

**FORENSIC DETECTION OF CANNABINOIDS IN BIOLOGICAL FLUIDS:  
TECHNIQUES AND SENSITIVITY****BARAKAYEV LAZIZBEK DJALOLOVICH****Expert of the forensic chemistry department of the Bukhara branch of the Republican  
Forensic Medical Expertise Scientific and Practical Center****JUMAYEVA SHARIFA JURAYEVNA****Expert of the forensic chemistry department of the Bukhara branch of the Republican  
Forensic Medical Expertise Scientific and Practical Center****QAYUMOV BAXTIYOR ALLAMURODOVICH****Expert of the forensic chemistry department of the Surkhandarya branch of the  
Republican Center for Forensic Medical Expertise****Abstract**

Forensic investigations often rely on the detection of cannabinoids in biological fluids to establish drug use and intoxication. While existing literature covers individual methods for cannabinoid analysis, a comprehensive guide detailing procedures for various sample types and their respective sensitivities remains absent. This study addresses this gap by presenting detailed protocols for isolating cannabinoids from saliva, blood, urine, and skin swabs. Extraction techniques with various solvents are employed, followed by thin-layer chromatography (TLC) for detection. The study demonstrates the effectiveness of urine as a readily accessible and informative sample for cannabinoid analysis. The TLC method boasts a sensitivity of 0.1 micrograms, providing a reliable means for identifying cannabinoids with high precision. These procedures and sensitivity data contribute significantly to advancing forensic science, offering valuable resources for practitioners and researchers in the field. The study emphasizes the importance of urine as a highly informative and readily accessible sample for cannabinoid analysis in forensic investigations. This comprehensive guide provides a valuable resource for forensic practitioners and researchers, contributing to the advancement of forensic science.

**Keywords:** Cannabinoid detection, Forensic science, Forensic toxicology, Drug analysis, Biological fluids, Urine analysis, Blood analysis, Saliva analysis, Skin swab analysis, Thin-layer chromatography (TLC), Tetrahydrocannabinol (THC), Cannabinol (CBN), Cannabidiol (CBD)<sup>9</sup>

**INTRODUCTION**

The detection of cannabinoids in biological fluids is crucial for forensic investigations, particularly in cases involving drug use, intoxication, and related criminal activity. While various analytical techniques for detecting cannabinoids exist, a comprehensive guide outlining specific procedures for different sample types and their respective sensitivities remains absent. This gap in knowledge hinders the efficiency and accuracy of forensic investigations relying on cannabinoid detection.

