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A SCIENTIFIC REVIEW ON FORENSIC TOXICOLOGY: CURRENT ADVANCES AND APPLICATIONS IN CRIME INVESTIGATION

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ABSTRACT

Forensic toxicology occupies a central position at the interface of analytical chemistry, pharmacology, and the law, providing the scientific evidence required to determine whether a drug, poison, or other xenobiotic substance contributed to death, injury, or impaired behaviour. Over the past three decades, the discipline has been transformed by rapid advances in analytical instrumentation, most notably liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and high-resolution mass spectrometry (HRMS), which have dramatically improved the sensitivity, specificity, and scope of substances that can be detected in biological specimens. This review synthesises current knowledge on the historical development, analytical methodologies, biological specimens, and interpretive frameworks used in forensic toxicology, with particular emphasis on its practical applications in criminal investigation, including postmortem toxicology, drug-facilitated crimes, driving-under-the-influence (DUI) casework, and the escalating challenge posed by novel psychoactive substances (NPS). Emerging areas such as alternative matrix testing (hair, oral fluid, vitreous humor), pharmacogenomic interpretation of drug toxicity, and the incorporation of artificial intelligence and high-throughput "toxicological -omics" approaches are discussed as frontiers that are reshaping the field. The review further examines quality assurance frameworks, validation requirements, and the legal admissibility standards that govern the use of toxicological evidence in court. Persistent challenges, including postmortem redistribution, interpretation of drug concentrations in the absence of case history, and the continuous emergence of new designer drugs, are critically analysed. The review concludes that continued investment in analytical innovation, harmonised international standards, and interdisciplinary collaboration between toxicologists, pathologists, and law enforcement will be essential to ensure that forensic toxicology continues to deliver accurate, timely, and legally defensible evidence in criminal investigations.

KEYWORDS: forensic toxicology; postmortem toxicology; mass spectrometry; novel psychoactive substances; drug-facilitated crime; forensic evidence; crime investigation; analytical chemistry

1. INTRODUCTION

Forensic toxicology is the branch of applied science that identifies and quantifies drugs, poisons, alcohol, and other chemical substances in biological specimens for legal purposes. It sits at the confluence of pharmacology, analytical chemistry, pathology, and criminal law, and it provides critical, often decisive, evidence in death investigations, impaired-driving prosecutions, workplace and human performance testing, and criminal cases involving poisoning or drug-facilitated offences (Levine, 2013; Flanagan, Taylor, Watson & Whelpton, 2007). Unlike clinical toxicology, which is primarily concerned with the immediate treatment of a poisoned or intoxicated patient, forensic toxicology must additionally satisfy rigorous evidentiary standards, including a documented chain of custody, validated analytical methods, and results that can withstand cross-examination in a court of law. The scope of modern forensic toxicology has expanded enormously since the discipline's origins in the nineteenth century. Where early forensic chemists relied on colour tests and simple distillation to detect classical poisons such as arsenic, contemporary laboratories routinely screen for hundreds of pharmaceuticals, drugs of abuse, novel psychoactive substances, pesticides, and volatile compounds using highly sensitive chromatographic and mass spectrometric platforms (Maurer, 2010). This growth has been driven both by advances in instrumentation and by the changing landscape of substance use, which has seen the proliferation of synthetic cannabinoids, synthetic opioids such as fentanyl analogues, and a constantly evolving array of "designer drugs" engineered to evade existing legislation and detection methods (Zawilska & Andrzejczak, 2015).

This review aims to provide a comprehensive and up-to-date overview of forensic toxicology as it is practised today. It examines the historical development of the field, the principal classes of biological specimens used in forensic analysis, the analytical techniques that underpin modern toxicological testing, and the interpretive challenges that arise when translating a laboratory result into a meaningful statement about impairment, cause of death, or criminal culpability. Particular attention is given to the discipline's direct applications in crime investigation, including postmortem toxicology, drug-facilitated sexual assault, driving under the influence of drugs and alcohol, and the detection of poisoning. The review closes with a discussion of quality assurance, legal admissibility, and emerging frontiers such as artificial intelligence-assisted data interpretation and pharmacogenomics, which are expected to shape the next generation of forensic toxicological practice.

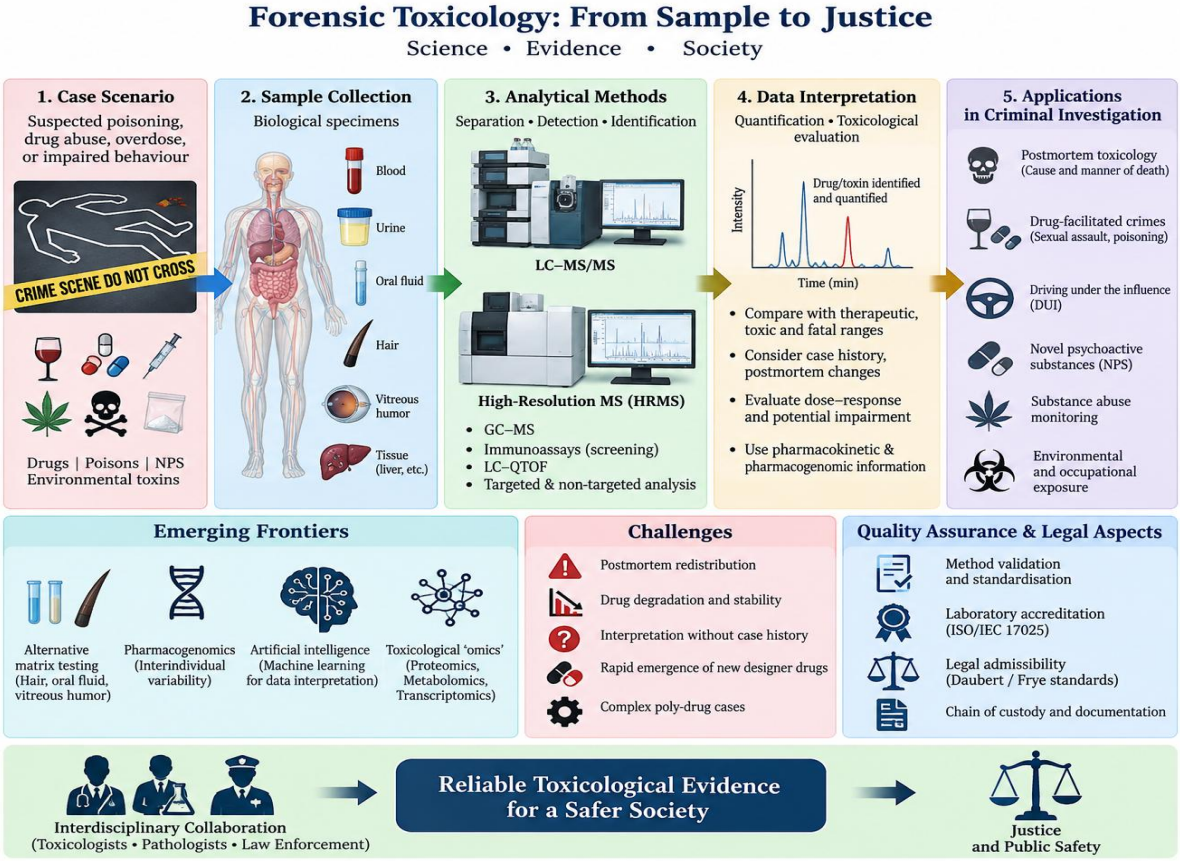

Figure 1: Schematic overview of forensic toxicology, illustrating the workflow from case assessment and biological specimen collection through advanced analytical techniques, toxicological interpretation, forensic applications, emerging technologies, quality assurance, and legal evaluation to the generation of reliable evidence for criminal investigation and public safety.

