

Biochemical Markers of Drug Abuse in Athletics

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Abstract

Drug abuse in athletics continues to threaten the integrity of competitive sports and the wellbeing of athletes worldwide. The development and validation of biochemical markers have revolutionized anti-doping science, offering objective, quantifiable indicators of illicit substance use that transcend traditional testing limitations. This review provides a comprehensive synthesis of current knowledge on biochemical markers associated with major classes of performance-enhancing drugs, including anabolic steroids, stimulants, peptide hormones, and erythropoietic agents. Emphasis is placed on the mechanistic basis of these markers, their metabolic and molecular signatures, and the analytical technologies such as high-resolution mass spectrometry, immunoassays, and omics-based approaches that enable their precise detection. The review further explores the evolution of the Athlete Biological Passport (ABP) as an individualized, longitudinal monitoring framework that integrates hematological, steroidal, and emerging molecular biomarkers to enhance detection sensitivity. Ethical, regulatory, and practical considerations surrounding biomarker application are discussed, highlighting the delicate balance between athlete privacy and the pursuit of fair competition. Emerging frontiers, including epigenetic, microRNA, and metabolomic signatures, are presented as promising avenues for next-generation doping surveillance. By consolidating recent advances and identifying future directions, this review underscores the pivotal role of biochemical markers in transforming doping control from reactive testing to predictive, evidence-based monitoring, thereby preserving the spirit and credibility of modern athletics.

Keywords: Drug abuse, Doping control, Biochemical markers, Athletics, Performance-enhancing drugs, and Anti-doping testing.

INTRODUCTION

The misuse of performance enhancing substances remains a persistent challenge within elite sport, eroding competitive integrity and compromising athlete health. Despite extensive anti-doping frameworks and globally implemented testing programmes, athletes continue to exploit anabolic-androgenic steroids, erythropoiesis-stimulating agents (ESAs), designer peptides and gene-related interventions (WADA, 2023; Khalil et al., 2024). These practices impose significant physiological and psychological burdens on athletes, including endocrine disruption, cardiovascular complications and long-term health risks (Khalil et al., 2024). Moreover, the evolution

of doping methodologies such as micro-dosing, synthetic designer molecules with minimal detection windows and adept use of masking agents—has heightened the demands placed on scientific detection and monitoring systems (WADA, 2023; Dragčević et al., 2024).

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In response to these challenges, biochemical markers have emerged as foundational elements in modern anti-doping science. Biochemical markers, defined as quantifiable molecular or cellular changes induced by drug exposure or physiological manipulation offer an indirect avenue for detecting illicit substance use (Dragčević et al., 2024; USADA, 2025). Traditional detection methods, which target the prohibited substance itself in blood or urine, are increasingly limited by shortened detection windows and the rapid evolution of doping agents (Khalil et al., 2024; WADA, 2024). By contrast, biomarker-based strategies capture downstream physiological or metabolic effects such as alterations in hormone ratios, metabolic fingerprints or gene expression changes providing a more persistent signal of doping behaviour (WADA, 2023; Dragčević et al., 2024). Technological breakthroughs including high-resolution mass spectrometry (HRMS), liquid-chromatography tandem mass spectrometry (LC-MS/MS) and comprehensive metabolomic profiling have significantly expanded laboratory detection capabilities and enabled deeper profiling of physiological perturbation (Dragčević et al., 2024; Khalil et al., 2024).

A key operational advance in the anti-doping domain is the implementation of the Athlete Biological Passport (ABP) framework, which monitors selected biomarkers longitudinally for each athlete to identify atypical patterns suggestive of doping rather than relying solely on substance detection. According to the World Anti-Doping Agency (WADA), the ABP tracks biological variables “over time that indirectly reveal the effects of doping” (WADA, 2023). The ABP consists primarily of two modules: the haematological module, which monitors parameters such as haemoglobin concentration, reticulocyte percentage and the OFF-score; and the steroidal module, which tracks endogenous steroid ratios to detect potential exogenous anabolic use (Zorzoli, 2011; USADA, 2025). However, the interpretation of ABP data presents significant complexities—confounding variables such as altitude exposure, dehydration, training load, illness and ethnicity may influence biomarker values and must be rigorously accounted for to avoid misclassification (WADA, 2023; Dragčević et al., 2024). Such challenges underscore the necessity for continual refinement of statistical models and monitoring protocols.

Simultaneously, the field of anti-doping biomarker research is advancing into novel molecular and omics-based territories. Emerging biomarkers such as circulating microRNAs (miRNAs), exosome-derived molecular cargo, epigenetic modifications and transcriptomic alterations are being investigated for their potential to extend detection windows, heighten sensitivity and capture

subtler physiological footprints of doping interventions (Loria et al., 2024; Oliveira et al., 2024). For example, circulating miRNAs have demonstrated stable presence in body fluids, making them promising candidates for non-invasive monitoring; in one study, miRNAs exhibited altered expression following human growth hormone (rhGH) administration (Leuenberger et al., 2010; Loria et al., 2024). Meanwhile, advanced analytical and statistical approaches including machine learning algorithms and multivariate biomarker profiling are increasingly applied to athlete longitudinal data, enhancing the detection of atypical patterns and reducing false positive risk (Eleftheriou et al., 2023; Oliveira et al., 2024). These developments represent a shift toward systems-biology frameworks in anti-doping, where multi-marker panels and integrated longitudinal analytics supersede single-analyte strategies.

