EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 13
Issue: 2
Year: 2026

FORENSIC DETECTION OF CANNABINOIDS IN URINE

Monika Thakur*[1], Chanakya Mehta [1], Dr. Dalbir Singh[2]

[1]Student, M.Sc. Forensic Science, USAHS Department, Rayat-Bahra University, Mohali, Punjab.
[2]Head of Department, Department of Forensic Science, Rayat-Bahra University, Mohali, Punjab.

***Corresponding Author: Monika Thakur**

Student, M.Sc. Forensic Science, USAHS Department, Rayat-Bahra University, Mohali, Punjab.

DOI: https://doi.org/10.5281/zenodo.18592222

How to cite this Article: Monika Thakur*[1], Chanakya Mehta[1], Dr. Dalbir Singh[2] (2026). Forensic Detection Of Cannabinoids In Urine. European Journal of Biomedical and Pharmaceutical Sciences, 13(2), 199–208.

Article Received on 16/01/2026 **Article Revised on 06/02/2026** **Article Published on 10/02/2026**

ABSTRACT

Cannabis is one of the most extensively abused psychoactive substances worldwide, which is primarily detected by its active compounds called cannabinoids. Cannabinoid detection in the biological matrices, such as urine, holds a significant importance in both forensically as well as clinical settings for monitoring drug abuse, establishing consumption of cannabis and its derivatives recently, and supporting the medico-legal investigations. This review aims to explore and evaluate various analytical methods used for the detection of various cannabinoids and their derivatives in various biological samples, particularly urine samples from the perspective of forensic. The main objective is to highlight the reliability, sensitivity, and applicability of various analytical techniques that are employed in forensic laboratories. The methodologies, including both preliminary as well as confirmatory techniques such as immunoassay-based screening tests (RIA, ELISA, etc.), chromatography (such as GC-MS, LC-MS, HPLC-MS, HPTLC), spectroscopy (FTIR, MS, etc.) have been studied in the review. Immunoassays being rapid and cost-effective for screening, are considered advantageous, but may often suffer cross-reactivity and false positives, therefore need confirmatory methods. Whereas the chromatographic and spectroscopic techniques, are more accurate, specific, and selective, but require a sophisticated instrumentation, skilled labor, and greater operational costs. According to a forensic perspective, use of confirmatory methods ensures both reliability as well as admissibility of an evidence in any legal proceeding. Overall, the review underlines the fact that the balanced application of the above analytical techniques is crucial to overcome limitations and to provide a scientifically-valid results.

KEY WORDS: THC, CBD, CBN, GC-MS, LC-MS, HPLC, HPTLC, NMR, FTIR, Immunoassays (RIA, EIA, and ELISA), urine analysis.

1. INTRODUCTION

Cannabis sativa is an annual herbaceous plant that is indigenous to Eastern Asia.[1] Cannabis plants contain chemical compounds called cannabinoids, which usually affect the human brain and body by suppressing its activity and functioning. Cannabis is the most commonly abused psychoactive substance globally in different forms like bhang, ganja, and charas.[2] It is grown globally for its seeds, fibers, and psychoactive properties as well. Cannabis contains chemical compounds called cannabinoids, which usually affect the human brain and body, suppressing their activity and functioning. The primary active compound in cannabis is delta-9-tetrahydrocannabinol (THC), responsible for its psychoactive effects, like “highs”, and can rapidly metabolize in the body into its metabolites, which can be detected in biological matrices such as blood, urine, saliva, sweat, and hair. Other important cannabinoids include cannabidiol (CBD) and cannabinol (CBN), which lack psychoactivity but have some potential medical benefits.[2] Cannabis interacts with the endocannabinoid system (ECS) in the human body, affecting neurological and psychological functions in the body.[3] Cannabis consumption leads to various effects such as euphoria, altered perception, memory impairment, and potential psychosis at high doses. Cannabis occasionally doesn’t cause serious harm, but excessive use or daily use could lead to physical, mental, and moral deterioration.[4] As per a study conducted in 2006, cannabis remains the most widely used drug

worldwide, with an estimated 166 million people who have used cannabis in 2006, which is equivalent to about 4 percent of the world's population aged 15–64 years.[5]

These compounds and their further metabolites formed in the body can be identified in various biological samples, such as urine, blood, and saliva. Urine is the most commonly used biological sample for cannabinoid analysis or other drug analysis due to its non-invasive collection, ease of storage, and relatively long detection window.[4] Cannabinoids in the human body undergo extensive metabolism, and some of their major metabolites act as key markers that can be used for urine analysis, such as 11-nor-9-carboxy-THC (THC-COOH), 11-hydroxy-THC. Cannabinoids can be identified in urine for days or weeks after usage due to high tissue retention, and it depends on the frequency and length of exposure.[12,13]

The elimination of cannabinoids from the body varies due to individual metabolism, frequency of use, and physiological factors.