Beyond its role in individual case investigation, forensic toxicology also functions as a critical source of aggregate public health intelligence, contributing to drug mortality surveillance, the early identification of emerging substance threats, and the evaluation of drug policy interventions. This dual role, serving both the individual case and the broader public interest, distinguishes forensic toxicology from many other forensic disciplines and underscores the importance of sustained investment in laboratory capacity, workforce training, and inter-agency data-sharing infrastructure, themes that are revisited throughout this review.

2. Historical Perspectives

The origins of forensic toxicology are generally traced to the work of the Spanish physician Mathieu Orfila, whose 1813 treatise Traité des poisons is widely regarded as the foundational text of the discipline (Wennig, 2009). Orfila systematised the detection of poisons in human tissue and demonstrated, through experimental studies, that chemical evidence of poisoning could be recovered from a corpse, a principle that underpins postmortem toxicology to this day. Throughout the nineteenth century, toxicological analysis remained dominated by wet chemistry: colour reactions, precipitation tests, and the Marsh test for arsenic (developed in 1836) were the principal tools available to investigators (Wennig, 2009).

The twentieth century brought successive waves of technological transformation. The development of spectrophotometry and, later, gas chromatography (GC) in the 1950s and 1960s allowed toxicologists to separate and identify complex mixtures of organic compounds with far greater resolution than classical wet chemistry (Maurer, 2010). The coupling of gas chromatography with mass spectrometry (GC-MS) in the 1970s and 1980s represented a watershed moment, providing both separation and structural confirmation in a single analytical run and establishing GC-MS as the historical "gold standard" for definitive identification of drugs and their metabolites (Drummer, 2007). The subsequent development of liquid chromatography-tandem mass spectrometry

(LC-MS/MS) in the late 1990s and 2000s extended this capability to thermally labile and polar compounds that were poorly suited to GC-based methods, including many contemporary drugs of abuse and their glucuronide metabolites (Maurer, 2010; Peters, 2011).

In parallel with these analytical advances, the twentieth century also saw the professionalisation of forensic toxicology as a distinct discipline, marked by the establishment of specialist societies such as The International Association of Forensic Toxicologists (TIAFT, founded 1963) and the Society of Forensic Toxicologists (SOFT, founded 1970), the development of formal certification schemes, and the publication of consensus guidelines on method validation and laboratory accreditation (Jenkins, 2008). These institutional developments have been instrumental in ensuring that forensic toxicological evidence meets the scientific and legal standards required for admissibility in criminal proceedings.

The late twentieth and early twenty-first centuries have additionally been marked by growing international harmonisation of forensic toxicological practice. Consensus guidelines on minimum detection cut-offs, recommended confirmatory techniques, and reporting terminology, published collaboratively through organisations such as TIAFT and national forensic science regulators, have helped to reduce inter-laboratory variability and to support the mutual recognition of toxicological findings across jurisdictions, an increasingly important consideration given the transnational nature of much contemporary drug trafficking and organised crime (Jenkins, 2008). At the same time, the ever-accelerating pace of new substance emergence, discussed in detail in Section 7, has ensured that forensic toxicology remains a continuously evolving discipline, in which laboratory practice must adapt in near real time to a constantly shifting drug landscape.

3. Scope and Branches of Forensic Toxicology

Forensic toxicology is commonly divided into several interrelated sub-disciplines, each addressing a distinct investigative question:

- Postmortem forensic toxicology: the analysis of tissues and fluids obtained at autopsy to determine whether drugs, alcohol, or poisons contributed to or caused death.
- Human performance (behavioural) toxicology: the assessment of impairment in living individuals, most commonly in the context of driving under the influence of alcohol or drugs (DUID) or workplace accidents.
- Forensic drug testing: the detection of illicit or prohibited substances in employment, correctional, sports, and probationary settings.
- Clinical/forensic toxicology of poisoning and drug-facilitated crime: identification of substances used in criminal poisoning, sexual assault facilitation, and child abuse or neglect.
- Environmental and occupational forensic toxicology: investigation of exposure to industrial chemicals, pesticides, and environmental contaminants with legal implications.

Each of these branches shares a common analytical foundation but differs in the specimens analysed, the substances targeted, and the interpretive framework applied to the results. A recurring theme across all branches is the necessity of correlating an analytical finding, typically a drug concentration in a biological matrix, with a functional or clinical outcome, a task that is often complicated by inter-individual variability in metabolism, tolerance, and post-sampling artefact (Drummer, 2007).

4. Specimen Collection and Types of Biological Matrices

The selection of an appropriate biological specimen is a foundational determinant of the reliability of forensic toxicological findings. Different matrices offer distinct windows of detection, are subject to different artefacts, and are more or less suited to particular analytical questions.

4.1 Blood

Peripheral (femoral) blood is regarded as the specimen of choice for quantitative interpretation in both antemortem and postmortem toxicology because concentrations in peripheral sites are less subject to postmortem redistribution than central blood obtained from the heart or great vessels (Pounder & Jones, 1990). Blood alcohol and drug concentrations obtained from femoral sites are considered most representative of the concentration at, or near, the time of death or sample collection.

4.2 Urine

Urine offers a longer detection window than blood for many substances because drugs and metabolites are concentrated in urine over time, making it especially valuable for qualitative screening in workplace, criminal justice, and driving-related testing. However, because urine concentrations reflect cumulative excretion rather than the concentration at a specific point in time, urine results are generally unsuitable for direct correlation with impairment (Moeller, Lee & Kissack, 2008).

4.3 Vitreous Humor

Vitreous humor, the gel-like fluid of the eye, is anatomically isolated and relatively resistant to putrefaction and postmortem redistribution, making it a valuable specimen for postmortem alcohol and electrolyte determination, particularly in decomposed remains (Pounder & Jones, 1990).

4.4 Hair

Hair analysis provides a uniquely long retrospective window of drug exposure, since drugs incorporated into the hair shaft during growth remain detectable for months after use, in contrast to the hours-to-days window offered by blood or urine. Segmental hair analysis, in which a hair shaft is cut into sequential segments and analysed separately, can be used to reconstruct an approximate exposure timeline, which is of particular value in cases of chronic poisoning, drug-facilitated crime, and child custody disputes (Pragst & Balikova, 2006).

4.5 Oral Fluid and Other Alternative Matrices

Oral fluid (saliva) has gained increasing acceptance in roadside and workplace drug testing because it is non-invasive, difficult to adulterate under observation, and reflects relatively recent drug use, correlating reasonably well with the pharmacologically active, unbound fraction of many drugs in blood (Vindenes et al., 2012). Other matrices, including nails, meconium, bone marrow, and adipose tissue, are used in specialised circumstances such as neonatal drug exposure or severely decomposed or skeletonised remains.

4.6 Approximate Detection Windows Across Matrices

The choice of specimen is frequently dictated by the detection window required for the investigative question at hand. Table 2 presents approximate, generalised detection windows for common substance classes across the principal matrices discussed above; actual detection times vary considerably depending on dose, frequency and chronicity of use, individual metabolism, and the analytical sensitivity of the method employed, and the values shown should be regarded as indicative rather than absolute.

Matrix	Typical Detection Window	Best Suited To
Blood	Hours to ~1–3 days	Recent use; impairment correlation
Oral fluid	Hours to ~1–2 days	Very recent use; roadside screening
Urine	1–4 days (longer for chronic cannabis use)	Qualitative screening; wide detection window
Hair (per cm segment)	Weeks to months (retrospective)	Chronic exposure history; DFC follow-up
Vitreous humor	Reflects late antemortem period	Postmortem/decomposed cases

Table 2. Approximate, generalised detection windows for common substances across principal forensic matrices.