The purpose of this review is to provide a comprehensive synthesis of current evidence concerning biochemical markers of drug abuse in athletics, articulating the mechanistic bases, analytical technologies and monitoring frameworks that underpin this evolving field. It examines established marker classes aligned with major doping categories including anabolic and ergogenic agents, stimulants, peptide hormones and erythropoietic compounds and elucidates their downstream metabolic, hormonal and molecular signatures. It evaluates the advanced analytical platforms and methodologies that enhance detection precision, with particular emphasis on high-resolution mass spectrometry, immunoassays and omics-based profiling. In addition, it critically explores the evolution, implementation and limitations of the Athlete Biological Passport, highlighting confounding variables, statistical interpretation challenges and opportunities for enhancement. Furthermore, the review delves into emerging frontiers in biomarker science, including epigenetic, microRNA and metabolomic signatures, and considers pathways toward integration of multi-marker, predictive monitoring systems. Ethical, regulatory and methodological challenges such as validation across diverse athlete populations, standardisation of pre-analytical workflows, intra- and inter-individual biological variability, and data-protection concerns are also addressed in depth. By consolidating empirical evidence, methodological advances and conceptual progress, this review seeks to offer researchers, sports scientists and anti-doping practitioners an evidence-based roadmap that supports the transition from reactive substance-based testing to holistic, biomarker-driven monitoring systems thereby enhancing athlete protection, upholding the integrity of sport and promoting clean competition.

CATEGORIES OF DRUG ABUSED IN ATHLETICS

The misuse of performance-enhancing substances in sports encompasses a complex spectrum of pharmacological agents, each targeting specific physiological systems to

augment strength, endurance, recovery, or focus. These substances are systematically classified by the World Anti-Doping Agency (WADA) under its annual List of Prohibited Substances and Methods (World Anti-Doping Agency, 2025).

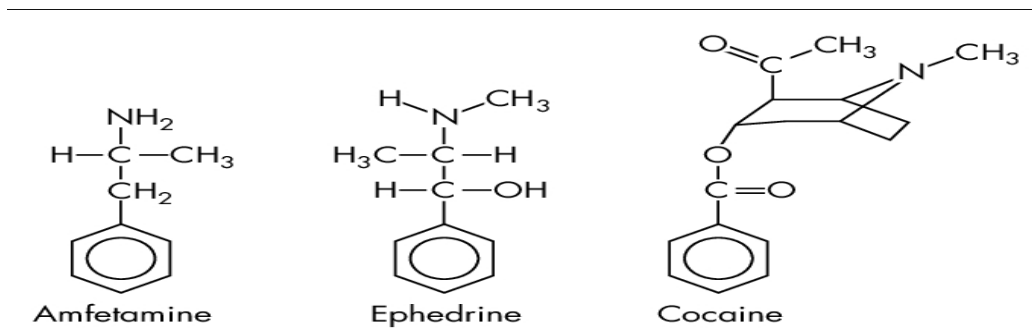


Figure 1. Chemical structures of amphetamine, ephedrine, and cocaine

Source: Avois et al. (2006)

1. Stimulants (Amphetamines, Ephedrine, Cocaine, etc.)

Stimulants such as amphetamines, ephedrine, and cocaine act on the central nervous system to heighten alertness, reduce fatigue, and enhance concentration. These effects result from increased catecholamine activity and are often sought to improve reaction time and endurance (Lu et al., 2023). However, misuse of stimulants can lead to cardiovascular strain, dependency, and psychological disturbances (Oliveira et al., 2024). Their accessibility and

rapid physiological impact have contributed to recurrent abuse in both professional and amateur sports.

2. Anabolic Agents (Anabolic-Androgenic Steroids, Selective Androgen Receptor Modulators)

Anabolic agents, including anabolic-androgenic steroids (AAS) and selective androgen receptor modulators (SARMs), are among the most frequently abused drugs in sports. They promote muscle hypertrophy and reduce recovery time, enabling greater training intensity (Bond

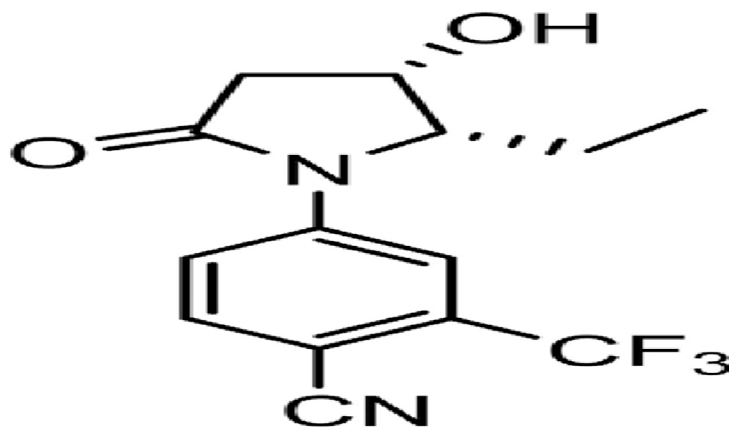


Figure 2. Chemical structure of selective androgen receptor modulator (SARM)-2f. This diagram illustrates compound developed to enhance anabolic effects while minimizing adverse androgenic activity, often associated with misuse in athletic performance enhancement

Source: Morimoto et al. (2019)

et al., 2022; Thevis et al., 2024). Long-term use, however, disrupts hormonal regulation and elevates cardiovascular and hepatic risks. Despite detection advances, AAS and SARMs continue to dominate global doping violations (Leciejewska et al., 2024).