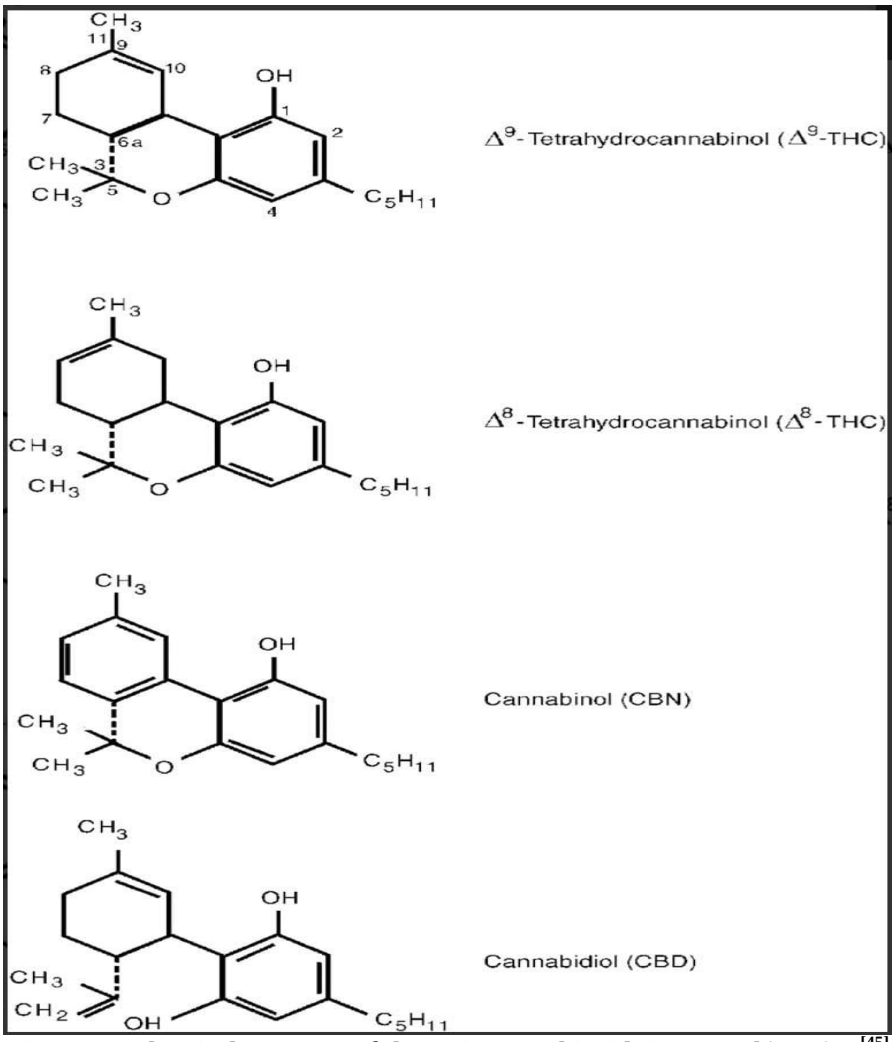

Figure 1.1 Chemical structure of the main cannabinoids in *Cannabis sativa*.[45]

This review describes various advanced analytical techniques, such as Gas Chromatography-Mass Spectrometry/Flame Ionization Detector (GC-MS/FID), LC-MS Liquid Chromatography-Mass Spectrometry), HPLC (High-Performance Liquid Chromatography paired with UV/DAD/MS), HPTLC (High-Performance Thin-Layer Chromatography), Fourier Transform Infrared Spectroscopy (FTIR), etc., among others, that offer improved sensitivity and specificity in the detection of cannabinoids in various types of biological samples.[6,7]

Forensic detection of cannabinoids in urine samples plays a very important role in identifying previous as well as recent drug use in criminal cases, as well as in workplace testing and rehabilitation programs. However, interpretation of results poses challenges due to factors such as passive exposure, variability of metabolism from person to person, and due to possible sample adulteration. Therefore, understanding various analytical methods, detection limits, and the interpretative considerations is a crucial part of establishing reliable forensic conclusions.[12]

2. Metabolism and excretion of cannabinoids

After the consumption of cannabis, the THC (primary active compound) is absorbed rapidly through inhalation or by ingestion, and it rapidly enters the systemic circulation and distributes extensively in tissues rich in lipids, such as the brain and adipose tissue. Smoking results in peak plasma concentrations of THC in about 3-7 minutes, whereas oral consumption of cannabis leads to slower absorption due to first-pass metabolism.[12, 38, 39, 40] It then undergoes an extensive hepatic metabolism via the cytochrome P450 enzymes (mainly the CYP2C9, CYP2C19, and CYP3A4). The major metabolites of THC include 11-hydroxy-THC (11-OH-THC), which is the main psychoactive component, and 11-nor-9-carboxy-THC (THC-COOH), which is basically a pharmacologically inactive compound.[12, 38, 39, 40]