5. Analytical Techniques and Instrumentation

Modern forensic toxicological analysis is typically conducted in two stages: an initial, rapid screening step designed to identify the presence or likely presence of a broad class of substances, followed by a confirmatory step that unambiguously identifies and quantifies the specific compound(s) present using a technique with a different detection principle from the screen (Maurer, 2010; Peters, 2011).

5.1 Immunoassay Screening

Immunoassays, including enzyme-linked immunosorbent assay (ELISA), enzyme multiplied immunoassay technique (EMIT), and kinetic interaction of microparticles in solution (KIMS), remain the most widely used first-line screening tools due to their speed, low cost, and amenability to automation. Immunoassays exploit antibody-antigen binding to detect a drug class (for example, opiates, benzodiazepines, or amphetamines) rather than a single compound, and are therefore prone to both false positives, arising from cross-reactivity with structurally related compounds, and false negatives, arising from poor cross-reactivity with novel or atypical analogues (Wu, McKay, Broussard, Hoffman & Moyer, 2017). For this reason, immunoassay results are considered presumptive and must be confirmed by an orthogonal chromatographic or mass spectrometric method before being reported as evidentiary findings.

5.2 Chromatographic Separation

Gas chromatography (GC) and high-performance/ultra-high-performance liquid chromatography (HPLC/UHPLC) provide the separation power required to resolve complex mixtures of drugs, metabolites, and endogenous compounds present in biological extracts. GC is particularly well suited to volatile and thermally stable compounds, including ethanol and other volatiles analysed by headspace GC, whereas LC, and particularly UHPLC with sub-2-micron particle columns, has become the preferred platform for polar, thermally labile, and high-molecular-weight analytes, including many contemporary drugs of abuse and their conjugated metabolites (Maurer, 2010).

5.3 Mass Spectrometry

Mass spectrometry (MS) provides structural identification based on the mass-to-charge ratio of ionised analytes and their fragmentation patterns, and its coupling with chromatographic separation (GC-MS and LC-MS/MS) constitutes the analytical backbone of contemporary confirmatory forensic toxicology. Triple-quadrupole LC-MS/MS operating in multiple reaction monitoring (MRM) mode offers exceptional sensitivity and specificity for targeted panels of known substances and is widely regarded as the current gold standard for confirmatory quantitation (Peters, 2011). High-resolution mass spectrometry (HRMS), including quadrupole time-of-flight (Q-TOF) and Orbitrap platforms, has become increasingly important because it enables retrospective, non-targeted data mining: a single acquisition can be re-interrogated against updated compound libraries as new substances, particularly novel psychoactive substances, emerge, without requiring re-analysis of the original specimen (Wu et al., 2017; Meyer & Maurer, 2016).

5.4 Emerging and Complementary Technologies

A range of emerging technologies are progressively being integrated into forensic toxicological workflows. Ambient ionisation mass spectrometry techniques, such as desorption electrospray ionisation (DESI) and direct analysis in real time (DART), allow near-instantaneous analysis of specimens with minimal sample preparation and are of growing interest for rapid triage in high-throughput or field settings (Jones & Kramer, 2020). Portable and handheld spectroscopic devices, including infrared and Raman spectroscopy units, are increasingly deployed by law enforcement for on-scene presumptive identification of seized substances, although their results require laboratory confirmation. Biosensor-based platforms and lab-on-a-chip microfluidic devices are also under active development, with the aim of providing rapid, low-cost, and decentralised toxicological screening, particularly relevant to roadside drug testing (Vindenes et al., 2012).

5.0 Sample Preparation and Extraction

Prior to instrumental analysis, biological specimens must undergo extraction and clean-up to isolate target analytes from the complex biological matrix and to remove interfering endogenous compounds such as proteins, lipids, and salts. Liquid-liquid extraction (LLE), in which analytes are partitioned into an immiscible organic solvent under controlled pH conditions, remains widely used for its simplicity and broad applicability, though it is comparatively labour-intensive and can be difficult to automate. Solid-phase extraction (SPE), which uses a sorbent cartridge to selectively retain analytes while interfering matrix components are washed away, offers greater reproducibility, cleaner extracts, and easier automation, and has become the predominant extraction approach in high-throughput forensic laboratories (Maurer, 2010).

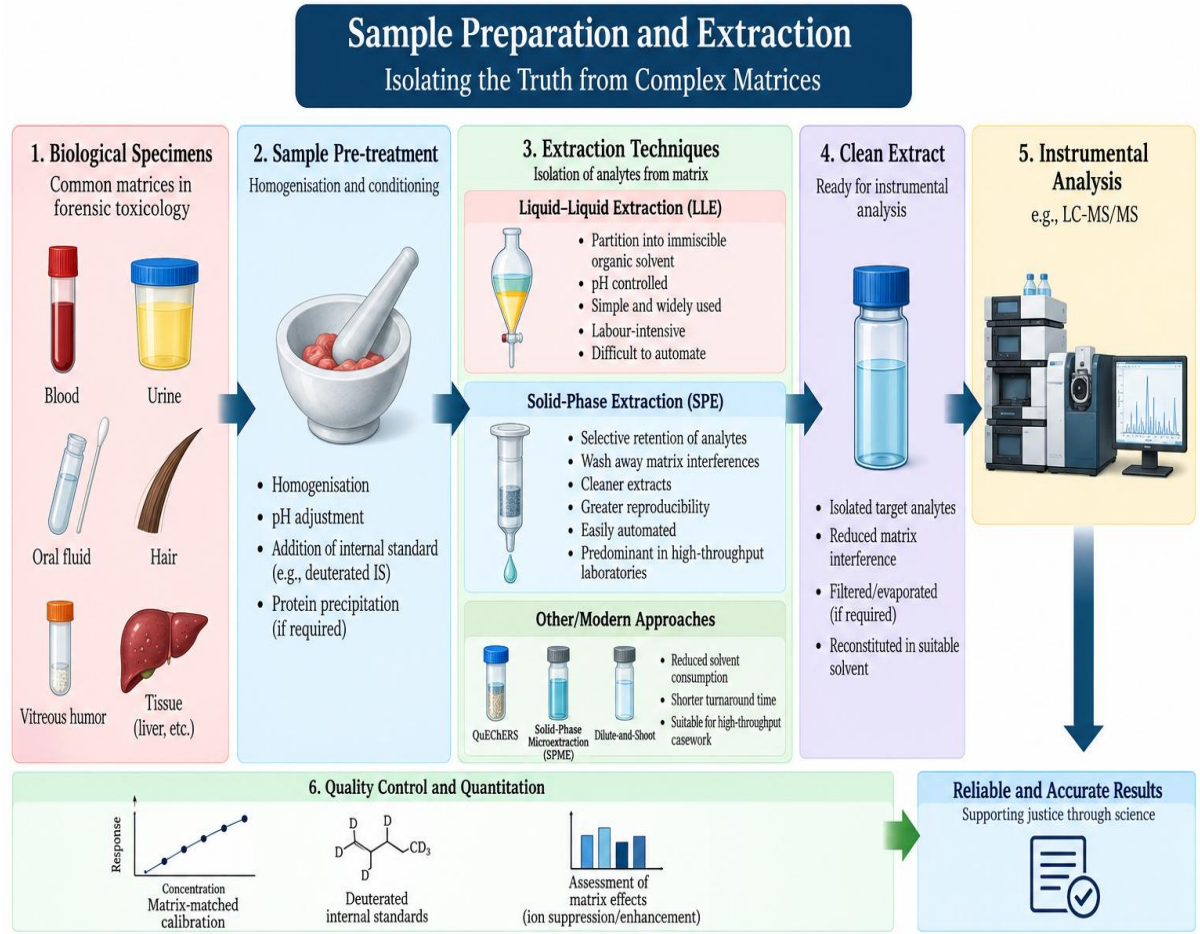

Figure 2: Overview of sample preparation and extraction techniques in forensic toxicology, illustrating biological specimen collection, sample pre-treatment, conventional extraction methods such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE), modern approaches including QuEChERS, solid-phase microextraction (SPME), and dilute-and-shoot, followed by extract clean-up, LC–MS/MS analysis, and quality-control measures for accurate and reproducible quantification