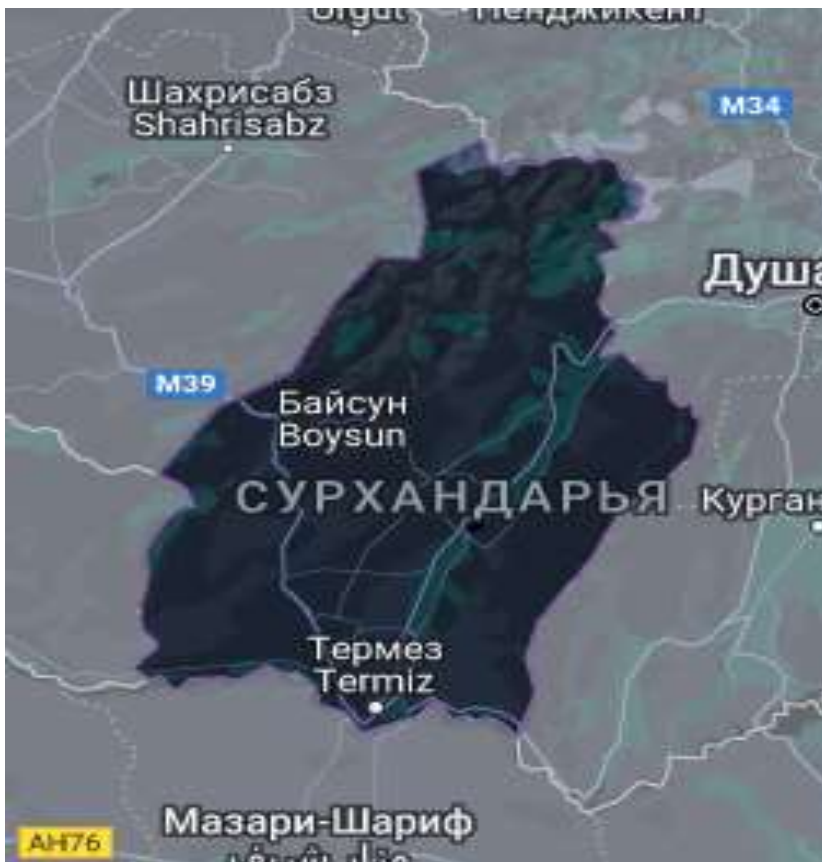
This study addresses this gap by presenting a practical guide to forensic chemical detection of cannabinoids in various biological fluids. We detail procedures for isolating cannabinoids from saliva, blood, urine, and skin swabs using extraction techniques with different solvents, followed by thin-layer chromatography (TLC) for detection. Our findings demonstrate the effectiveness of urine as a readily accessible and informative sample for cannabinoid analysis, emphasizing the sensitivity and reliability of the TLC method for achieving precise and conclusive results. This study provides valuable insights into the practical aspects of forensic cannabinoid detection, offering a valuable resource for forensic practitioners and researchers to enhance their investigative capabilities.

The Republican Scientific and Practical Center for Forensic Medical Examination of the Surkhandarya branch is located in the Surkhandarya region in the city of Termez. Surkhandarya region is the southernmost region of Uzbekistan

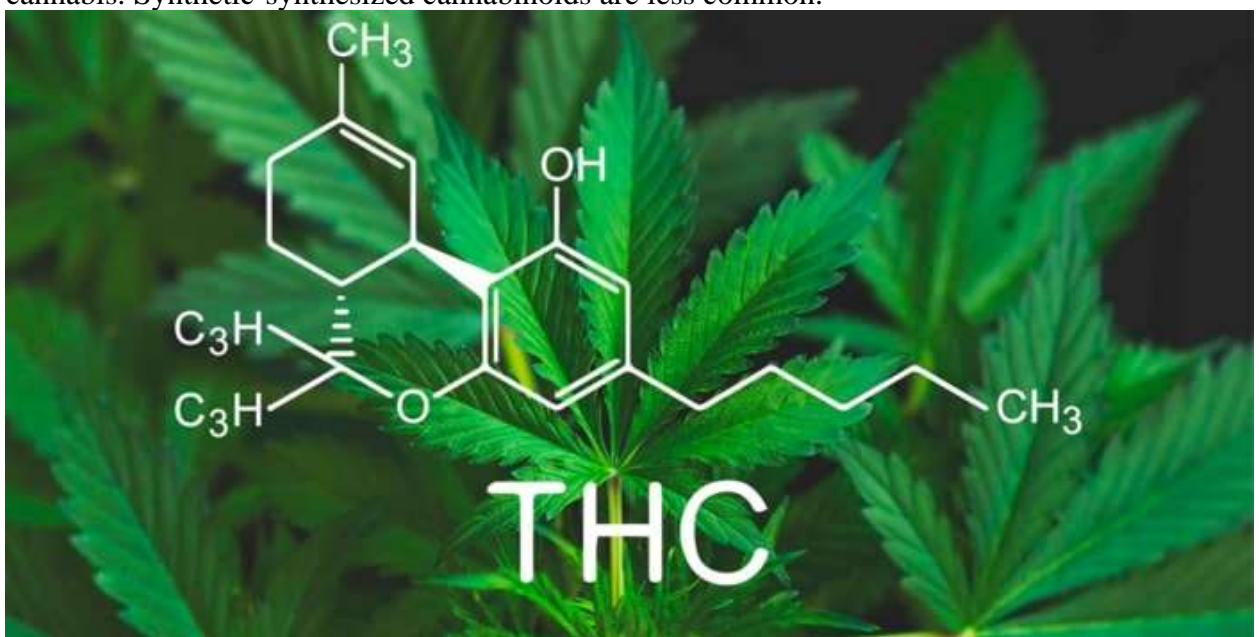


The administrative center is the city of Termez.