In urine, even THC itself is rarely detected because of its highly lipophilic nature, due to which it is sequestered in the fat tissues. Whereas, THC-COOH and its glucuronide conjugates are excreted over several days through the kidneys. The detection window mainly depends on the frequency of use of the drug or any psychoactive substance. In case of cannabinoids, or an occasional user, the detection window is mainly about 1-3 days, and for chronic consumers, it's up to several weeks. These pharmacokinetic features make the urine analysis an effective tool for retrospective inspection rather than impairment assessment.[12, 39, 40]

3. Examination of cannabinoids in biological samples

In the field of forensic chemistry and toxicology, various biological samples are routinely utilized for drug detection, including blood, urine, saliva, hair, and sweat. Blood provides a real-time concentration of drugs, which are crucial for determining drug impairment and alcohol consumption. For example, blood tests for THC levels can indicate acute impairment, which is critical in forensic toxicology. Hair samples offer insight into long-term drug use history, which has the potential to reveal the pattern of use by a person over months or even years. Conversely, saliva samples are efficient due to their ease of collection and effectiveness in detecting recent drug use. Additionally, sweat can also serve as a viable option for drug monitoring in recent use, but not for long-term detection. Urine samples are most commonly used in drug testing due to their reliability for long-term testing, ease of collection, and low cost.[4, 7, 12] Cannabinoids can be analysed by both biological and non-biological samples, which helps choose the right matrix for performing analysis.[11] Different biological samples provide various other information regarding the time of use and extent of use of any drug.[17]

Table 1.1: shows a comparison of biological samples for cannabinoid detection.

Feature	Urine	Blood	Saliva	Hair
Collection	Easy	Invasive	Moderate	Non-invasive
Detection	Days-weeks	Hours-days	Hours-1 day	Weeks-months
Stability of analyte	High	Moderate	Low	High
Cost	Low	High	Moderate	High
Commonly used	Most common	Moderate	Less common	Least common

Urine is the liquid waste excreted by the kidneys through the urinary duct. Urine is mainly made up of water and various dissolved substances like urea, creatinine, electrolytes, and metabolic byproducts. It is essential for maintaining a balanced concentration of electrolytes and bodily fluids, as well as removing toxins and waste products, for the optimal metabolism of the body. Typically, human urine has a pH between 4.5-8. It contains all the traces of the substances that are being consumed and metabolized by the body, such as drugs, toxins, and others, which are useful in medical as well as forensic testing. Cannabinoids and their metabolites are concentrated in urine, therefore making their detection easier. Urine has a similar distribution and higher detection windows compared to blood and oral fluid, but requires simpler pre-treatment.[9, 12, 13] Rosendo et al. (2022) were the first team to extract THC, THC-OH, THC-COOH, CBN, and CBD from urine samples by using the microextraction by packed sorbent (MEPS) technique for pre-concentrating the compounds, which GC-MS later analysed.[13] The method was applied successfully to authentic samples and was proven efficient.[4] Urine is the preferred sample for cannabinoid detection because of its higher concentration and longer detection time of cannabinoids and their metabolites in urine.[7, 12]

AUTHORS	SAMPLES	RESULT
Kishore et al. [1999][23]	351 adolescents from urban South Delhi and rural villages of Uttar Pradesh (10 to 19 years)	48 out of 351 (23.07%) used cannabis during the previous year
Mohan et al. [2001][24]	Two samples of 26,792 and 19,436 individuals from 72 colonies in New Delhi (above 10 years of age).	Among 22,349 males, only 45 (0.3%) used cannabis during the previous 30 days.
Goel and Chakrabarti [2010][25]	103 individuals from 118 households in Sikkim (86.4% males; 15 to 44 years).	14 out of 103 participants (13.6%) reported cannabis use.
Tsering et al. [2010][26]	416 grade 8,9, and 10 students from West Bengal (mean age = 15 years).	13 out of 416 (0.03%) students reported cannabis use during the previous year.

