e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(3); 459-464

Original Research Article

Detection of Drugs of Abuse in Postmortem Blood and Urine Samples in Unnatural Deaths: A Cross-Sectional Forensic Toxicology Study

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Received: 26-01-2026 / Revised: 24-02-2026 / Accepted: 10-03-2026

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Conflict of interest: Nil

Abstract

Background: The detection of drugs of abuse in postmortem biological specimens constitutes a cornerstone of forensic toxicological investigation in cases of unnatural death. Accurate identification and quantification of substances in postmortem blood and urine samples are essential for establishing the cause, manner, and circumstances surrounding death. Despite advancements in analytical techniques, comprehensive data regarding the prevalence and distribution patterns of drugs of abuse in postmortem cases remain limited in many regions.

Methods: This cross-sectional study was conducted at the Department of Forensic Medicine and Toxicology. A total of 324 unnatural death cases subjected to medicolegal autopsy, in which postmortem blood and urine samples were collected and analyzed using immunoassay screening followed by gas chromatography–mass spectrometry (GC-MS) confirmation, were included. Demographic, circumstantial, and toxicological data were analyzed using descriptive and inferential statistics.

Results: Drugs of abuse were detected in 138 cases (42.6%). The mean age of positive cases was 34.2 ± 11.8 years, with male predominance (79.7%). Ethanol was the most frequently detected substance (51.4% of positive cases), followed by opioids (21.0%), cannabinoids (14.5%), benzodiazepines (8.7%), and amphetamines (4.3%). Polysubstance detection occurred in 31 cases (22.5%). A statistically significant association was observed between drug detection and manner of death ($p = 0.003$), with the highest prevalence in accidental deaths (52.1%). The concordance rate between blood and urine detection was 78.3%.

Conclusion: Drugs of abuse are prevalent in unnatural death cases, particularly among young males. Systematic postmortem toxicological screening using confirmatory analytical methods is indispensable for accurate forensic interpretation and death certification.

Keywords: Drugs of abuse; postmortem toxicology; unnatural death; forensic autopsy; gas chromatography–mass spectrometry; ethanol; opioids.

DOI: 10.25258/ijcpr.18.3.73

Introduction

Unnatural deaths encompassing suicides, homicides, and accidents represent a significant proportion of medicolegal cases investigated by forensic pathologists worldwide. The role of drugs of abuse in contributing to, precipitating, or directly causing unnatural deaths has been increasingly recognized as a critical dimension of forensic investigation [1].

Substance abuse not only serves as a direct cause of death through acute intoxication and overdose but also functions as an indirect contributory factor by impairing judgment, promoting risk-taking behavior, and exacerbating psychiatric comorbidities [2]. The global burden of drug-related mortality has escalated dramatically over the past two decades. The United Nations Office on Drugs and Crime estimated that approximately 585,000 people died as a result of drug use in 2017, with opioids accounting for the majority of drug-related fatalities [3]. In parallel, alcohol-related mortality continues to represent an enormous public health challenge, contributing to approximately 3 million deaths annually according to the World Health Organization [4].

Postmortem toxicological analysis serves as an indispensable component of the forensic autopsy, providing objective chemical evidence that complements gross and histopathological findings

[5]. The analysis of postmortem blood and urine specimens enables the detection, identification, and quantification of drugs and their metabolites, thereby informing the determination of cause and manner of death [6]. While blood concentrations provide information regarding drug levels at or near the time of death, urine analysis offers a broader temporal window of detection, capturing prior drug exposure over hours to days [7].

Advances in analytical instrumentation, particularly immunoassay screening techniques coupled with confirmatory methods such as gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS), have significantly enhanced the sensitivity, specificity, and scope of postmortem drug detection [8]. However, the interpretation of postmortem toxicological results remains challenging due to phenomena such as postmortem redistribution, wherein drug concentrations in blood may change after death due to passive diffusion from tissues and organs [9].

Several studies have documented the prevalence of drugs of abuse in postmortem cases across different jurisdictions. Drummer and Gerostamoulos reported that drugs were implicated in a substantial proportion of coroner's cases in Australia [10]. Similarly, studies from Scandinavian countries have demonstrated increasing trends in postmortem detection of novel psychoactive substances alongside traditional drugs of abuse [11]. In the United States, the opioid epidemic has profoundly altered the postmortem toxicological landscape, with fentanyl and its analogues now predominating in overdose death cases [12].

