substrates for rapid and reliable on-site detection of THC in complex biological matrices, with direct relevance to forensic drug screening and law enforcement.

Figure 4 illustrates the findings of Sivashanmugan et al. (2019) on the effectiveness of combining TLC with SERS for detecting THC in urine samples. Before separation, the Raman spectrum of urine was dominated by strong signals from abundant metabolites such as urea, which masked the characteristic THC peaks and prevented reliable identification. After TLC separation on diatomaceous substrates,



the background interference was reduced, allowing clear THC-specific peaks to emerge. These results demonstrate that integrating TLC with SERS enables cannabinoids to be isolated from complex biological fluids and reliably detected, highlighting the forensic value of this approach for analysing drug metabolites in real-world samples.



**Figure 4:** SERS analysis of a urine sample from a marijuana user, showing spectra collected prior to and following TLC separation (adapted from Sivashanmugan et al., 2019).

In order to further understand the molecular basis of detection, Islam et al. (2020) investigated the interactions of THC, CBD, CBN, and the synthetic analogue HU-210 with silver surfaces using SERS supported by density functional theory (DFT). The study showed that cannabinoids generally adsorb parallel to the silver surface via benzene rings and oxygen atoms, which yielded stable Raman enhancement. At the same time, small variations in adsorption geometry and band intensities allowed the compounds to be distinguished from one another. By comparing experimental vibrational bands with theoretical assignments, the study provided molecular-level insight into cannabinoid-surface interactions and demonstrated that spectral fingerprints can be linked directly to chemical structure. To further improve sensitivity, the authors evaluated different salts as enhancers, identifying  $\text{MgCl}_2$  as the most effective due to its ability to increase local electric fields and generate more active SERS sites. Taken together, these findings advanced both the fundamental understanding of cannabinoid behaviour on metallic substrates and the practical refinement of SERS as a reliable tool for forensic drug detection. Açıkgöz and Çolak (2023) developed a novel approach for the detection of illicit drugs in human blood by combining SERS with advanced multivariate analysis. In their study, silver nanoparticles were synthesised and mixed with blood samples obtained from both drug users and controls to enhance the Raman signal of analytes. Spectra were collected using a 785 nm excitation laser, which provided strong enhancement of characteristic molecular vibrations. However, because blood is a chemically complex matrix with overlapping signals, traditional spectral interpretation alone was insufficient for reliable classification. To address this, the authors employed chemometric tools, specifically PCA for exploratory visualisation and PLS-DA for supervised classification. They applied

leave-one-out cross-validation (LOOCV) to verify that the classification model remained reliable and avoided overfitting. The method successfully separated blood samples spiked with drugs like MDMA and THC from negative controls, achieving this even at low concentration levels. These results highlight the potential of SERS, especially when combined with chemometric modelling, to deliver rapid and accurate drug screening in complex biological fluids. Importantly, this approach reduces the need for extensive sample preparation compared to traditional chromatographic methods and is well suited for forensic applications, where quick and reliable drug identification in biological samples is critical for investigations, toxicology assessments, and legal proceedings.

With regard to human sample analysis, Raman-based methods - in particular SERS - have been demonstrated to offer significant advantages in the context of forensic toxicology. The capacity to detect cannabinoids with high sensitivity in saliva, plasma, and even blood, utilising portable devices and minimal preparation, is well-suited to the requirements of roadside testing, workplace screening, and post-mortem analysis. Raman spectroscopy shows promise in overcoming persistent challenges in forensic drug testing, offering a rapid and accurate way to detect drugs in complex biological matrices with minimal sample handling. Consequently, it offers a pathway toward practical deployment in both investigative and judicial settings. It is important to highlight that only drops of blood, saliva or seminal fluid can often be found at a crime scene, and it is usually preferred to use these samples for DNA analysis, which can identify the person to whom the found sample belongs. Utilising Raman non-destructive methodology, it has become feasible to ascertain the state of the individual who left the trace, that is to say, whether they were in a state of intoxication. Table 2 compiles the outcomes of studies utilising Raman for detecting cannabinoids in human biological samples.