More recently, simplified and miniaturised extraction approaches, including QuEChERS ("quick, easy, cheap, effective, rugged, and safe") methods originally developed for pesticide residue analysis, solid-phase microextraction (SPME), and "dilute-and-shoot" protocols enabled by the high sensitivity of modern LC-MS/MS instrumentation, have gained popularity for their reduced solvent consumption, shorter turnaround times, and suitability for high-throughput casework. Regardless of the extraction approach selected, appropriate use of matrix-matched calibration, deuterated internal standards, and assessment of matrix effects (ion suppression or enhancement) is essential to ensure accurate and reproducible quantitation, particularly for LC-MS/MS methods, which are especially susceptible to matrix-related signal interference (Peters, 2011).

5.5 Comparative Overview of Core Analytical Platforms

Table 1 summarises the principal analytical platforms used in contemporary forensic toxicology, together with their characteristic applications, strengths, and limitations. No single platform is universally optimal; rather, laboratories typically deploy a combination of these technologies in a tiered screening-to-confirmation workflow tailored to the case type and the substances of interest.

Technique	Primary Use	Strength	Limitation
Immunoassay (ELISA/EMIT)	First-line screening	Rapid, low-cost, automatable	Class-level detection; cross-reactivity
GC-MS	Confirmation of volatile/stable compounds	Robust, extensive libraries	Limited for polar/thermolabile drugs
LC-MS/MS (triple-quad)	Targeted quantitative confirmation	High sensitivity/specificity	Requires known target compound list
HRMS (Q-TOF/Orbitrap)	Non-targeted/NPS screening	Retrospective data mining	High cost; complex data review
ICP-MS	Trace metal/element quantitation	Extremely low detection limits	Metals/elements only
Headspace GC-FID	Volatiles (ethanol) quantitation	Validated, legally robust	Limited to volatile compounds

Table 1. Comparative summary of principal analytical platforms used in forensic toxicology laboratories.

6. Postmortem Toxicology and Interpretive Challenges

Postmortem toxicology seeks to establish whether a drug, poison, or alcohol played a causative or contributory role in death. This determination is rarely straightforward, and interpretation must account for several phenomena unique to the postmortem state.

6.1 Postmortem Redistribution

Postmortem redistribution (PMR) refers to the diffusion of drugs from tissues of high concentration, such as the lungs, liver, and myocardium, into adjacent blood vessels following death, which can cause central blood concentrations to markedly exceed those present at the time of death (Pounder & Jones, 1990). This phenomenon is particularly pronounced for basic, lipophilic drugs with large volumes of distribution, such as many antidepressants and opioids, and can lead to substantial over-estimation of the antemortem drug concentration if central rather than peripheral blood is analysed. Awareness of PMR has led forensic pathologists to prioritise femoral blood sampling and to interpret central-to-peripheral concentration ratios as an indicator of redistribution artefact (Drummer, 2007).

6.2 Postmortem Drug Redistribution from Decomposition

Putrefaction introduces additional interpretive complexity, including microbial production of ethanol (neoformation), degradation of certain drugs such as cocaine and some benzodiazepines, and diffusion of gastric drug content into surrounding tissues. In decomposed remains, alternative specimens such as vitreous humor, bone marrow, or muscle tissue may be necessary where blood is unavailable or unreliable (Pounder & Jones, 1990).

6.3 Interpreting Drug Concentrations

Even when a reliable peripheral blood concentration is obtained, interpretation must consider tolerance, polydrug interactions, pre-existing disease, and the wide inter-individual variability in the concentration-effect relationship. Published "therapeutic" and "toxic" reference ranges provide useful context but cannot be applied mechanically to an individual case; forensic toxicologists must integrate case history, scene findings, and autopsy results with the analytical data to reach a defensible interpretation (Drummer, 2007; Levine, 2013).

6.4 The Multidisciplinary Death Investigation Team

Accurate interpretation of postmortem toxicological findings rarely rests with the toxicologist alone. Effective death investigation depends on close collaboration between the forensic pathologist, who correlates autopsy and histopathological findings with the toxicological results; the death investigator or coroner's officer, who documents scene circumstances, medication availability, and witness accounts; and, where relevant, the forensic anthropologist or odontologist in cases involving decomposed or skeletonised remains. This multidisciplinary integration is essential because an identical drug concentration may represent an incidental therapeutic finding in one case and a contributory cause of death in another, depending entirely on the surrounding clinical and circumstantial context (Drummer, 2007; Levine, 2013).

Certifying a death as drug-related additionally requires the toxicologist and pathologist to weigh the plausibility of natural disease processes, mechanical trauma, and asphyxial or positional factors as alternative or contributory causes, since polydrug toxicity frequently coexists with underlying cardiovascular, respiratory, or hepatic disease that may independently predispose an individual to a fatal outcome at a drug concentration that would not otherwise be considered lethal.

6.5 Cause Versus Manner of Death

It is important to distinguish the toxicologist's contribution to establishing the medical cause of death, the specific disease or injury process, such as "combined drug toxicity" or "acute fentanyl intoxication", from the pathologist's or coroner's ultimate determination of the manner of death, the broader legal/administrative classification of a death as natural, accident, suicide, homicide, or undetermined. Toxicological findings inform, but do not by themselves determine, the manner of death; a fatal drug concentration is equally consistent with accidental overdose, deliberate self-administration, or third-party administration, and distinguishing between these possibilities requires integration of toxicological data with scene investigation, psychiatric and medical history, and, where relevant, forensic pathology findings regarding the route and pattern of drug administration (for example, injection site distribution) (Drummer, 2007). This distinction is of direct relevance to criminal investigation, since a finding of homicide, as opposed to accident or suicide, is what triggers a criminal prosecution, and the toxicologist may be called to testify specifically on the scientific plausibility of alternative explanations for a given concentration profile.

7. Novel Psychoactive Substances (NPS)

The proliferation of novel psychoactive substances (NPS), often marketed as "legal highs", "research chemicals", or sold under deliberately misleading branding, represents one of the most significant challenges confronting contemporary forensic toxicology. NPS encompass synthetic cannabinoids, synthetic cathinones, novel synthetic opioids (including fentanyl analogues and nitazenes), phenethylamines, and novel benzodiazepines, among other structural classes, many of which are chemically modified at a rapid pace specifically to circumvent existing legislation and standard toxicological screening panels (Zawilska & Andrzejczak, 2015).

The rapid turnover of NPS compounds means that reference standards, validated methods, and pharmacological/toxicological data often lag substantially behind the appearance of a new substance on the illicit market, creating a persistent "detection gap" for forensic laboratories (Meyer & Maurer, 2016). High-resolution mass spectrometry has become an essential tool in addressing this gap, since its non-targeted, full-scan acquisition mode allows retrospective screening of stored data against continually updated NPS spectral libraries without requiring physical re-analysis of the specimen. International information-sharing initiatives, such as the European Union Early Warning System and national forensic drug intelligence networks, play a complementary role by rapidly disseminating information on newly detected substances to laboratories and law enforcement agencies (Zawilska & Andrzejczak, 2015).