Tikko et al. [2014][27]	4024 children from a nationwide survey (95.8% males; mean age = 15.6 years).	1423 (35.4%) reported ever use, 1375 (34.2%) last year use, and 1163 (28.9%) last month use of cannabis.
Kumar et al. [2015][28]	3437 participants between 25 and 70 years of age from Lucknow (82.9% males; 25 to 70 years).	42 out of 3437 (1.2%) reported current use of cannabis (ganja, bhang).
Arora et al. [2016][29]	230 medical students from Meerut.	18 out of 230 (7.82%) reported current use of cannabis or bhang.
Ashtankar and Talapalliwar [2017][30]	280 individuals from an urban slum in Nagpur (92.5% males)	20 (7.2%) individuals were found to be "addicted" to cannabis and brown sugar
Panwar and Prakash [2017][31]	1200 participants from community and treatment centers in Indore city between 2009 and 2013.	Cannabis use was reported in 30 (2.5%) participants from the community sample and 22 (11%) from the treatment center population.
Uppal et al.[2018][32]	175 participants from rural Bangalore (86.9% males; 13 to 30 years).	5 out of 175 (2.9%) reported current use of cannabis.

4. Sample Collection and Preparation

4.1 Sample Collection

Urine samples are mainly collected under controlled conditions to ensure the authenticity of the sample. Approx 30-60 mL volume of sample is collected in the sterile, tamper-evident containers to avoid any tampering. The chain of custody must be maintained to ensure the admissibility and authenticity of the sample for forensic purposes. Some important parameters, such as the temperature (32-38^{0}C checked immediately after voiding, pH (mainly 4.5-8.0), creatinine concentration, and specific gravity of the sample, are routinely checked to detect dilution and adulteration in the sample.[6]

4.2 Sample Preparation

For an effective analysis, efficient sample preparation is a crucial step for eliminating the interfering substances and the concentrated analytes. The most common pre-treatment steps include:

- **Hydrolysis:** It includes the enzymatic (beta-glucuronidase) or alkaline hydrolysis for converting the conjugated THC-COOH to its free form.
- **Extraction:** It mainly involves:
  - *Liquid-liquid extraction (LLE)***:** It mainly uses the mixtures of n-hexane: ethyl acetate.
  - *Solid-phase extraction (SPE):* It employs the use of sorbent columns (C18, silica) for cleaner extracts and automation compatibility.
  - *Solid-phase microextraction (SPME):* It is a solvent-free alternative.

5. Preliminary screening of cannabis using color tests

Color tests for cannabinoids are most specific because only a few plants can give false positive results, and these plants are henna, mace, agrimony, nutmeg etc. The positive results provide an indication of the possible presence of cannabinoids, but do not confirm or give a definite identification of the cannabinoids present. Therefore, it is mandatory to confirm the results by using discriminative techniques such as TLC, GC-MS, LC-MS/MS, etc.

- *Fast Blue B salt test*

Reagents: Petroleum ether, Fast Blue B salt (0.1% diluted with methanol or water), Sodium bicarbonate or sodium hydroxide (10% w/w aqueous solution).[6, 41,42]

Procedure: The urine sample is first subjected to hydrolysis can be alkaline or enzymatic, to release the conjugated metabolites. After hydrolysis, the cannabinoids are extracted using liquid-liquid extraction using suitable reagents such as hexane or ethyl acetate. The extract is then evaporated to dryness and then reconstituted in methanol. The few drops of the Fast-Blue B reagent are added, and the mixture is then alkalinized using sodium carbonate or sodium hydroxide solution.[6, 41, 42]

Results: Reddish-purple/violet color indicates the presence of cannabinoids in the sample.[6, 41, 42]

- *Duquenois-Levine test*

Reagents: Duquenois reagent (Acetaldehyde, ethanol, vanillin), Concentrated hydrochloric acid (HCl), Chloroform.[6, 41, 42]

Procedure: The urine sample is first subjected to hydrolysis and then extracted using organic solvent extraction. The extract is then kept in the test tube. A few drops of the duquenois reagent are added to the extract. Concentrated HCl is added and mixed into the extract, then the chloroform is added and the tube is shaken gently.[6, 41, 42]

Results: Purple/violet color in the chloroform layer indicates the presence of cannabinoids.[6, 41, 42]

6. Analytical techniques for the detection of cannabinoids

Many research studies were conducted earlier, which concluded that for analyzing and detecting the presence of cannabinoids in multiple types of biological samples, like blood, saliva, hair, and urine, multiple types of analytical techniques can be applied, which are effective as well as reliable. This review also provides a comprehensive evaluation of current and emerging analytical techniques, sample preparation strategies, validation criteria, and interpretive considerations used in forensic cannabinoid detection.