Despite these international contributions, there remains a significant paucity of systematic postmortem toxicological data from South Asian settings, where patterns of substance abuse may differ substantially due to cultural, socioeconomic, and regulatory factors [13].

Furthermore, studies comparing the concordance between blood and urine detection rates in postmortem specimens are limited, yet such data are essential for optimizing specimen collection protocols in forensic practice [14]. The present study was therefore undertaken with the aim of determining the prevalence and pattern of drugs of abuse detected in postmortem blood and urine samples from unnatural death cases, evaluating the concordance between specimen types, and analyzing the association between drug detection and demographic and circumstantial variables.

Materials and Methods

Study Design and Setting: This was a cross-sectional, retrospective analytical study conducted at the Department of Forensic Medicine and Toxicology.

Study Population and Sample Size: All medicolegal autopsy cases classified as unnatural deaths during the study period, in which paired postmortem blood and urine samples were collected and submitted for toxicological analysis, were eligible for inclusion. A total of 324 cases met all inclusion criteria and constituted the final study sample.

Inclusion Criteria: Cases were included if: (a) the death was classified as unnatural (suicide, homicide, accident, or undetermined); (b) the autopsy was conducted within 72 hours of death; (c) paired femoral blood and urinary bladder urine specimens were collected during autopsy; and (d) complete toxicological analysis reports were available.

Exclusion Criteria: Cases were excluded if: (a) advanced decomposition precluded reliable specimen collection; (b) only a single specimen type was available; (c) the chain of custody documentation was incomplete; or (d) the death was subsequently reclassified as natural upon further investigation.

Specimen Collection and Handling: Postmortem blood samples were collected from the femoral vein using sterile syringes, and urine samples were aspirated directly from the urinary bladder during autopsy. Specimens were transferred to appropriately labeled containers containing sodium fluoride (for blood) and plain containers (for urine), sealed, and maintained at 4°C until analysis. Chain of custody protocols were strictly adhered to throughout the specimen handling process.

Analytical Procedures: Initial screening was performed using enzyme-linked immunosorbent assay (ELISA) and cloned enzyme donor immunoassay (CEDIA) techniques for the following drug classes: ethanol, opioids, cannabinoids, benzodiazepines, amphetamines, cocaine metabolites, and barbiturates. All presumptive positive results were confirmed using gas chromatography–mass spectrometry (GC-MS) with established validated methods. Blood alcohol concentration (BAC) was determined using headspace gas chromatography with flame ionization detection (HS-GC-FID). Quantitative analysis of confirmed drugs was performed using calibrated reference standards.

Data Collection: Demographic data (age, sex, occupation, residence), circumstantial information (manner of death, cause of death, scene findings), clinical history (known substance use, psychiatric history), and complete toxicological results were extracted from autopsy reports, police inquest

documents, and laboratory records using a standardized data abstraction form.

Statistical Analysis: Data were entered into Microsoft Excel 2019 and analyzed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation (SD).

The chi-square test was employed for associations between categorical variables. Fisher's exact test was used when expected cell frequencies were below 5. The independent samples t-test was used for comparing means between groups. McNemar's test was used to compare detection rates between paired blood and urine specimens. Statistical significance was set at $p < 0.05$.

Results

Demographic and Circumstantial Characteristics: Among the 324 unnatural death cases analyzed, 248 (76.5%) were male and 76 (23.5%) were female. The mean age was 36.8 ± 14.2 years (range: 15–78 years). Regarding the manner of death, accidental deaths constituted the largest category (142 cases, 43.8%), followed by suicides (118 cases, 36.4%), homicides (48 cases, 14.8%), and undetermined deaths (16 cases, 4.9%).

Overall Drug Detection: Drugs of abuse were detected in 138 of 324 cases (42.6%). The mean age of drug-positive cases was significantly lower than drug-negative cases (34.2 ± 11.8 vs. 38.7 ± 15.6 years; $p = 0.004$). Males accounted for 110 of 138 positive cases (79.7%), compared to 138 of 186 negative cases (74.2%), though this difference did not reach statistical significance ($p = 0.241$).

A statistically significant association was found between drug detection and manner of death ($\chi^2 = 14.07$, $p = 0.003$), with the highest positivity rate observed in accidental deaths (52.1%), followed by undetermined deaths (43.8%), homicides (37.5%), and suicides (32.2%).