Synthetic opioids, and fentanyl analogues in particular, warrant specific mention given their extreme potency, which is frequently orders of magnitude greater than that of morphine or heroin, and their central role in the ongoing opioid-related overdose crisis observed in North America and, increasingly, other regions. Their potency necessitates analytical methods with very low limits of detection, and their involvement in cases can be easily missed by immunoassay screens not specifically configured to detect them, underscoring the importance of targeted and non-targeted mass spectrometric confirmation in suspected overdose deaths (Peters, 2011).

8. Forensic Toxicology in Drug-Facilitated Crime

Drug-facilitated crimes (DFC), most notably drug-facilitated sexual assault (DFSA) and drug-facilitated robbery, present a distinctive analytical challenge because the substances involved, commonly sedatives such as benzodiazepines, gamma-hydroxybutyrate (GHB), and certain antihistamines, are rapidly metabolised and eliminated from blood and urine, often within a window of 12 to 72 hours, while victims frequently delay reporting or seeking a forensic examination (Negrusz & Gaensslen, 2003).

This narrow detection window has driven increased reliance on hair analysis in DFC investigations, since a single dose of a sedative incorporated into the hair shaft can, in favourable circumstances, be detected weeks to months after the incident, allowing retrospective confirmation of exposure even when blood and urine specimens were not collected in time (Pragst & Balikova, 2006). Nonetheless, hair analysis in the DFC context is technically demanding, requiring extremely sensitive methods capable of detecting single, low-dose exposures against a backdrop of potential external contamination, and results must be interpreted cautiously in conjunction with segmental analysis and case circumstances.

Investigative protocols for suspected DFC cases typically emphasise the earliest possible collection of blood and urine specimens, alongside a delayed hair sample collected several weeks after the alleged incident to allow the relevant hair segment to grow out sufficiently for analysis. Toxicological findings in DFC cases must always be interpreted alongside medical, forensic, and circumstantial evidence, since a negative toxicology result does not exclude drug facilitation given the pharmacokinetic constraints described above (Negrusz & Gaensslen, 2003).

The forensic response to NPS has also driven innovation in seized-drug (as opposed to biological specimen) analysis, since the rapid characterisation of a new substance in seized powders, tablets, or blotter material is often the first step in enabling laboratories to subsequently detect that same substance in biological specimens. Presumptive colour and immunoassay tests are of limited value for most NPS classes, and definitive identification typically depends on a combination of GC-MS, LC-HRMS, and, where available, nuclear magnetic resonance (NMR) spectroscopy to fully elucidate novel chemical structures, particularly positional and stereo-isomers that may fall outside existing controlled substance schedules on technical grounds (Zawilska & Andrzejczak, 2015).

Synthetic cannabinoids illustrate the interpretive difficulty posed by NPS particularly well: unlike delta-9-tetrahydrocannabinol, many synthetic cannabinoid receptor agonists are full, rather than partial, agonists at the cannabinoid CB1 receptor and can produce severe, occasionally fatal, toxicity at low doses, including seizures,

acute kidney injury, and psychosis, despite structural similarity to compounds perceived by users as comparatively benign (Meyer & Maurer, 2016). This divergence between perceived and actual risk underscores the public health, as well as forensic, importance of rapid and accurate NPS identification.

9. Alcohol and Driving Under the Influence Toxicology

Ethanol remains the single most prevalent substance implicated in impaired-driving casework worldwide, and blood alcohol concentration (BAC) analysis, typically performed by headspace gas chromatography with flame ionisation detection, is one of the most extensively validated and legally standardised forensic toxicological measurements (Jones, 2010). Per se legal limits, which define a specific BAC threshold above which driving is a criminal offence irrespective of demonstrable impairment, provide prosecutors with a relatively straightforward evidentiary pathway, although toxicologists may additionally be called upon to perform retrograde extrapolation, estimating an individual's BAC at an earlier time point (such as the time of an accident) based on a later measured concentration and the pharmacokinetics of alcohol elimination, a practice that carries significant scientific caveats and inter-individual variability (Jones, 2010).

Driving under the influence of drugs (DUID) presents a substantially more complex interpretive challenge than alcohol-impaired driving, because, unlike alcohol, there is no straightforward, universally accepted concentration-impairment relationship for most drugs, owing to variability in tolerance, chronic versus acute use, and the pharmacodynamics of the substance in question (Vindenes et al., 2012). Many jurisdictions have therefore adopted "per se" or "zero-tolerance" legislative approaches for certain controlled substances, under which any detectable concentration above an analytical cut-off constitutes an offence, circumventing the need to establish behavioural impairment on a case-by-case basis. Roadside oral fluid testing devices have become an increasingly important investigative tool in DUID enforcement, offering rapid, non-invasive presumptive screening for common drug classes, including cannabinoids, amphetamines, and opioids, at the roadside, with confirmatory laboratory analysis performed subsequently on a corresponding blood or oral fluid specimen (Vindenes et al., 2012).

9.1 Cannabis and the Challenge of a Non-Linear Concentration-Impairment Relationship

Cannabis-impaired driving illustrates the interpretive limitations of per se drug-driving thresholds particularly clearly. Because delta-9-tetrahydrocannabinol (THC) is highly lipophilic and redistributes rapidly from blood into fatty tissue, blood THC concentrations fall precipitously within the first hour or two after inhalation, often well before subjective and behavioural impairment has fully resolved, while chronic, heavy cannabis users may exhibit persistently measurable THC concentrations for days to weeks after last use, long after any acute impairment has subsided (Vindenes et al., 2012). This mismatch between blood concentration and functional impairment has driven ongoing research into alternative approaches, including oral fluid THC testing, which better reflects recent use, and standardised field sobriety and drug recognition expert (DRE) protocols, which assess behavioural and physiological indicators of impairment independent of a specific concentration threshold.

10. Poisoning Investigations and Heavy Metal Analysis

Deliberate or accidental poisoning cases require forensic toxicologists to consider an extremely broad differential of possible agents, ranging from classical inorganic poisons, such as arsenic, thallium, and antimony, to pharmaceuticals, pesticides, carbon monoxide, and naturally occurring toxins. Because the range of possible poisons vastly exceeds what can be covered by any single targeted screening panel, comprehensive or "general unknown" screening strategies, typically combining immunoassay, GC-MS, and increasingly HRMS-based non-targeted approaches, are employed when the causative agent is not suspected on clinical or circumstantial grounds (Maurer, 2010).

10.1 Pesticides and Agrochemicals

Pesticide poisoning, whether accidental, occupational, suicidal, or homicidal, remains a significant cause of death in many agricultural regions globally, with organophosphate and carbamate insecticides of particular forensic importance owing to their mechanism of action as acetylcholinesterase inhibitors, producing a characteristic cholinergic toxidrome. Forensic analysis of suspected pesticide poisoning typically combines measurement of blood or plasma cholinesterase activity, which provides indirect biochemical evidence of exposure, with direct chromatographic identification and quantitation of the parent compound and its metabolites in blood, gastric contents, and, where relevant, the suspected source material (Maurer, 2010). Paraquat and other herbicides with distinct, often severe and delayed, toxic mechanisms require separate, compound-specific analytical approaches and are of continuing forensic relevance in regions where access to these agents remains comparatively unrestricted.