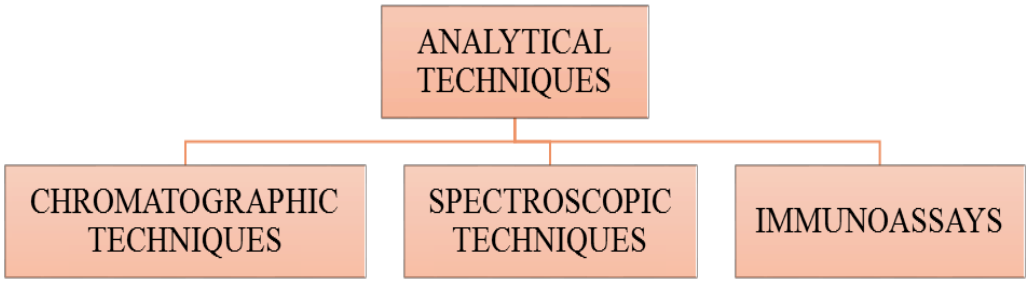

Figure 1.2: illustrates the various analytical techniques employed for detecting cannabinoids in a urine sample.

5.1 IMMUNOASSAY SCREENING

Immunoassays are one of the most common and widely used screening methods for the detection of cannabinoids in urine samples. Immunoassay screening is mainly based on the antigen-antibody interaction specific to THC-COOH and some related metabolites. Some of the most commonly used immunoassays are EIA (Enzyme Immunoassay) and RIA (Radio Immunoassay), ELISA (Enzyme-linked immunosorbent assay) etc. EIA is the most commonly used method nowadays, although the RIA has lost favour in recent years.[38] Immunoassays are mainly rapid, cost-effective, and have high throughput therefore, serve as preliminary screening tool in forensic as well as workplace testing programs. Immunoassays due to their cross-reactivity with non-cannabinoids compounds like ibuprofen or naproxen, are susceptible to false positives. Therefore, need for the confirmatory analysis is mandatory following the positive immunoassay results.

Radio Immunoassay (RIA): Radio immunoassays are very sensitive, have been widely used for years, but have lost favour in recent years due to their inherent disadvantages. They provide limited stability of the radiolabelled compounds that are used for screening, and need specific disposal for the radioactive materials used, and their specific handling to avoid any health hazards. Radio-labelling is mainly carried out by using either ^{3}H or ^{125}I, whereas ^{125}I radiolabels are more preferred due to their higher specificity than ^{3}H.[38] **Law et al. (1984)** described the ^{125}I tracer RIA method, which required a small volume of sample and allowed detection of cannabinoids and their metabolites even after many days of consumption. RIA was then coupled with high-performance liquid chromatography (HPLC), and the combined method, HPLC-RIA, was used for THC metabolites detection and analysis in blood and urine samples.[9]

Enzyme Immunoassay (EIA): Enzyme Immunoassays are the most widely and commonly used screening methods for cannabinoid detection in urine samples nowadays. These are rapid, simple, and do not require specific handling and disposal as in the radio immunoassays. Many studies have described the utilization of enzyme-multiplication immunoassay techniques (EMIT) for determining cannabinoids and their metabolites in urine samples have been reported. The initial screening by EMIT was then followed by confirmation using TLC, HPTLC, or mostly by GC-MS.[38]

Enzyme-linked immunosorbent assays (ELISA): This technique was used to carry out the microanalysis of cannabis components and their metabolites. This method was applicable for the analysis of THC metabolites in blood, urine, saliva, and plasma. This immunoassay technique detects the presence of specific drugs and their metabolites by using antibodies and a color change reaction. It is mainly ideal for initial screening and needs confirmatory analysis using chromatography techniques like GC, HPLC, etc.[38] **Fraser et al. (2001)** used ELISA and EIA assays for cannabinoid screening in urine samples, followed by GC-MS confirmation.[39]

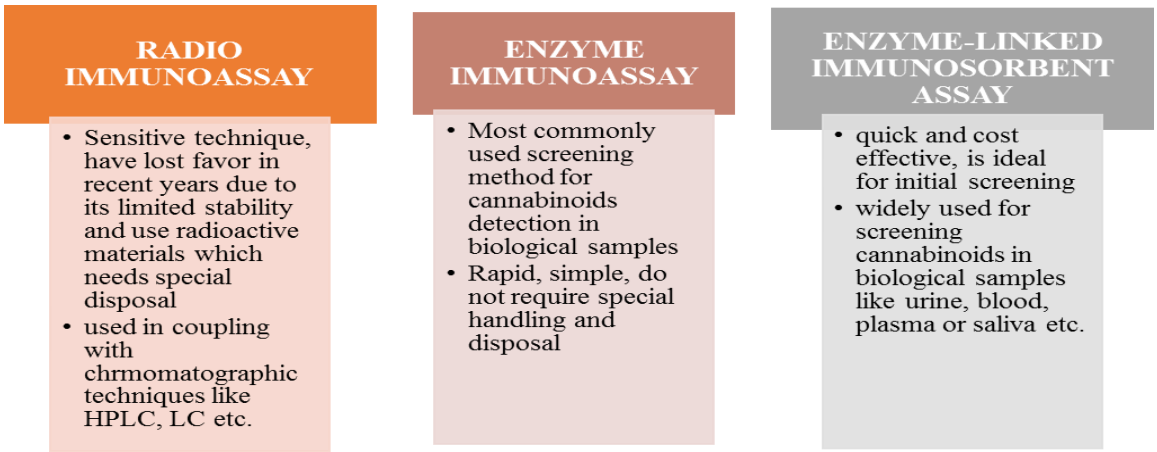

Figure 1.3 shows immunoassay techniques used for cannabinoid detection.