3. Peptide Hormones and Growth Factors (EPO, GH, IGF-1)

Peptide hormones and growth factors such as erythropoietin (EPO), growth hormone (GH), and insulin-

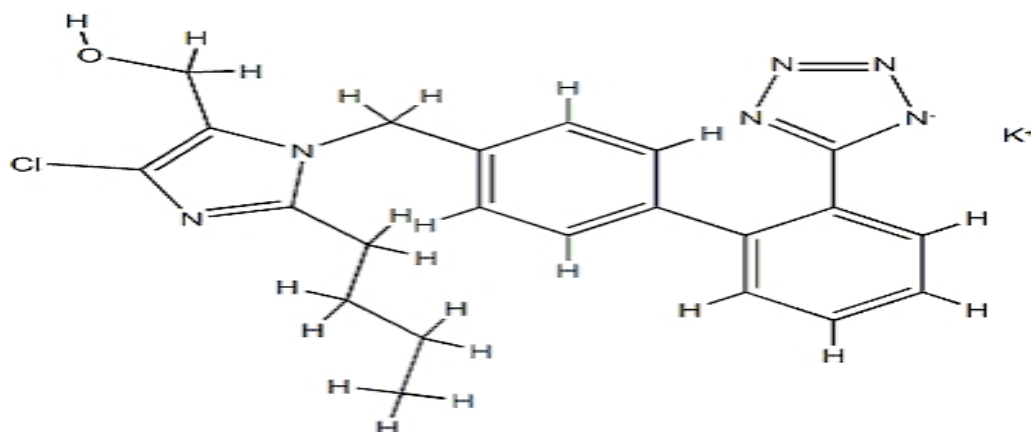


Figure 3.Chemical structure of EPO

Source: Del-Aguila-Arcentales et al. (2022)

like growth factor-1 (IGF-1) are misused to enhance oxygen delivery and tissue regeneration. These substances confer a considerable advantage in endurance sports but are difficult to regulate due to their structural similarity to natural hormones (Dragčević et al., 2024).

4. Beta-2 Agonists

Beta-2 agonists, including salbutamol, clenbuterol, and formoterol, are therapeutic agents for asthma but are abused for their anabolic and fat-reducing effects (Hagström et al., 2023). Chronic misuse can alter metabolic

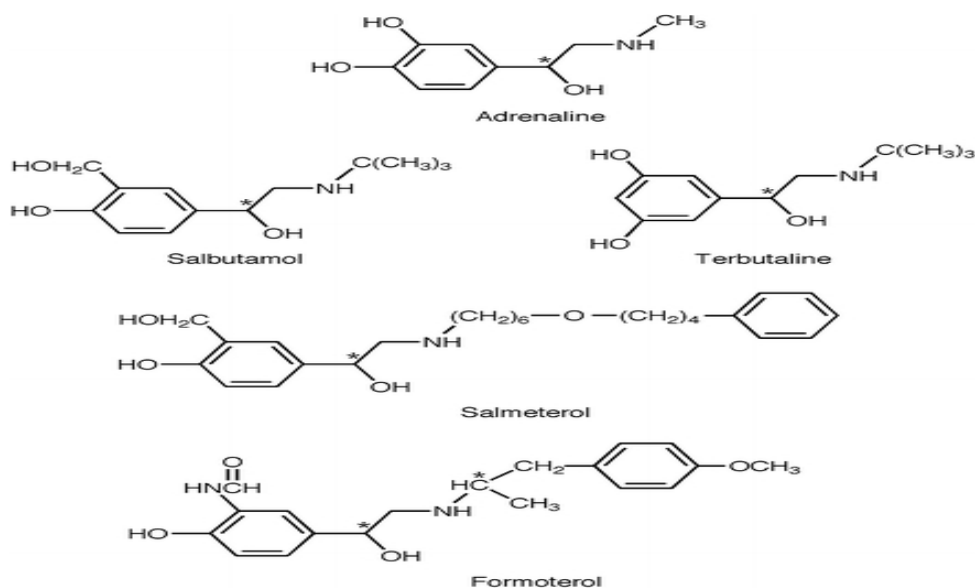


Figure 4.Chemical structure of Beta-2 Agonists.

Source: Prentice et al. (2014)

balance and muscle function, prompting WADA to regulate their permissible use (World Anti-Doping Agency, 2025). Similarly, diuretics and masking agents such as furosemide and hydrochlorothiazide are employed to manipulate urinary concentration or obscure the presence of other substances, representing a form of indirect doping (Krumm and Faiss, 2021; Thevis et al., 2024).