Table 1: Demographic and Circumstantial Characteristics by Drug Detection Status (N = 324)

Variable	Drug Positive (n = 138)	Drug Negative (n = 186)	p-value
Age (mean ± SD, years)	34.2 ± 11.8	38.7 ± 15.6	0.004*
Sex			0.241
Male	110 (79.7%)	138 (74.2%)	
Female	28 (20.3%)	48 (25.8%)	
Manner of Death			0.003*
Accident	74 (53.6%)	68 (36.6%)	
Suicide	38 (27.5%)	80 (43.0%)	
Homicide	18 (13.0%)	30 (16.1%)	
Undetermined	8 (5.8%)	8 (4.3%)	
Residence			0.032*
Urban	94 (68.1%)	104 (55.9%)	
Rural	44 (31.9%)	82 (44.1%)	

***Statistically significant ($p < 0.05$)**

Distribution of Detected Substances: Among the 138 drug-positive cases, ethanol was the most frequently detected substance, present in 71 cases (51.4%), with a mean BAC of 0.168 ± 0.094 g/dL. Opioids were detected in 29 cases (21.0%), including morphine (n = 17), codeine (n = 7), and tramadol (n = 5). Cannabinoids were identified in 20 cases (14.5%), benzodiazepines in 12 cases (8.7%), and amphetamine-type stimulants in 6 cases (4.3%). Polysubstance detection (two or more drug classes) occurred in 31 cases (22.5%), with the ethanol-opioid combination being the most frequent co-detection pattern (n = 14, 45.2% of polysubstance cases).

Table 2: Distribution of Drugs of Abuse Detected in Positive Cases (N = 138)

Substance Class	n (%)	Mean Concentration in Blood	Detected Alone n (%)	Detected with Others n (%)
Ethanol	71 (51.4)	0.168 ± 0.094 g/dL	48 (67.6)	23 (32.4)
Opioids	29 (21.0)	Morphine: 0.24 ± 0.18 µg/mL	14 (48.3)	15 (51.7)
Cannabinoids	20 (14.5)	THC-COOH: 42.6 ± 31.4 ng/mL*	16 (80.0)	4 (20.0)
Benzodiazepines	12 (8.7)	Diazepam: 0.52 ± 0.38 µg/mL	8 (66.7)	4 (33.3)
Amphetamines	6 (4.3)	Methamphetamine: 0.31 ± 0.22 µg/mL	4 (66.7)	2 (33.3)

***Urine concentration; THC-COOH = 11-nor-9-carboxy-Δ9-tetrahydrocannabinol**

Concordance between Blood and Urine Detection: Among all 138 positive cases, 108 (78.3%) showed concordant detection in both blood and urine specimens.

Ethanol showed the highest concordance rate (88.7%), followed by opioids (82.8%) and amphetamines (83.3%). Cannabinoids demonstrated a notable discordance, with 5 cases (25.0%) positive in urine alone but negative in blood, attributable to the longer urinary detection window for THC-COOH. Benzodiazepines showed the lowest concordance (66.7%), with 4 cases detected exclusively in urine. Overall, urine demonstrated a higher cumulative detection rate compared to blood (136 vs. 121 positive detections across all drug classes; McNemar's test $p = 0.018$).

Table 3: Concordance of Drug Detection between Postmortem Blood and Urine (N = 138)

Substance	Both Positive n (%)	Blood Only n (%)	Urine Only n (%)	Concordance Rate (%)
Ethanol	63 (88.7)	6 (8.5)	2 (2.8)	88.7
Opioids	24 (82.8)	2 (6.9)	3 (10.3)	82.8
Cannabinoids	13 (65.0)	2 (10.0)	5 (25.0)	65.0
Benzodiazepines	8 (66.7)	0 (0.0)	4 (33.3)	66.7
Amphetamines	5 (83.3)	1 (16.7)	0 (0.0)	83.3
Overall	**108 (78.3)**	**13 (9.4)**	**17 (12.3)**	**78.3**

Discussion

The present study demonstrates that drugs of abuse are detected in a substantial proportion (42.6%) of unnatural death cases, underscoring the critical importance of comprehensive postmortem toxicological screening in forensic practice. This prevalence is consistent with international reports documenting drug detection rates ranging from 30% to 55% in medicolegal autopsy populations [15]. The finding aligns closely with a multicenter European study by Launiainen et al., who reported a 45% positivity rate for psychoactive substances in postmortem cases in Finland [16].