The central and southern parts of the region are flat. The Gissar range is located in the north, in the west and northwest are its spurs - the Baisuntau Mountains (4425 m) and Kugitangtau (3139 m), in the east is the Babatag range (up to 2290 m), in the south is the valley of the Amu Darya River. In the south it borders with the Balkh province of Afghanistan, in the southwest - with the Lebap Velayat of Turkmenistan, in the northwest - with the Kashkadarya region, in the east - with the Nosiri-Khusrav and Shaartuz districts of the Khatlon region of Tajikistan, in the northeast - Tursunzadev, Rudaki and Shakh rinavsky districts of Tajikistan, in the north - with Ainin and Penjikent districts of the Sughd region of Tajikistan. As of August 1, 2022, the population of the Surkhandarya region numbered 2,771,100 people - this is the 7th place (out of 14) among the regions of Uzbekistan. The region consists of 1 city of regional subordination (Termez) and 14 districts (Angora, Bandikhan, Baysun, Denau, Dzharkurgan, Kizirik, Kumkurgan, Muzrabad, Altynsay, Sariasi, Sherabad, Shurchinsky, Termez, Uzun)



In our branch of the Scientific and Practical Center for Forensic Medical Examination of the chemical department, various analyses are carried out, in most cases, this is a study on cannabinoids (hashish, anasha, etc.) Cannabinoids are a group of chemical compounds that have a psychoactive effect on the human body. Most of them are of natural origin and are found in cannabis. Synthetic-synthesized cannabinoids are less common.



The main sources of cannabinoids are marijuana, hashish, and anasha (ganja). They differ from each other in the concentration of the active substance and the method of preparation.

Cannabinoids are drugs and are of plant origin. The main psychoactive effects of hashish and marijuana are associated with 9-delta-tetrahydrocannabinol (THC), a narcotic substance contained in the resinous substance, stems and leaves of female specimens of wild cannabis (*Cannabissativa*). A variety of wild-growing cannabis, Indian hemp (*Cannabisindica*), is especially rich in THC. Forms of cannabis used in illegal sale: marijuana; hashish; hashish oil. Marijuana is the dried and crushed upper part of the cannabis plant with leaves and flowers. The content of psychoactive substances in marijuana reaches 13-15%. Hashish resin (tar) released by cannabis during the growing season has a green, dark brown or black color. The content of the main psychoactive substance (THC) is usually about 2%, but can reach 9-10%. Hashish oil is a concentrated dark liquid and viscous cannabis resin extract with a THC content of 10 to 30-60%. Hashish oil is added to regular tobacco cigarettes or cigarettes with marijuana (in 1 cigarette 500-750 mg of marijuana with a THC content of 1-4%).

#### **The effect of cannabinoids on the human body**

The effect of cannabinoids is to stimulate the active release of a neurotransmitter in the brain called dopamine, which causes a feeling of euphoria. Although the effect of a psychoactive substance on the body is individual and can vary from one person to another, most people who use cannabinoids experience the following sensations:

euphoria;  
mental and physical relaxation;  
altered perception of time;  
increased sensory perception;  
pain reduction;  
increased appetite.

The side effects of cannabis include:

the occurrence of coughing attacks; anxiety;  
chest and lung discomfort; the feeling of "buzzing" of the body;  
redness and dryness of the eyes; rapid heartbeat;  
dizziness, fainting; increased sweating;  
impaired concentration;  
hallucinations, paranoia and psychosis (in case of overdose).