Heavy metal poisoning, though comparatively rare in contemporary forensic casework relative to pharmaceutical and drug-related deaths, remains a recurring feature of high-profile criminal poisoning investigations and requires specialised analytical approaches, most commonly inductively coupled plasma mass spectrometry (ICP-MS), which offers exceptional sensitivity for trace element quantitation in blood, urine, hair, and tissue specimens. Segmental hair analysis is of particular value in chronic heavy metal poisoning, as it can reveal a temporal pattern of exposure corresponding to intermittent or escalating dosing (Pragst & Balikova, 2006). Carbon monoxide poisoning, whether accidental, suicidal, or homicidal, is typically assessed by co-oximetry or spectrophotometric determination of carboxyhaemoglobin saturation in postmortem blood, and remains an important cause of unrecognised poisoning death, particularly in cases involving fire, faulty heating appliances, or enclosed-space suicide.

10.2 Common Drug Classes Encountered in Forensic Casework

Table 3 summarises several of the major pharmacological classes most frequently encountered in forensic toxicological casework, together with representative examples and their principal forensic relevance. This list is illustrative rather than exhaustive, given the very large number of individual compounds encountered in practice.

Drug Class	Representative Examples	Principal Forensic Relevance
Opioids/opiates	Morphine, heroin, fentanyl, oxycodone	Overdose death; DUID; poisoning
Benzodiazepines	Diazepam, alprazolam, flunitrazepam	DFC; polydrug toxicity; DUID
Stimulants	Cocaine, amphetamine, methamphetamine	DUID; sudden cardiac death; abuse
Cannabinoids	THC; synthetic cannabinoid agonists	DUID; NPS toxicity
Antidepressants/antipsychotics	Amitriptyline, quetiapine	Overdose; postmortem redistribution
Volatiles	Ethanol, methanol, toluene	DUID; occupational exposure; poisoning
Metals/elements	Arsenic, thallium, lead	Chronic/acute homicidal poisoning

Table 3. Major pharmacological classes commonly encountered in forensic toxicology casework.

11. Pharmacogenomic and Molecular Advances

An increasing body of research has highlighted the role of genetic polymorphisms in cytochrome P450 (CYP) enzymes and other drug-metabolising proteins in explaining unexpectedly high or low postmortem drug concentrations, particularly in cases involving opioids and psychotropic medications metabolised via CYP2D6 and CYP2C19 (Jannetto & Bratanow, 2014). Poor metabolisers may accumulate a parent drug to toxic concentrations at therapeutic dosing, while ultra-rapid metabolisers may convert a prodrug, such as codeine, into its active metabolite at an accelerated rate, occasionally with fatal consequences. Incorporating pharmacogenomic testing into the postmortem toxicological work-up, where genetic material is available, offers forensic toxicologists an additional line of evidence to explain atypical concentration findings and to distinguish a genuinely toxic exposure from one that reflects an individual's inherent metabolic phenotype (Jannetto & Bratanow, 2014).

Beyond pharmacogenomics, molecular and "-omics" based approaches, including metabolomics and proteomics, are being explored as complementary tools for estimating the postmortem interval, distinguishing antemortem from postmortem drug administration, and identifying novel biomarkers of specific intoxications, though these approaches remain predominantly at the research stage and have not yet been widely adopted into routine forensic casework (Meyer & Maurer, 2016).

Practical implementation of pharmacogenomic testing in forensic laboratories remains limited by cost, turnaround time, and, critically, by the current paucity of large, well-characterised postmortem datasets correlating genotype with observed drug concentrations across diverse case populations, which constrains the statistical confidence with which a genetic finding can be invoked to explain an individual case. Professional guidance in this area continues to evolve, and most laboratories currently reserve pharmacogenomic testing for cases in which an unusual or unexplained concentration finding cannot be adequately accounted for by case history, co-administered substances, or known postmortem artefact alone (Jannetto & Bratanow, 2014).

12. Advances in Alternative Matrix Analysis

Continuing refinement of hair, oral fluid, and dried blood spot (DBS) analytical methods has expanded the practical utility of alternative matrices in forensic casework. Micro-sampling approaches, such as DBS, require only a few microlitres of blood obtained from a simple finger-prick, offer advantages in specimen stability, storage, and transport, and are of particular interest in resource-limited settings and in cases where conventional venous blood collection is impractical (Wu et al., 2017). Improvements in extraction efficiency and instrument sensitivity have also lowered detection limits for hair analysis to the point where single, low-dose drug exposures, of direct relevance to DFC investigations, can increasingly be reliably distinguished from environmental contamination, although this remains an area of active methodological debate, particularly with respect to decontamination protocols and the interpretation of external versus incorporated drug residues (Pragst & Balikova, 2006).

13. Applications in Criminal Investigation

Forensic toxicology contributes to criminal investigation across a wide spectrum of case types, and the toxicologist's findings frequently serve as pivotal, sometimes case-determining, evidence.

13.1 Homicide and Suspicious Death Investigations

In deaths where the cause is not immediately apparent, or where a homicide by poisoning is suspected, toxicological analysis can establish the presence of a lethal or contributory substance, assist in distinguishing homicide from suicide or accident based on the substance and route of administration identified, and support the reconstruction of the circumstances and approximate timing of intoxication (Drummer, 2007).

13.2 Sexual Assault Investigations

As discussed in Section 8, toxicological evidence of sedative or incapacitating substance exposure can corroborate a victim's account in drug-facilitated sexual assault investigations, while also assisting in evaluating a suspect's claims regarding a complainant's voluntary intoxication.

13.3 Impaired Driving and Vehicular Homicide

Toxicological confirmation of alcohol or drug impairment underpins prosecutions for driving under the influence and, in cases resulting in death or serious injury, vehicular homicide or aggravated dangerous driving charges.

13.4 Child Abuse and Neglect

Toxicological analysis, including hair and meconium testing, can reveal evidence of deliberate poisoning, medication misuse, or environmental exposure to illicit substances in cases of suspected child abuse or neglect, providing objective corroboration in circumstances where the child is unable to provide a reliable history.

13.5 Mass Fatality and Public Health Toxicological Incidents

Forensic toxicology laboratories also play a critical role in mass fatality events with a suspected toxicological component, including outbreaks of contaminated or adulterated illicit drug supplies, mass poisoning incidents involving food or beverage contamination, and chemical or industrial disasters. In such incidents, rapid, coordinated toxicological screening across multiple decedents or affected individuals can identify a common causative agent, informing both the forensic determination of cause of death and the public health response, including product recalls, public warnings, and epidemiological investigation of the source and distribution of the contaminated substance. The recent proliferation of highly potent synthetic opioids within illicit drug supplies has made this surveillance function increasingly important, with several jurisdictions establishing rapid toxicological alert systems that flag clusters of overdose deaths sharing a common, particularly dangerous, adulterant (Peters, 2011; Zawilska & Andrzejczak, 2015).

13.6 Doping and Sports-Related Investigations

Although governed by a somewhat distinct regulatory framework, anti-doping forensic toxicology shares substantial methodological overlap with criminal forensic toxicology, relying on similarly sensitive LC-MS/MS and HRMS platforms to detect prohibited performance-enhancing substances and their metabolites in athlete specimens.

13.7 Illustrative Case Vignettes

The following composite vignettes, constructed from patterns commonly described in the forensic toxicology literature, illustrate how the principles discussed above are applied in practice. They are illustrative in nature and do not describe specific real individuals or investigations.

Vignette 1 – Unexplained Sudden Death: An apparently healthy adult is found deceased at home with no obvious signs of trauma. Autopsy reveals no definitive anatomical cause of death. Peripheral blood toxicology identifies a combination of a sedating antihistamine and an opioid analgesic at concentrations individually within a range reported in therapeutic use, but jointly capable of producing additive central nervous system and respiratory depression. Integration of the toxicological findings with the autopsy findings and scene investigation supports a certification of death due to combined drug toxicity, illustrating the importance of considering polydrug interaction effects rather than assessing each substance in isolation.