5. Diuretics and Masking Agents

Diuretics and masking agents are misused to dilute urine or conceal the presence of banned substances during doping

tests. Drugs such as furosemide and hydrochlorothiazide increase urine output, while plasma expanders like hydroxyethyl starch modify blood composition to disguise doping-related changes (Krumm and Faiss, 2021; World Anti-Doping Agency, 2023; Thevis et al., 2024). Their misuse undermines detection accuracy and compromises the integrity of anti-doping procedures.

6.Cannabinoids and Narcotics

Cannabinoids and narcotics including Δ^9 -tetrahydrocannabinol (THC) and morphine derivatives

are prohibited in-competition for their psychoactive and pain-masking effects (Reardon and Creado, 2014). Although some cannabinoids have recognized therapeutic uses, their influence on cognitive function and motivation remains concerning for athlete safety and performance

integrity (Thevis et al., 2024).

7. New and Designer Drugs (SARMs, Gene Doping Agents, Microdosing Strategies)

A growing concern is the emergence of new and designer

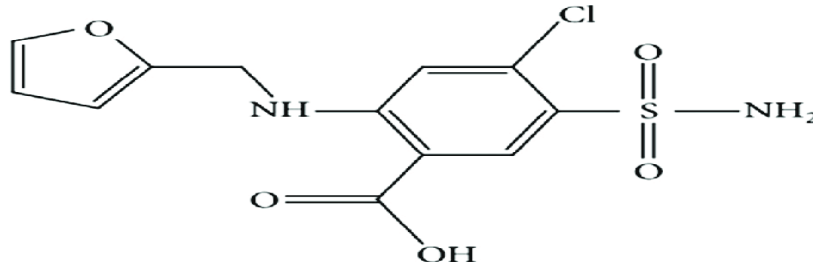


Figure 5. Chemical structure of the diuretic furosemide

Source: Söderholm et al. (2010)

drugs, such as next-generation SARMs, gene-doping vectors, and microdosing strategies. These substances are engineered to evade traditional testing and exploit biological processes that govern muscle growth, recovery, and erythropoiesis (Unal and Ozer Unal, 2004; Lu et al.,

2023). The continued evolution of such methods highlights the dynamic nature of doping and the ongoing need for research-driven countermeasures (Dragčević et al., 2024; Thevis et al., 2024).

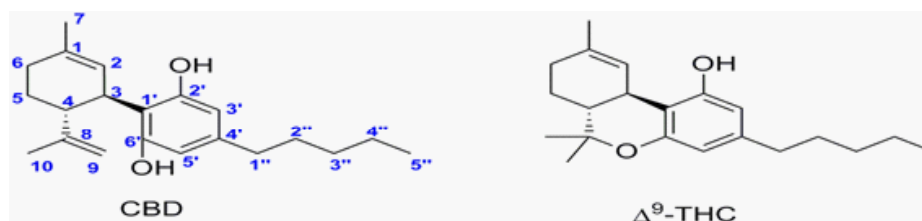


Figure 6. Cannabidiol (CBD) and (-)-trans- Δ^9 -tetrahydrocannabinol (Δ^9 -THC)

Source: Morales et al. (2017)

BIOCHEMICAL MARKERS OF DRUG ABUSE IN ATHLETICS AND THEIR MECHANISMS OF DETECTION

Biochemical markers serve as the cornerstone of modern anti-doping science, allowing investigators to detect both direct and indirect evidence of drug use. Direct biomarkers represent the parent substance or its metabolites in biological matrices, such as urine, plasma, or dried blood spots (Oliveira et al., 2024). Indirect biomarkers, by contrast, reflect the physiological or biochemical consequences of drug exposure, such as altered hormone ratios, hematological shifts, or gene expression changes (Krumm and Faiss, 2021). The combination of these two approaches enhances detection sensitivity and broadens the temporal window for identifying doping events. Drug detection relies on the understanding of pharmacokinetic and metabolic signatures, encompassing absorption, biotransformation, and elimination pathways. For instance, anabolic steroids undergo hepatic reduction and

conjugation, producing a series of 5 α - and 5 β -reduced metabolites that serve as reliable biochemical indicators of exogenous steroid use (Thevis et al., 2024). Similarly, recombinant peptide hormones display distinct isoform distributions compared with endogenous analogues, enabling differentiation through immunoassays and liquid chromatography–tandem mass spectrometry (LC-MS/MS) (International Testing Agency, 2024).

However, major challenges persist. Biomarker detection must account for sensitivity and specificity limitations, inter-individual biological variability, and temporal detection windows that vary by compound and administration route (Krumm and Faiss, 2021; World Anti-Doping Agency, 2023). Factors such as genetic polymorphisms, circadian rhythms, altitude exposure, and training load can influence biomarker interpretation, emphasizing the need for longitudinal monitoring through the Athlete Biological Passport (ABP) framework (Dragčević et al., 2024).