#### **Forensic definition of cannabinoid intoxication**

When examining living persons for the presence of cannabinoids, the main object of research is urine. Urine is at the same time the most accessible and convenient for chemical and toxicological examination.

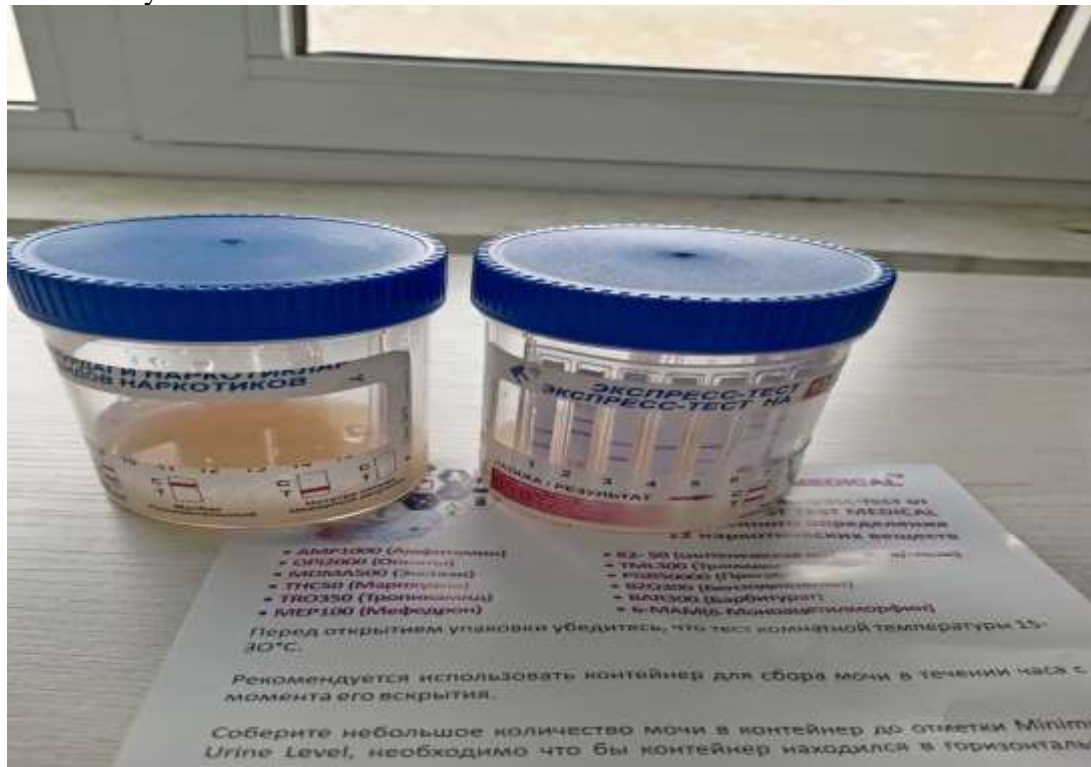
The main component determining the narcotic psychoactive properties of marijuana is trans-delta-(9)-tetrahydrocannabinol (THC). The components that confirm the use of marijuana are THC and its main metabolites: the primary psychoactive metabolite 11-hydroxy-delta-(9)-tetrahydrocannabinol (11-OH-THC) and the final biologically inactive product 11-nor-9-carboxy-delta-(9)-tetrahydrocannabinol (THC-COON), as well as in smaller amounts of cannabidiol and cannabidiol .

The primary metabolite can be detected in blood plasma; in urine, it can be detected only in the first few hours after ingestion and in much smaller amounts.

To determine cannabinoids in urine, we initially performed an analysis using immunochromatographic urine analysis, which is a test for the detection of a number of psychoactive compounds.