5.2 CHROMATOGRAPHIC TECHNIQUES

Chromatographic techniques are separation-based techniques that are used to isolate and analyze different individual components present in a complex mixture. These techniques work by distributing the components of the mixture between two phases: a stationary phase (solid or liquid on solid support), the other mobile phase (can be liquid or gas). These techniques are based on the principle of separation due to the interaction of different components of a mixture with both stationary and mobile phases, causing them to travel at different speeds and come out at different times due to their retention for each phase, causing their separation.

Gas Chromatography (GC): Gas chromatography is one of the most commonly used chromatographic methods for quantitative analysis of cannabinoids.[15, 6] It is a widely used analytical technique used to separate and analyze gaseous and volatile compounds. It works on the principle of differential partition, where the sample is first converted to vapors and then mixed with a gaseous mobile phase. The components that have a greater affinity towards the stationary phase travel slowly, and those with less affinity towards the stationary phase travel faster, thus separating according to their partition coefficient. Acidic cannabinoids undergo decarboxylation inside the column due to the high temperature inside the column required for analysis; therefore, they are required to be derivatized before analysis for accurate determination.[41, 42, 43, 44] It is mainly coupled with Mass Spectrometry (MS) or Flame Ionization Detector (FID) to detect and quantify cannabinoids or any other drug.[22, 6] Mass spectrometry helps in identifying the parent analyte by employing standardized electron ionization to fragment analytes. Flame Ionization Detector (FID) uses relatively cheap authentic standards, which help provide accurate quantification of cannabinoids in a sample, whereas Mass Spectrometry (MS) mainly requires equivalent deuterated standards for quantification, which are very expensive and are not available for all cannabinoids.[21, 15, 22, 6, 41] **Rosendo et al. (2022)** were the first to extract THC, THC-OH, THC-COOH, CBN, and CBD from the urine samples by using the microextraction by packed sorbent (MEPS) technique for the pre-concentration of the compounds, which were later analysed using GC-MS. This method was successfully applied to all the authentic samples and was proven efficient. Modern microextraction techniques are way cheaper, faster, and require only a smaller amount of sample and organic solvents, and also have good extraction efficiencies.[13] **Ameline et al. (2020)** developed a GC-MS/MS methodology for detecting Cannabidiol (CBD) in biological samples like urine, hair, and sweat after administering a cannabidiol capsule to a human volunteer. They expected low concentrations of the CBD analyte, so they decided not to use the corresponding deuterated compound. The team then managed to extend the detection window of Cannabidiol from 48 h (in case of urine sample) to 144 h (in sweat sample) with the analysis of all the biological samples. Concerning the urine sample, the non-hydrolysed samples were tested negative, but the enzymatic hydrolysis gave positive results, which proved this step vital for analysing CBD-gluc, which is the excreted form of cannabidiol.[14]

Liquid Chromatography (LC): Liquid chromatography (LC) is a highly sensitive and selective separation technique that allows analysis of both major and minor cannabinoids in biological matrices such as urine, blood, plasma, and oral fluids.[8] The separated components of the mixture cannot be identified alone by LC; therefore, Mass Spectrometry is also used for the detection of unknown and known compounds to analyze the chemical structure of the components in the mixture.

High-performance liquid chromatography (HPLC) is a commonly used liquid chromatography technique for the quantitative analysis of cannabinoids in biological samples.[15, 41] Most commonly used columns in this technique include C18 columns due to their high resolution and ability to differentiate the cannabinoids; these columns mainly consist of C18 stationary phase, whereas water or methanol with 0.1% formic acid is the mobile phase.[41, 42, 43, 44] Various detection techniques can be used with HPLC. Some common detection techniques include ultraviolet (UV) and Mass Spectrometry (MS), as well as diode array detection (DAD). UV detection is less expensive than mass spectrometry detection and is more straightforward.[35, 36, 41] **Vikingsson et al. (2022)** investigated 6000 de-identified urine samples for concentrations of cannabinoids for workplace drug testing. The samples were first screened using a Fast LC-MS-MS screening assay for 7-OH-CBD and delta-9-THC-COOH. The positive samples from Fast LC-MS-MS screening were then tested using an immunoassay technique. The samples then underwent confirmation by the LC-MS-MS method for 11 cannabinoids and their further metabolites. This study evaluated the presence of both ether-linked and acid-linked conjugated metabolites in the urine samples. Out of 6000 samples analysed, 522 were positive for 7-OH-CBD and delta-9-THC-COOH.[34]