SPECIFIC MARKER CATEGORIES

1. Steroid Biomarkers

Steroid profiling remains the most established biochemical strategy for detecting exogenous anabolic-androgenic steroid (AAS) use. The endogenous steroid profile, including the testosterone-to-epitestosterone (T/E) ratio and ratios of 5 α -/5 β -reduced metabolites, provides critical information on androgen metabolism and possible suppression of endogenous testosterone production (Holterhus et al., 2002). Deviations from an athlete's baseline steroidal pattern are tracked within the ABP Steroidal Module, which applies adaptive Bayesian models to identify atypical longitudinal variations. When steroid profile irregularities are observed, Isotope Ratio Mass Spectrometry (IRMS) is employed as a confirmatory method to distinguish endogenous from synthetic steroids based on carbon isotope composition (13C/12C ratio). This high-precision analytical approach provides unequivocal evidence of exogenous administration (Thevis et al.,

2024). The integration of steroidomics, comprehensive analysis of steroid metabolites using high-resolution mass spectrometry has further expanded the marker panel for identifying designer or microdosed steroids (Lu et al., 2023).

2. Peptide Hormone Biomarkers

Peptide hormones such as growth hormone (GH), insulin-like growth factor-1 (IGF-1), and erythropoietin (EPO) are among the most challenging doping agents to detect due to their structural similarity to endogenous counterparts and short biological half-lives. Direct detection utilizes immunoassays or LC-MS/MS to distinguish recombinant from endogenous isoforms based on differences in glycosylation or molecular weight (International Testing Agency, 2024; Oliveira et al., 2024). Indirect markers including IGF-1 and procollagen type III N-terminal propeptide (P-III-NP) serve as biochemical indicators of

Table 1. Key steroidal biomarkers and their diagnostic relevance

Marker/Ratio	Physiological meaning	Analytical techniques	Anti-doping interpretation
T/E ratio	Testosterone/Epitestosterone balance	GC-MS, LC-MS/MS	Elevated > 4.0 suggests exogenous T
5 α -/5 β -androstanediol ratio	Metabolic pathway indicator	GC-MS	Altered profile indicates steroid abuse
17 α -methylated metabolites	Exogenous steroid signature	HRMS	Confirms oral anabolic agent use

Sources: Holterhus et al. (2002); Thevis et al. (2024)

GH administration, reflecting the downstream anabolic response of tissues (Dragčević et al., 2024). These indirect biomarkers are incorporated into longitudinal monitoring programs, where deviations from physiological baselines are evaluated statistically within the ABP (Krumm and Faiss, 2021). The integration of omics technologies, particularly proteomics and metabolomics, promises to uncover new molecular signatures of peptide hormone misuse (Lu et al., 2023).

3. Erythropoietic Agents

The detection of erythropoiesis-stimulating agents (ESAs)

and blood manipulation strategies relies primarily on hematological and molecular biomarkers. Key hematological parameters such as hemoglobin concentration ([Hb]), reticulocyte percentage (Ret%), and the composite OFF-score form the basis of the ABP Haematological Module (Dragčević et al., 2024). These metrics collectively reflect erythropoietic balance, allowing indirect identification of EPO use or blood transfusion. Molecular biomarkers enhance detection sensitivity through the analysis of EPO isoform patterns, mRNA expression profiles, and other regulatory molecules such as hepcidin and ferritin (Creangă et al., 2025). Recombinant and long-acting EPO

Table 2. Biochemical and analytical markers for GH and IGF-1 misuse

Marker	Types	Detection methods	Typical change after ministration
GH isoform ratio	Direct	Immunoassay	Increased 22 kDa fraction
IGF-1	Indirect	LC-MS/MS	Elevated plasma levels
P-III-NP	Indirect	Immunoassay	Increased collagen turnover

Sources: Dragčević et al. (2024); ITA (2024)

analogues can be differentiated from endogenous isoforms using isoelectric focusing or LC-MS-based proteomic methods (Thevis et al., 2024). Recent studies have explored transcriptomic and metabolomic indicators of erythropoietic stress, offering promising avenues for next-generation ABP marker development (Lu et al., 2023).

4. Stimulant and Narcotic Markers

Stimulants (e.g., amphetamines, methylphenidate, cocaine) and narcotics (e.g., morphine derivatives) are primarily

Table 3. Hematological and molecular biomarkers in the ABP haematological module

reflecting central nervous system adaptation to chronic stimulant exposure. Integration of neurochemical and metabolomic data may therefore extend detection beyond the pharmacological window of the parent compound (Oliveira et al., 2024).