The choice of a liquid medium such as urine for analysis has certain advantages. The result can be obtained quickly and with a high degree of informativeness and reliability due to the rather high

concentration of the studied substances in the urine. Having enough urine for analysis is also not a problem. In addition, this method has been worked out and is the leading one in the primary and emergency examination of a person for drug use, including cannabinoids. The method of analysis is immunochromatography, which allows detecting 14 types of narcotic substances in urine with an accuracy of 90%.



Forensic chemical detection of cannabinoids in biological fluids. Description of the procedure:

- Technique for isolating cannabinoids from saliva  
A. The resulting sample is mixed with 50 ml of saturated aqueous sodium chloride solution B. cannabinoids are extracted with ethyl acetate (or ethanol) three times 10 ml for 5 minutes. The extract is dried by adding 1-1.5 g of anhydrous sodium sulfate for 30 minutes. The extract is filtered through a paper filter and evaporated into several drops
- The technique of isolating cannabinoids from the blood  
A. 5 ml of blood plasma is extracted four times with a mixture consisting of petroleum ether and 1.5% pentanol by volume (5 ml each) B. The combined organic layer is evaporated to a volume of several drops and transferred to a plate. B. Chromatographed under the same conditions as the extract from saliva.
- The technique of isolating cannabinoids from urine.  
A. 1 ml of methanol and 150 ml of 50% caustic soda solution are added to 2 ml of urine and mixed.  
B. The bottle is tightly sealed and placed in a thermoblock for 10 minutes at 60 degrees B. After cooling, the vial is opened and 6 n. hydrochloric acid is added to pH = 2-3 g. Twice extracted with a mixture of B (n-hexane and ethyl acetate (7:1) mixed in a ratio of 7:1) 5 ml each. The top layer is separated, transferred to a vial, evaporated to a dry residue in a stream of warm air
- The technique of isolating cannabinoids from flushing  
A. Sampling (flushing) from the surface of the fingers and lips is carried out separately, by wiping with a cotton or gauze swab moistened with ethyl alcohol B. Alcohol from the samples is evaporated at room temperature. B. Cannabinoids are extracted from the tampon material with

ethyl acetate (ethanol) twice in 10 ml for 1 min . The combined extract is evaporated to a final volume of 0.1-0.3 ml

- Detection of cannabinoids by thin-layer chromatography

A. The dry residue obtained after evaporation of the final volume of the extract is dissolved in chloroform and applied to zone 1 of the chromatographic plate, pre-activated at 80 degrees for 30 minutes. As a “witness”, 2-3 µl of a standard tetrahydrocannabinol solution is applied to zone 2.

B. The plate is placed in a chromatography chamber saturated with vapors of a mixture of solvents of the system :

- Petroleum ether and diethyl ether are mixed in a ratio (4:1) The length of the solvent front path is 10 cm B. Dried at room temperature or in a current of warm air until the odor of solvents is completely removed. The plate is evenly sprayed with 0.5% solution of durable blue B in 10% aqueous solution of sodium carbonate D. In the area of the chromatogram of the “witness”, red-purple spots with  $R_f = 0.8$  are observed, which indicates the presence of tetrahydrocannabinol (reaction sensitivity is 0.1 micrograms).

#### Conclusion

This study addresses a critical gap in forensic science by providing a comprehensive guide to the chemical detection of cannabinoids in biological fluids. The research highlights the effectiveness of urine as a readily accessible and informative sample for cannabinoid analysis, emphasizing the sensitivity and reliability of thin-layer chromatography (TLC) with a sensitivity of 0.1 micrograms for achieving precise and conclusive results. The detailed procedures for isolating cannabinoids from various biological fluids, including saliva, blood, urine, and skin swabs, provide valuable resources for forensic practitioners and researchers. This study contributes significantly to advancing forensic science by offering a practical and reliable approach for detecting cannabinoids in different biological samples. Further research should explore the application of these methods in various case scenarios and investigate the potential for developing more sensitive and automated techniques for cannabinoid detection.

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