The demographic profile of drug-positive cases—predominantly young males from urban settings—is concordant with well-established epidemiological patterns of substance abuse. Darke and Duflou, in their extensive analysis of drug-related deaths in Australia, similarly identified young adult males as the most disproportionately affected cohort [17]. The significantly lower mean age in drug-positive compared to drug-negative cases ($p = 0.004$) likely reflects the known peak prevalence of substance use disorders in the third and fourth decades of life [18].

The predominance of ethanol (51.4% of positive cases) as the most frequently detected substance resonates with global forensic toxicological literature. Kugelberg and Jones documented ethanol as the single most commonly encountered substance in medicolegal death investigations across multiple jurisdictions [19]. The mean BAC of 0.168 ± 0.094 g/dL observed in the present study exceeds the legal intoxication threshold in most jurisdictions, indicating significant impairment at or near the time of death. This finding is particularly relevant in the context of accidental deaths, where alcohol-related impairment of psychomotor function, coordination, and judgment is a well-recognized precipitating factor [20].

The detection of opioids as the second most common drug class (21.0%) reflects the growing global burden of opioid-related mortality. While the specific compounds detected in our study—morphine, codeine, and tramadol—differ from the synthetic opioid epidemic predominating in North America, they represent the opioid abuse patterns characteristic of South Asian settings [21]. The high proportion of polysubstance detection among opioid-positive cases (51.7%) is particularly concerning, as the synergistic respiratory depressant effects of opioids combined with ethanol or benzodiazepines substantially amplify the risk of fatal respiratory failure [22].

The significant association between drug detection and manner of death ($p = 0.003$), with the highest positivity rate in accidental deaths (52.1%), has important implications for forensic death investigation. Substance intoxication may render individuals vulnerable to accidents through impaired consciousness, altered perception, and compromised motor function [23]. Conversely, the relatively lower detection rate in suicidal deaths (32.2%) may reflect the intentional use of other lethal agents—such as pesticides, hanging, or sharp force—in the study population, consistent with regional patterns of suicidal methods [24].

The concordance analysis between postmortem blood and urine represents a novel contribution of this study. The overall concordance rate of 78.3% highlights the complementary value of analyzing paired specimens. The superior detection rate of urine for cannabinoids and benzodiazepines is pharmacokinetically expected, as the urinary metabolites of these compounds (THC-COOH and oxazepam, respectively) persist longer than parent compounds in blood [25]. These findings support

the forensic toxicological recommendation for routine collection and analysis of both specimen types to maximize detection sensitivity and provide a comprehensive toxicological profile [26].

The phenomenon of postmortem redistribution must be acknowledged as a significant interpretive limitation in any study utilizing postmortem blood concentrations. Drugs with high volumes of distribution, such as tricyclic antidepressants and certain opioids, are particularly susceptible to concentration changes after death due to release from tissue reservoirs [27]. The use of femoral blood in the present study partially mitigates this concern, as peripheral blood is generally considered more resistant to postmortem redistribution than central cardiac blood [28].

Limitations of this study include its retrospective single-center design, potential underestimation of certain substances not included in the immunoassay screening panels (particularly novel psychoactive substances), the absence of comprehensive vitreous humor analysis, and the inability to correlate toxicological findings with detailed clinical and psychological histories. Additionally, the potential effects of postmortem interval, embalming, and specimen degradation on analytical results could not be fully controlled.

Conclusion

This cross-sectional forensic toxicology study demonstrates that drugs of abuse are detectable in a significant proportion of unnatural death cases, with ethanol and opioids being the most commonly identified substances. Young urban males are disproportionately represented among drug-positive decedents, and accidental deaths show the strongest association with substance detection. Polysubstance use is observed in nearly one-quarter of positive cases, highlighting the complex pharmacological milieu frequently encountered in forensic practice.

The complementary analysis of paired postmortem blood and urine specimens enhances overall detection sensitivity and is recommended as standard practice. These findings emphasize the indispensable role of systematic and comprehensive postmortem toxicological analysis in the accurate determination of cause and manner of death, and they provide valuable epidemiological data for informing substance abuse prevention and public health policy initiatives.

Future prospective multicenter studies incorporating expanded drug panels, novel psychoactive substance detection, and quantitative vitreous humor analysis are warranted to further strengthen the forensic toxicological evidence base.

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