5. Beta-2 Agonists and Diuretics

Beta-2 agonists (e.g., salbutamol, clenbuterol) and diuretics (e.g., furosemide, hydrochlorothiazide) present

detected through their urinary and plasma metabolites, which serve as direct biochemical markers of use (Lu et al., 2023). Modern LC-MS/MS workflows enable detection of these metabolites at trace levels, with confirmatory quantification based on characteristic fragmentation patterns (Thevis et al., 2024). Emerging evidence suggests that stimulant misuse may also induce neurotransmitter pathway perturbations, affecting dopaminergic and serotonergic turnover (Reardon and Creado, 2014). These alterations could serve as potential indirect biomarkers

unique analytical challenges due to therapeutic overlap and rapid elimination. Metabolomic fingerprinting of urinary profiles enables differentiation between permitted therapeutic doses and supra-therapeutic misuse (Pluim et al., 2011). Biochemical ratio markers such as the parent-to-metabolite concentration ratio help distinguish inhaled therapeutic administration from systemic or oral abuse (Thevis et al., 2024). For diuretics and masking agents, indicators such as altered electrolyte concentrations,

Table 4. Major stimulant and narcotic metabolites used in doping detection

Substance	Primary metabolite	Biological matrix	Detection methods
Amphetamine	p-hydroxyamphetamine	Urine	LC-MS/MS
Cocaine	Benzoyllecgonine	Urine/Plasma	GC-MS
Morphine	Morphine-3-glucuronide	Urine	LC-MS/MS

Source: Thevis et al. (2024)

urinary specific gravity, and creatinine levels serve as indirect markers of manipulation (Krumm and Faiss, 2021). Advanced LC-MS/MS and HRMS approaches now permit simultaneous detection of multiple diuretics and masking agents within a single analytical run, increasing efficiency and reliability (World Anti-Doping Agency, 2025).

ADVANCED ANALYTICAL TECHNIQUES IN BIOMARKER DETECTION

Table 5. Beta-2 agonist and diuretic detection summary

Compound	Typical Biomarker/Ratio	Analytical techniques	Interpretation
Salbutamol	Parent/metabolite ratio	LC-MS/MS	Distinguish therapeutic vs. abuse
Clenbuterol	Parent detection	HRMS	Indicates anabolic misuse
Furosemide	Parent+altered electrolyte profile	LC-MS/MS	Masking agent

Sources: Thevis et al. (2024); WADA (2025)

laboratories, the practical challenges they encounter, and the innovations shaping next-generation detection systems.

CHROMATOGRAPHY-MASS SPECTROMETRY PLATFORMS

Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) continue to serve as the foundational technologies for direct detection of parent compounds and metabolites in urine and blood (Khalil et al., 2024). High-resolution mass spectrometry (HRMS) extends this capability to

non-targeted screening, enabling laboratories to identify unanticipated or designer substances retrospectively (Thevis et al., 2024). Nonetheless, these methods require stringent calibration, matrix matching and expert interpretation to avoid analytical artefacts and false positives. An overview of an LC-MS/MS workflow applied to urinary peptidomic analysis is illustrated in Figure 7.

ISOTOPE RATIO MASS SPECTROMETRY (IRMS) AND CONFIRMATORY ANALYSES

In the steroidal domain, IRMS is the confirmatory standard for differentiating endogenous from exogenous steroids by measuring $^{13}\text{C}/^{12}\text{C}$ ratios (Cawley and Flenker, 2008). When applied following an atypical steroid profile, IRMS provides high specificity evidence of synthetic steroid administration (Dragčević et al., 2024). However, due to its cost and complexity, IRMS is typically reserved for confirmatory rather than routine screening. The analytical workflow for the isolation and isotopic characterization of endogenous steroids using multi-immunoaffinity

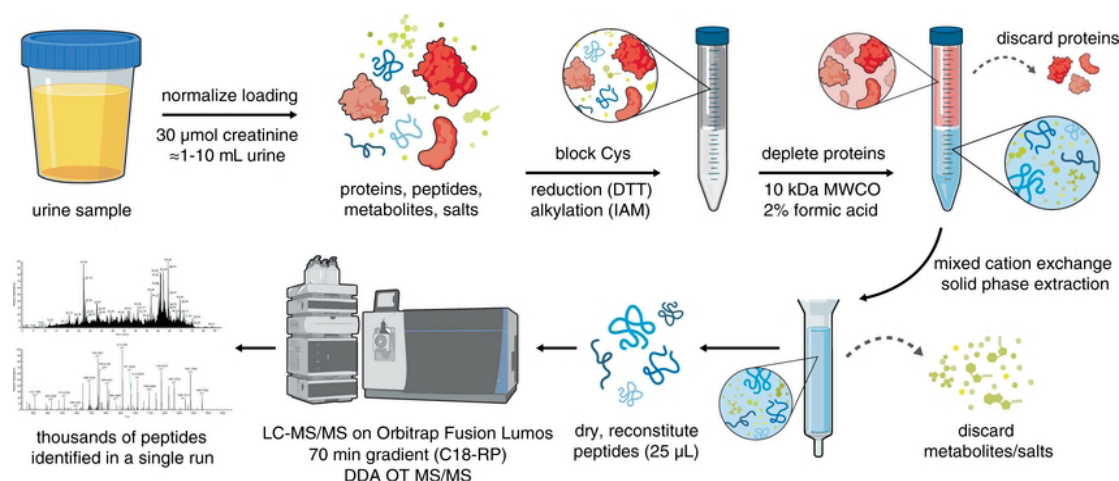


Figure 7. An efficient chemical extraction and LC-MS/MS workflow for urinary peptidomic analysis. This diagram illustrates the LC-MS/MS method, detailing the sequential steps of sample preparation, peptide extraction, chromatographic separation, and tandem mass spectrometric detection used for comprehensive urinary peptide profiling