**Table 2:** Summary Raman Spectroscopy for the Detection of Cannabinoids in Human Samples

| Study                      | Sample                            | Method                                                       | Excitation (nm) | Chemometrics                                 | Accuracy                                                    | Key Findings                                                                                            | Forensic Relevance                                                                  |
|----------------------------|-----------------------------------|--------------------------------------------------------------|-----------------|----------------------------------------------|-------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Farquharson et al., 2017   | Saliva                            | Portable SERS analyser                                       | NR              | Comparative evaluation vs. urinalysis        | Undocumented                                                | Demonstrated handheld SERS feasibility for on-site drug screening (model drug: buprenorphine)           | Non-invasive, rapid roadside/workplace screening concept applicable to cannabinoids |
| Sivashanmugan et al., 2019 | Saliva and urine                  | Portable SERS (Au NPs on diatomaceous earth) + TLC for urine | NR              | PCA for validation                           | THC peaks in saliva; TLC+SERS confirms urine                | THC peaks (~1601 & 1681 $\text{cm}^{-1}$ ) in saliva; TLC+SERS enabled unambiguous THC ID in urine      | Rapid on-site THC detection; workflow handles complex urine matrix                  |
| Islam et al., 2020         | THC, CBD, CBN, HU-210 (standards) | SERS (Ag) + DFT                                              | NR              | DFT band assignment; enhancer salt screening | DFT-assisted markers; enhancer optimisation (no single LoD) | Parallel adsorption via rings/oxygen; $\text{MgCl}_2$ best enhancer; markers differentiate cannabinoids | Guides substrate/condition choice for sensitive, selective SERS assays              |
| Açıkgöz & Çolak, 2023      | Whole blood (users vs. controls)  | SERS (Ag NPs), 785 nm                                        | 785             | PCA, PLS-DA, LOOCV                           | Class separation at low levels (PLS-DA)                     | Separated spiked/positive from controls at low levels                                                   | Toward rapid drug screening in complex biofluids with minimal prep                  |



## INNOVATIONS AND EMERGING TRENDS IN CANNABIS ANALYSIS BY RAMAN SPECTROSCOPY

As the field of forensic cannabis analysis has evolved, there has been a notable increase in the complexity of the analysis. This has resulted in the integration of advanced analytical and computational approaches into Raman procedure. The foundation for interpreting spectral data was laid by traditional chemometric tools. In more recent studies, the scope of Raman spectroscopy has been expanded through the use of computational modelling, machine learning, and artificial intelligence. Collectively, these innovations signify a transition from purely descriptive applications towards predictive, automated, and field-deployable forensic solutions.

In the early contributions, the importance of chemometric techniques for resolving complex spectral datasets from forensic samples was emphasised. As demonstrated in the relevant literature, such methods as PCA, partial least squares (PLS) regression, and OPLS-DA have been shown to be essential tools for exploratory analysis, classification, and quantification (Sánchez et al., 2020; Santos et al., 2022; Mojica et al., 2022). Refinements such as Competitive Adaptive Reweighted Sampling combined with PLS (CARSPLS) further enhanced quantification of drug and adulterant concentrations, thereby strengthening the statistical reliability of forensic evaluations (Santos et al., 2022).

The subsequent phase of development entailed the application of Raman spectroscopy to the differentiation of cannabinoids, utilising computational modelling. Ramos-Guerrero et al. (2022) combined Raman microscopy with OPLS-DA and quantum chemical simulations to classify cannabis varieties and distinguish THC, CBD, and CBN, improving spectral assignment and interpretability. Concurrently, Coombs et al. (2022) expanded Raman application to synthetic cannabinoids, demonstrating the detection of receptor agonists impregnated into paper substrates. This capability is directly pertinent to forensic screening in postal and correctional settings. In the context of this trajectory, Wolfe et al. (2023) undertook a comparative analysis of the experimental Raman spectra of eleven cannabinoids using density functional theory (DFT) simulations. This analysis led to the identification of reliable vibrational markers that differentiate between cannabinoids. Furthermore, it resulted in the refinement of spectral libraries for forensic and regulatory purposes.

Machine learning and artificial intelligence have since emerged as central to innovation in Raman-based forensic workflows. Laetsch (2024) applied Raman microscopy with transfer learning and linear discriminant analysis, achieving high accuracy in the classification of cannabinoid standards. Expanding on this, Laetsch et al. (2024) introduced convolutional neural networks (CNNs) trained on scalogram-transformed Raman spectra, achieving 100% classification accuracy and illustrating the potential for high-throughput, non-invasive analysis. Grijalva et al. (2024) combined Raman microscopy, DFT, and AI, demonstrating both PCA/LDA (99.83% accuracy) and CNN-based classification of seven cannabinoids, with scalogram-based image analysis yielding 100% accuracy in less than ten minutes of CPU training. These advances underscore the value of AI-enhanced Raman procedures in overcoming challenges posed by overlapping spectra and complex mixtures.

Recent studies have also addressed the quantification of cannabinoids using novel excitation approaches. Chuchuen et al. (2024) utilised near-infrared (NIR)-excited Raman spectroscopy for the quantification of THC, CBD, and CBN, achieving sub-0.5 mg/mL detection limits and strong linear correlations ( $R^2 > 0.98$ ) between intensity and concentration. This work demonstrates the potential for Raman spectroscopy to provide sensitive, quantitative analysis in complex forensic contexts.