Vignette 2 – Suspected Drug-Facilitated Assault: A complainant reports a sexual assault approximately 36 hours after the alleged incident, describing a period of amnesia inconsistent with the reported quantity of alcohol consumed. Blood and urine specimens collected at presentation are negative for common sedatives, consistent with the rapid elimination of many drugs used in drug-facilitated assault. A hair sample collected six weeks later, once sufficient growth has occurred, is analysed by segmental LC-MS/MS and identifies a single low-level exposure to a short-acting benzodiazepine in the segment corresponding to the approximate time of the alleged incident, providing corroborating toxicological evidence despite the initial negative blood and urine results.

Vignette 3 – Roadside Drug-Impaired Driving: A driver involved in a single-vehicle collision exhibits signs of impairment inconsistent with a negative roadside breath alcohol result. An oral fluid screening device returns a presumptive positive for a synthetic cannabinoid. Confirmatory LC-HRMS analysis of a subsequently obtained blood specimen identifies a specific synthetic cannabinoid metabolite not included in the laboratory's standard targeted panel, which is only recognised through retrospective interrogation of the non-targeted acquisition against an updated NPS spectral library, illustrating the practical value of high-resolution, non-targeted screening in cases involving emerging substances.

14. Quality Assurance, Method Validation, and Legal Admissibility

The evidentiary weight of forensic toxicological findings depends fundamentally on the scientific rigor of the underlying analytical process. Laboratory accreditation to international standards such as ISO/IEC 17025, together with adherence to discipline-specific guidelines published by bodies such as SOFT/AAFS and TIAFT, establishes the minimum quality framework within which forensic toxicology laboratories are expected to operate (Jenkins, 2008).

Method validation, encompassing assessment of accuracy, precision, specificity, sensitivity (limit of detection and limit of quantitation), linearity, recovery, matrix effects, and stability, is a prerequisite for the deployment of any analytical method in casework, and validation data must be documented and available for review by opposing experts and the court. Chain-of-custody documentation, from specimen collection through to final reporting, must be maintained without gaps to preserve the integrity and admissibility of the evidence (Jenkins, 2008).

In many common-law jurisdictions, the admissibility of forensic toxicological expert testimony is governed by standards such as the Daubert standard in the United States, which requires that the underlying scientific methodology be testable, subject to peer review and publication, possess a known or potential error rate, and be generally accepted within the relevant scientific community (or, in some jurisdictions, the older Frye "general acceptance" standard). Forensic toxicologists serving as expert witnesses must therefore be prepared to articulate not only their findings but also the scientific validity and limitations of the methods used to obtain them, and to clearly distinguish established, well-validated interpretive principles from areas of ongoing scientific uncertainty (Levine, 2013).

14.1 Proficiency Testing and Peer Verification

In addition to initial method validation, accredited forensic toxicology laboratories are required to participate in regular external proficiency testing programmes, in which blind specimens of known composition are analysed alongside routine casework to verify ongoing analytical accuracy. Internal quality control measures, including the analysis of calibrators, blank specimens, and quality control samples within each analytical batch, provide a further layer of assurance that instrument performance remains within validated parameters. Many laboratories additionally employ a peer-review or technical review process, in which a second qualified toxicologist independently reviews raw data, calculations, and interpretive conclusions prior to the release of a final report, reducing the risk of transcription, calculation, or interpretive error reaching the courtroom (Jenkins, 2008).

14.2 Expert Testimony and Communication of Uncertainty

Effective expert testimony requires forensic toxicologists to communicate complex analytical and pharmacological concepts in a manner accessible to a lay jury while remaining scientifically precise. This includes clearly distinguishing quantitative certainty, such as the analytically determined concentration of a substance, from the considerably greater uncertainty inherent in extrapolating that concentration to a specific functional outcome, such as impairment or a precise time of ingestion. Overstatement of the certainty of an interpretive conclusion, for example presenting retrograde extrapolation of blood alcohol concentration as a precise value rather than a range with defined assumptions, has been identified as a recurring source of miscarriage-of-justice risk and is an area of active concern for professional bodies overseeing forensic toxicological practice (Jones, 2010; Levine, 2013).

15. Current Challenges and Limitations

- Interpretive uncertainty: the absence of universally applicable concentration-effect relationships for most non-alcohol substances complicates the translation of an analytical finding into a statement about impairment or cause of death.
- Postmortem redistribution and specimen artefact: variability in specimen site, collection timing, and decomposition state continues to challenge the reliability of postmortem drug concentration interpretation.
- The NPS detection gap: the continuous emergence of new synthetic substances outpaces the availability of reference standards, validated methods, and toxicological data.
- Resource and capacity constraints: many forensic laboratories, particularly in low- and middle-income countries, lack access to advanced instrumentation such as LC-MS/MS or HRMS, creating global disparities in forensic toxicological capability.
- Polydrug use and interaction effects: the increasing prevalence of polysubstance use complicates attribution of a specific cause of impairment or death to a single agent.
- Standardisation and harmonisation: variability between jurisdictions in cut-off concentrations, legal thresholds, and reporting practices can complicate cross-jurisdictional comparison and international casework.

Several of these challenges intersect in ways that compound their overall impact on forensic practice. For example, the combination of resource constraints and the NPS detection gap is particularly acute in smaller or under-resourced laboratories, which may lack both the instrumentation and the informatics infrastructure needed to perform retrospective non-targeted data review, meaning that cases involving an undetected novel substance may be closed without the causative agent ever being identified, with downstream consequences for both individual case outcomes and broader public health surveillance of emerging drug threats.

Addressing these challenges will require sustained investment in laboratory capacity, continued research into the pharmacokinetics and pharmacodynamics of emerging substances, and closer international collaboration in data-sharing and method standardisation (Meyer & Maurer, 2016; Zawilska & Andrzejczak, 2015).

15.5 Global Perspectives and Capacity Building

The distribution of forensic toxicological capability is markedly uneven across the globe. High-income countries typically maintain accredited laboratories equipped with LC-MS/MS and, increasingly, HRMS platforms, supported by well-established professional societies, certification pathways, and legal frameworks governing the admissibility of forensic evidence. By contrast, many low- and middle-income countries continue to rely predominantly on immunoassay screening and basic chromatographic methods, with limited access to reference standards, confirmatory mass spectrometric instrumentation, or the technical expertise required to interpret complex casework, constraining both the range of substances that can be reliably detected and the overall throughput of casework (Jenkins, 2008).

International organisations, including the United Nations Office on Drugs and Crime (UNODC) and TIAFT, have supported capacity-building initiatives aimed at narrowing this gap through training programmes, proficiency testing schemes open to laboratories in resource-limited settings, and the promotion of harmonised minimum

standards for forensic toxicological practice. Regional forensic science networks have similarly played an important role in facilitating knowledge exchange, shared reference libraries, and collaborative validation studies, helping to extend advanced analytical capability beyond the small number of laboratories that would otherwise possess it in isolation. Continued progress in this area is likely to be essential not only for equitable access to justice within affected countries, but also for effective international collaboration in transnational drug trafficking and organised crime investigations, which depend on broadly comparable toxicological evidentiary standards across jurisdictions.

15.6 Ethical and Privacy Considerations

Forensic toxicological testing raises a number of ethical considerations that merit explicit acknowledgement. Biological specimens obtained for toxicological analysis, particularly hair, urine, and blood, can incidentally reveal highly sensitive personal information extending well beyond the specific substances relevant to the investigation at hand, including evidence of pregnancy, chronic illness, or unrelated lawful medication use, raising questions about the appropriate scope of testing and the handling and eventual disposition of residual specimens and data (Wu et al., 2017).