Source: Palanski et al. (2025)

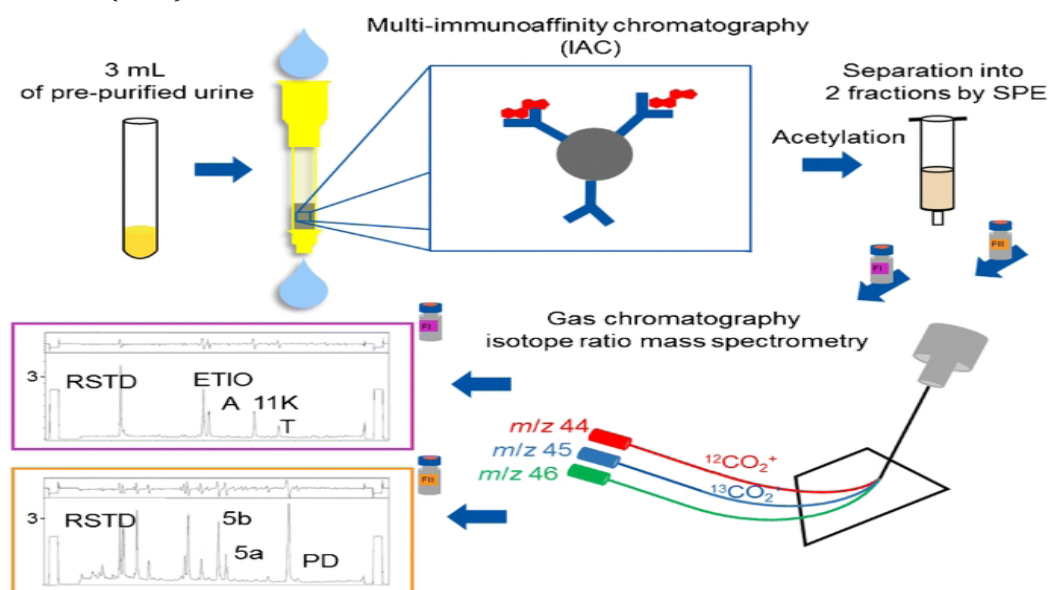


Figure 8. Workflow for the isolation and analysis of endogenous steroids in urine using multi-immunoaffinity chromatography and isotope ratio mass spectrometry (IRMS). The diagram illustrates the sequential process of selective steroid extraction, purification, and isotopic analysis for precise characterization of endogenous steroid profiles.

Source: Putz et al. (2019)

chromatography coupled with IRMS is illustrated in Figure 8

IMMUNOASSAYS, TARGETED MS AND HORMONE/PEPTIDE BIOMARKER DETECTION

Peptide hormones and growth factors such as GH and IGF-1 present unique analytical challenges owing to structural similarity with endogenous molecules. Immunoassays offer initial screening, but targeted LC-MS/MS or immunoaffinity-enrichment LC-HRMS are increasingly employed to improve specificity and isoform discrimination (International Testing Agency, 2024; Khalil et al., 2024). For longitudinal monitoring, assays must demonstrate low analytical and biological variability to reliably detect deviations from an individual athlete's baseline.

METABOLOMICS, PROTEOMICS AND MULTIVARIATE LONGITUDINAL PROFILING

Untargeted metabolomics and proteomics offer a systems-level perspective of doping, enabling detection of perturbations in metabolic networks following substance use (Mohannad et al., 2025). Longitudinal profiling of athletes' baselines and subsequent deviations reduces noise from inter-individual variability and enhances sensitivity to subtle manipulations. However, standardisation of collection, processing and statistical thresholds remains a significant hurdle. The integration

of these omics-based methodologies with artificial intelligence for enhanced detection and interpretation within anti-doping frameworks is illustrated in Figure 9.

ARTIFICIAL INTELLIGENCE AND BIOINFORMATICS FOR PATTERN RECOGNITION

Machine learning (ML) and advanced bioinformatics are rapidly gaining traction in the anti-doping domain as tools for longitudinal data integration and anomaly detection. For example, an AI-driven model using athlete performance passports (APP) achieved a 53.8% prediction rate for sanctioned weightlifters, demonstrating the potential to identify suspicious patterns beyond traditional biomarker thresholds (Ryoo et al., 2024). Bioinformatics frameworks