In the future, it is anticipated that advances will be made through the refinement of specialised Raman techniques and instrumentation. SERS continues to enhance sensitivity for trace-level detection, while SORS facilitates non-invasive analysis through packaging and containers (Akçan & Yildirim, 2021;



Mojica et al., 2022). The convergence of deep learning, portable instrumentation, and advanced chemometric modelling is expected to solidify the role of Raman as a versatile forensic tool.

Innovations in the domains of chemometric analysis, computational modelling, artificial intelligence, and advanced sampling geometries are reshaping the forensic use of Raman spectroscopy for cannabis. These developments are transforming Raman from a confirmatory laboratory technique into a predictive, portable, and legally defensible method capable of addressing the growing demands of forensic and regulatory cannabis analysis. Table 3 summarises the innovations and current trends in cannabis analysis using various Raman spectroscopy methods described above.

**Table 3: Summary of Innovations and Emerging Trends in Cannabis Analysis by Raman Spectroscopy**

| Study                                                     | Sample                                 | Method                                                          | Excitation (nm)           | Chemometrics                              | Accuracy                                       | Key Findings                                                                           | Forensic Relevance                                              |
|-----------------------------------------------------------|----------------------------------------|-----------------------------------------------------------------|---------------------------|-------------------------------------------|------------------------------------------------|----------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| Spectroscopy Online (2020); AZoM (2019); Spicher (2016)   | Various seized drugs/materials         | Handheld Raman (785–1064 nm); SERS add-ons; reference libraries | 785–1064                  | Library matching; peak-based confirmation | Presumptive ID; LoD varies by device/substance | Accurate presumptive ID of narcotics; broad applicability (fluids, fibres, explosives) | Rapid field triage; preserves chain-of-custody                  |
| Akçan & Yildirim, 2021; Mojica et al., 2022               | Materials in opaque containers         | Spatially Offset Raman Spectroscopy (SORS)                      | Offset geometry (various) | Spectral deconvolution / offset selection | Through-container ID (qualitative)             | Non-invasive identification through packaging                                          | Safer handling of hazardous materials/ clandestine lab items    |
| Sánchez 2020; Santos 2022; Ramos-Guerrero 2022; Goff 2022 | Plant materials, extracts, standards   | Raman (532–1064 nm)                                             | 532–1064 (various)        | PCA, PLS/PLS-DA, OPLS-DA; CARS-PLS        | High-accuracy classification / improved quant  | High-accuracy classification; improved quant of drugs/adulterants                      | Auditable decision-making for legal contexts                    |
| Laetsch et al. 2024; Grijalva 2024                        | Spectral datasets (cannabinoids)       | Raman + CNNs/transfer learning; Raman+D-FT+AI                   | Various                   | Deep learning (CNNs), transfer learning   | Near-perfect (reports up to 100%)              | Near-perfect to 100% classification; fast training/inference                           | Automated, high-throughput workflows with strong generalisation |
| Chuchuen 2024; Porcu 2022                                 | Extracts/solutions; plant material     | 785–1064 nm Raman; quantitative calibration                     | 785–1064                  | Regression (e.g., PLS), peak ratios       | Qualitative ID (reduced fluorescence)          | Sub-0.5 mg/mL LoDs; strong linearity ( $R^2 > 0.98$ )                                  | Sensitive, fluorescence-resistant quant in complex samples      |
| Coombs et al., 2022                                       | Paper/porous substrates (mail, prison) | Raman (bench/portable)                                          | Various                   | Peak-based ID                             | Qualitative detection on substrates            | Detects synthetics on impregnated substrates                                           | Targeted screening in mailrooms and prisons                     |

## CONCLUSION

In both plant and human analysis, Raman spectroscopy has become a core part of the forensic workflow which is characterised by rapidity of this method, its reagents-independence, and its compatibility with chain-of-custody requirements. In cannabis studies, Raman spectroscopy can distinguish hemp from drug-type plants, classify varieties, monitor stress, and quantify cannabinoids such as THC, CBD, and CBN. In human samples, SERS enables sensitive cannabinoid detection while keeping material intact for DNA analysis. This allows investigators to connect identity with intoxication. Advances in chemometrics, computational modelling, and artificial intelligence further improve accuracy and classification. Together, these developments confirm Raman spectroscopy as a key method



for modern forensic investigations, with cannabinoids providing a strong example of its value. In conjunction with portable instrumentation and advanced sampling strategies, these advances serve to consolidate Raman spectroscopy as a field-deployable, courtroom-defensible technique for cannabis analysis. The ongoing refinement of data-driven workflows and instrumentation is set to consolidate its position further, thereby ensuring that Raman continues to serve as a pragmatic and authoritative component of contemporary forensic practice.

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