In the specific context of drug-facilitated crime investigations, careful, trauma-informed communication with complainants regarding the limitations of toxicological testing, including the realistic likelihood of a negative result given typical reporting delays, is an important complement to the analytical process, since an unexplained negative toxicology result can otherwise be misinterpreted, by investigators, the courts, or the complainant, as evidence that an assault did not occur (Negrusz & Gaensslen, 2003). More broadly, forensic toxicologists have a professional and ethical obligation to report findings objectively and within the bounds of established scientific validity, resisting pressure, whether from prosecution or defence, to overstate the certainty or specificity of an interpretive conclusion beyond what the underlying science supports (Levine, 2013).

16. Future Directions

Several converging trends are expected to shape the future trajectory of forensic toxicology. The continued adoption of high-resolution and non-targeted mass spectrometric workflows is likely to further reduce the detection gap for novel psychoactive substances by enabling retrospective re-interrogation of stored data as new compounds are characterised (Meyer & Maurer, 2016). Artificial intelligence and machine learning approaches are increasingly being explored for automated spectral interpretation, pattern recognition across large toxicological datasets, and predictive modelling of drug interactions and postmortem redistribution, offering the potential to improve both the speed and consistency of data interpretation, although rigorous validation and transparency will be essential before such tools can be relied upon in an evidentiary context.

Point-of-care and portable analytical devices, including handheld mass spectrometers and biosensor platforms, are expected to see expanded deployment in field and roadside settings, complementing rather than replacing laboratory-based confirmatory analysis. Advances in micro-sampling technologies, such as dried blood spots and microneedle-based sampling, may further improve specimen stability and reduce the logistical burden of specimen transport, particularly in remote or resource-limited settings. Finally, the continued integration of pharmacogenomic and "-omics" based approaches promises to enhance the interpretive precision of postmortem toxicology, helping to explain atypical findings and to individualise the interpretation of drug concentrations in a manner not previously possible (Jannetto & Bratanow, 2014).

16.2 Recommendations for Practice and Policy

Based on the foregoing review, several practical recommendations can be advanced for forensic toxicology laboratories, death investigation systems, and policymakers seeking to strengthen the discipline's contribution to criminal investigation:

- Prioritise the acquisition and maintenance of LC-MS/MS and, where feasible, HRMS capability, recognising these platforms as the current standard for reliable confirmatory identification, particularly for novel and emerging substances.
- Institute or strengthen participation in external proficiency testing and laboratory accreditation schemes as a precondition for the acceptance of toxicological evidence in criminal proceedings.
- Adopt standardised protocols for specimen collection in postmortem cases, prioritising peripheral (femoral) blood collection and retention of alternative matrices such as vitreous humor to support robust interpretation.
- Develop or participate in rapid information-sharing networks for novel psychoactive substances to reduce the detection gap between the emergence of a new compound and the availability of validated reference methods.
- Ensure victim-centred, trauma-informed protocols for the collection of specimens in suspected drug-facilitated crime cases, including timely blood/urine collection and appropriately delayed hair sampling.
- Invest in training and continuing education for forensic toxicologists in the communication of scientific uncertainty, to support accurate and appropriately caveated expert testimony.
- Support international capacity-building initiatives to reduce disparities in forensic toxicological capability between jurisdictions, particularly in low- and middle-income countries.

16.4.1 Toward a Learning Forensic Toxicology System

A recurring theme throughout this review is that forensic toxicology functions most effectively not as an isolated analytical service but as part of a broader, adaptive "learning" system, one in which findings from individual cases are aggregated, analysed, and fed back into public health surveillance, drug policy, and laboratory practice. Jurisdictions that have invested in linking forensic toxicological data with public health and law enforcement

information systems have generally proven more able to detect and respond rapidly to emerging drug threats, such as a sudden cluster of deaths associated with a newly circulating fentanyl analogue or an adulterated drug batch, than those in which toxicological data remains siloed within individual case files. Strengthening these feedback loops, through improved laboratory informatics, standardised data coding, and formal data-sharing agreements between forensic laboratories, public health authorities, and law enforcement agencies, represents an important, and currently under-realised, opportunity to enhance the societal value of forensic toxicological practice beyond its traditional case-by-case evidentiary role.

16.3 Abbreviations

Abbreviation	Meaning
BAC	Blood Alcohol Concentration
DBS	Dried Blood Spot
DFC / DFSA	Drug-Facilitated Crime / Drug-Facilitated Sexual Assault
DUID	Driving Under the Influence of Drugs
ELISA / EMIT	Enzyme-Linked Immunosorbent Assay / Enzyme Multiplied Immunoassay Technique
GC-MS	Gas Chromatography-Mass Spectrometry
HRMS	High-Resolution Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
NPS	Novel Psychoactive Substance(s)
PMR	Postmortem Redistribution
SPE / SPME	Solid-Phase Extraction / Solid-Phase Microextraction
THC	Delta-9-Tetrahydrocannabinol
TIAFT / SOFT	The International Association of Forensic Toxicologists / Society of Forensic Toxicologists

16.1 Scope and Limitations of This Review

As a narrative rather than systematic review, this article synthesises broad thematic trends drawn from the forensic toxicology literature rather than applying a formal, pre-registered search and inclusion methodology; readers seeking a comprehensive, exhaustively catalogued account of any single sub-topic, such as the pharmacokinetics of a specific substance class, are directed to the specialised primary and reference literature cited throughout. Jurisdiction-specific legal thresholds, scheduling of controlled substances, and admissibility standards vary considerably around the world and are described here only in general terms; practitioners should consult the specific statutory and regulatory framework applicable to their own jurisdiction for case-specific guidance.

16.4 Epidemiological Context

The demand placed upon forensic toxicology services is closely tied to broader epidemiological trends in substance use and drug-related mortality. Many jurisdictions have reported substantial increases in drug-related deaths over the past two decades, driven in large part by the proliferation of potent synthetic opioids within illicit drug supplies, alongside continuing, and in some regions rising, mortality associated with stimulants, polysubstance combinations, and, in certain populations, alcohol-related disease and injury. These trends have placed sustained pressure on forensic toxicology laboratories and death investigation systems, contributing to casework backlogs in some jurisdictions and reinforcing the case for continued investment in analytical capacity, staffing, and informatics infrastructure capable of supporting both individual case interpretation and aggregate public health surveillance (Peters, 2011; Zawilska & Andrzejczak, 2015). Robust, timely forensic toxicological data also underpins broader drug policy and public health responses, including harm reduction programming, drug-checking services, and targeted public health alerts, illustrating the discipline's relevance well beyond the individual criminal case.

17. CONCLUSION

Forensic toxicology has undergone remarkable transformation since its nineteenth-century origins in classical wet chemistry, evolving into a highly sophisticated discipline underpinned by advanced chromatographic and mass spectrometric technologies. Its contributions to criminal investigation are broad and consequential, spanning postmortem death investigation, impaired-driving enforcement, drug-facilitated crime, and poisoning cases, and

its findings frequently constitute pivotal evidence in criminal proceedings. Nonetheless, the discipline continues to face substantial challenges, including the interpretive complexities of postmortem redistribution, the rapid proliferation of novel psychoactive substances, and persistent disparities in laboratory capacity between jurisdictions. Continued investment in analytical innovation, rigorous method validation and quality assurance, international collaboration and data-sharing, and interdisciplinary engagement between toxicologists, pathologists, and the legal system will be essential to ensure that forensic toxicology continues to provide accurate, reliable, and legally defensible evidence in support of criminal justice.

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