Thin-layer Chromatography (TLC)/High Performance Thin-Layer Chromatography (HPTLC): Thin-layer chromatography (TLC) has been extensively utilized over the years because of its simplicity and low-cost screening test for drugs, including cannabinoids, but sometimes it often gives poor resolution results due to systematic errors. High-performance thin-layer chromatography (HPTLC) has a desired potential for eliminating systematic errors and also providing better resolution of cannabinoids.[17] It has emerged as a simple, cost-effective, and efficient technique offering minimal sample preparation, high precision, and multiple sample analysis simultaneously, consuming less amount of solvent than other chromatography techniques.[18] HPTLC is a powerful

analytical technique suitable for both qualitative and quantitative analysis. It is a robust, rapid, and efficient technique for quantitative analysis of compounds.[19] It works on the principle of partial differentiation of components of a mixture based on their relative affinities towards the adsorbent. The component with more relative affinity towards the stationary phase travels slower, and the component with less relative affinity travels faster, and thus are separated.[20] **Sharma et al. (2010)** conducted a study on 102 male patients suspected of cannabis abuse. Urine samples were collected from all 102 male patients, and liquid-liquid extraction from urine was done; color tests were performed before confirmatory analysis by HPTLC. The results were then confirmed using HPTLC, which indicated that out of 102 patients, 64 were detected positive for cannabis abuse.[10] **Djalolovich et al. (2025)** in their study demonstrated the effectiveness of urine as a readily available and informative sample for analysis and detection of cannabinoids in forensic investigations. The study employed various extraction techniques with different solvents, and thin-layer chromatography was used for the detection of cannabinoids from various biological samples such as saliva, urine, blood, etc.[12]

5.3 SPECTROSCOPIC TECHNIQUES

Spectroscopic techniques are the methods or techniques that are used to analyse the interaction of electromagnetic radiation with the matter present in a sample and to determine the composition and structure of a sample. These techniques are useful in detecting cannabinoids, other drugs, and their metabolites in various biological as well as non-biological samples based on their interaction with the EMR.

Fourier Transform Infrared Spectroscopy (FTIR): It is a spectroscopic technique that employs the use of Infrared radiation for the identification of the functional groups in the structure of the cannabinoids and for generating the spectral fingerprint of the structural components. It is based on the absorption and emission of light in the infrared region, causing vibrations in the chemical bonds of the sample's components, which allows for the determination of the chemical composition of various samples. It provides quick and non-destructive analysis of samples, but offers limited detection in complex mixtures, and is less sensitive for trace detection; therefore, it needs to be coupled with separation techniques like chromatography, which provide efficiency, selectivity, and specificity to the technique. It helps in the identification of functional groups in cannabinoids like THC, CBD, and CBN by their characteristic IR peaks.[6] **Hazekamp et al. (2005)** measured the cannabinoids with Fourier Transform Infrared Spectroscopy. They used potassium bromide (KBr), which was added to the ethanolic solution of cannabinoids, which was then followed by vacuum ethanol evaporation, because KBr in the IR region does not show any kind of absorption. IR spectra were then measured in the range of 500-4000 cm^{-1}. It showed that IR spectra presented more peaks compared to UV spectra. Therefore, considered more reliable than UV.[21]

Nuclear Magnetic Resonance (NMR): It is a powerful analytical technique that employs the use of magnetic properties of atomic nuclei (such as ^{1}H or ^{13}C) to determine the structure and concentration of chemical compounds present in the sample. It helps analyze the molecular fingerprint of a chemical compound, which is useful in determining the components of drugs present in the biological samples. It helps identify and differentiate various major as well as minor cannabinoids, for example, THC, CBD, CBN, and CBG. It provides information about the structural purity of the cannabinoid standards, thus maintaining integrity. It acts as another alternative for GC and HPLC.[16, 46] NMR, unlike chromatographic techniques like GC and HPLC, is not sensitive to impurities; it is reproducible and more accurate than GC and HPLC.[6] NMR has a major advantage in that this technique does not require reference standards; therefore, it can also quantify cannabinoids that lack pre-existing reference standards, which other techniques cannot.[16] **Hazekamp** and **Choi**[37] developed a cannabinoid detection and quantification method using ^{1}H-NMR, which does not require any chromatographic separation and purification. It was found that their technique was appropriate for cannabinoids quantification, mainly CBDA, THCA, CBG, CBGA, and possibly other cannabinoids.[16, 46]

UV-Visible Spectroscopy: It is one of the most commonly used spectroscopic techniques, which works on the principle of measuring the absorption of ultraviolet or visible light by a compound at a specific wavelength based on the electronic structure of the compound. It is useful in the screening and quantification of cannabinoids in plant extracts. Cannabinoids like THC and CBD have conjugated double bonds that absorb light in the UV region (200-400nm). It is a simple, fast, and cost-effective technique with low specificity; therefore, less suitable for forensic applications, only used in preliminary screening.[38]

Mass Spectrometry: Mass spectrometry (MS) is an advanced spectroscopic technique that helps identify and quantify chemical compounds based on their mass-to-charge ratio (m/z). It is one of the most widely used spectroscopic techniques for the detection and quantification of cannabinoids and their metabolites in biological samples such as urine, blood, saliva, and hair. It can detect even low levels (nanogram range) of cannabinoids and is suitable for confirmatory testing. It is useful in identifying cannabinoids like THC, CBD, CBN, and their metabolites like 11-OH-THC and THC-COOH. It is always used in conjunction with chromatographic techniques for the detection, quantification, and confirmation of the presence of components of drugs (such as cannabinoids) in the samples. When coupled with GC (Gas Chromatography), it helps in the detection of volatile cannabinoids in

biological samples, whereas when coupled with LC (Liquid Chromatography), it is useful in detecting the non-volatile and thermally labile cannabinoids. **Rosendo et al. (2022)** extracted THC, THC-OH, THC-COOH, CBN, and CBD from urine samples with the help of microextraction by packed sorbent (MEPS) technique for concentrating the compounds, and then the samples were analysed using GC-MS for confirmatory analysis.[13] **Vikingsson et al. (2022)** evaluated the presence of both ether-linked and acid-linked conjugated metabolites in the urine samples by using a Fast LC-MS-MS screening assay for 7-OH-CBD and delta-9-THC-COOH.[34]

6 DISCUSSION

Cannabis has been used over the years both recreationally as well as medicinally, due to the presence of psychoactive constituents in it like THC, due to which its cultivation and use are prohibited or regulated in many countries. After consumption, the cannabinoids undergo extensive metabolism in the human body and form metabolites such as THC-COOH, which are commonly excreted through urine. Among the various biological samples, urine is most preferred for the detection of cannabinoids because of its non-invasive nature, cost-effectiveness, high stability, and ease of collection. As it provides a longer detection window than other samples, cannabinoids can be detected even after days or weeks of consumption. Various analytical techniques have been developed and used for the qualification as well as quantification of cannabinoids in various biological samples, as well as in the compounds of the cannabis plant.

Chromatographic techniques are considered more reliable for the detection of cannabinoids. GC-MS is highly sensitive and is often used for confirmatory testing, especially in the case of volatile components where derivatization is needed. Studies using GC-MS have demonstrated that it is effective in the detection of THC and its metabolites, even in trace amounts, especially when used in pairs with advanced sample preparation methods like MEPS. LC-MS and HPLC provide high precision and selectivity in analysing non-volatile and thermally unstable compounds without derivatization. HPTLC is suitable for mass testing in rehabilitation centres due to its ability to screen multiple samples at once with minimal solvent use, making it cost-effective.

Spectroscopic techniques such as FTIR are non-destructive and are useful in identifying the functional groups present in cannabinoid compounds, but are less frequently used due to their lower sensitivity.

Immunoassays, particularly ELISA, are widely used for preliminary screening. They are rapid, simple, and user-friendly, but are prone to cross-reactivity, which can cause false-positive results. Therefore, positive results often require confirmation using chromatographic methods.

7 CONCLUSION

In forensic toxicology, the reliable detection of cannabinoids and their metabolites in urine is vital for cases including drug abuse related to workplace drug policies and in legal investigations.

Chromatographic techniques, such as GC-MS and LC-MS, provide high accuracy, precision, and specificity for the detection of cannabinoids and their metabolites. HPTLC, due to its efficiency and lower cost, stands out as a practical tool for routine screening. While immunoassays are very beneficial for quick and initial screenings, followed by confirmatory analysis using chromatographic techniques. As advancements in techniques continue, combining these techniques enhances the sensitivity, selectivity, speed, suitability, and reliability of drug testing. Therefore, understanding and applying the right analytical approach is essential for accurate detection of cannabinoids in urine samples.

Many advancements in the chromatographic and spectrometric techniques have improved the accuracy and reliability of the detection of cannabinoids in urine samples. Howsoever, challenges in the interpretation of results persist due to the prolonged excretion windows as well as metabolic variability from person to person. The future advancements should prioritize portable confirmatory techniques, microfluidic sample preparation, and an integrated AI-based interpretation model.

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