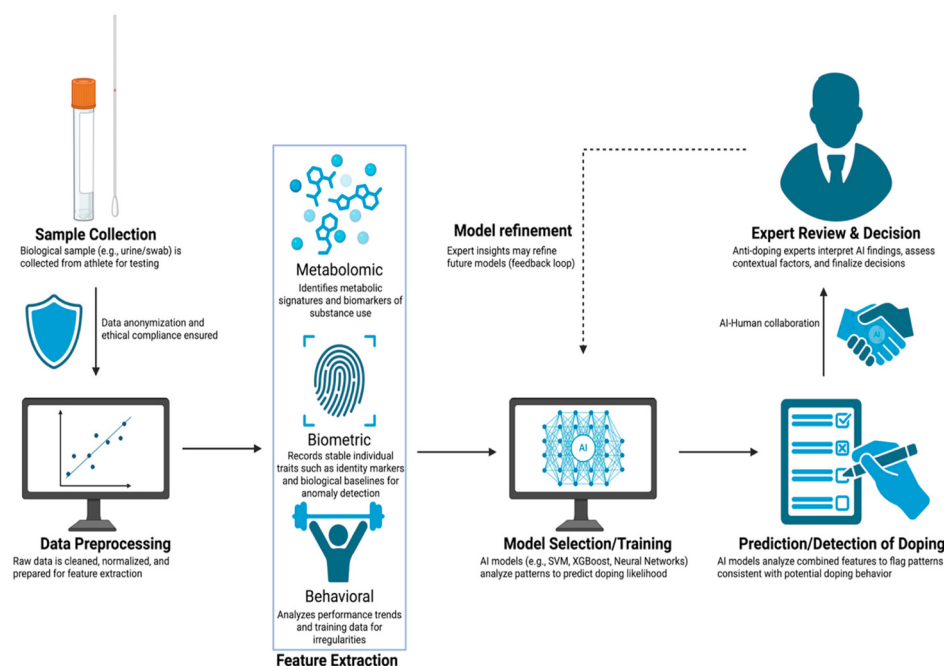


Figure9. Integrated workflow of metabolomics and artificial intelligence in modern anti-doping strategies. The diagram illustrates the combined application of metabolomic profiling and AI-driven data analysis for the detection, interpretation, and prediction of doping-related biochemical patterns

Source: AbuHaweeleh et al. (2025)

such as multivariate copula-based Bayesian models are also under development for interpreting complex biomarker dependencies across time (Deliu and Liseo, 2024). Key challenges include building representative training datasets, ensuring model transparency, avoiding bias and aligning computational outputs with regulatory decision-making. The integration of artificial intelligence and bioinformatics within metabolomic-based anti-doping workflows is illustrated in Figure 9.

THE ATHLETE BIOLOGICAL PASSPORT (ABP)

The Athlete Biological Passport (ABP) is a cornerstone of modern anti-doping science, shifting the emphasis from direct detection of prohibited substances to the longitudinal monitoring of biomarkers reflecting physiological effects of doping. Developed under the World Anti-Doping Agency (WADA), the ABP enables individualised assessment of an athlete's biological data over time to identify abnormal fluctuations suggestive of manipulation (WADA, 2023).

This strategy enhances detection sensitivity and supports

Table 6. Comparison of analytical techniques for biomarker detection

Techniques	Target Analyte	Sample type	Detection Limit	main Advantage	Limitations	keyReference
GC-MS	Steroids, stimulants	Urine	ng/mL	High specificity	Thermal degradation	Khalial., 2024
LC-MS/MS	Peptides, narcotics	Plasma	pg/mL	Broad range	Matrix effects	Oliveira <i>et al.</i> , 2024
IRMS	Steroids	Urine	n/a	Source discrimination	Costly	Dragčević, <i>et al.</i> , 2024
Immunoassay	GH, IGF-1	Serum	ng/mL	High throughput	Cross-reactivity	ITA, 2024
AI / Bioinformatics	Multivariate biomarkers	Integrated data	n/a	Pattern recognition	Data dependency	Ryoo <i>et al.</i> , 2024

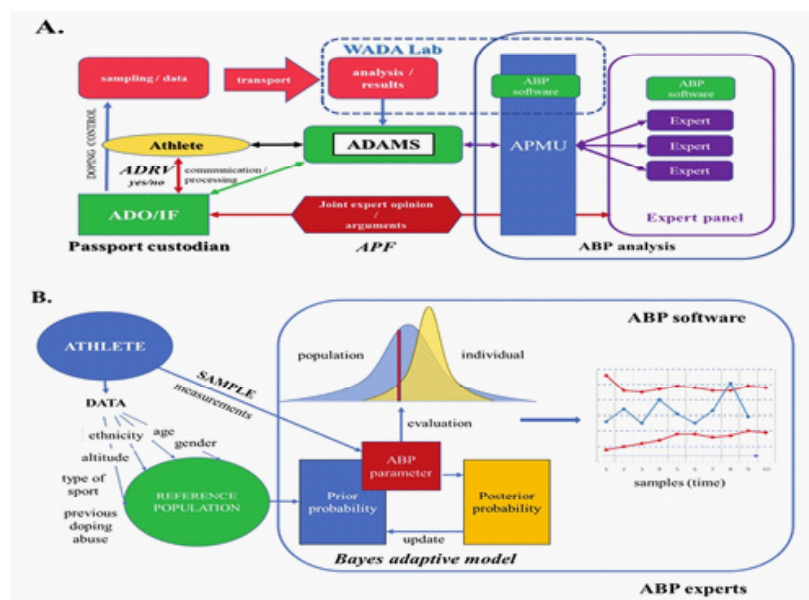


Figure 10. Athlete Biological Passport (ABP) workflow illustrating the overall process (A) and analytical procedures (B). Abbreviations: ABP – Athlete Biological Passport; ADAMS – Anti-Doping Administration and Management System; ADO – Anti-Doping Organisation; ADRV – Anti-Doping Rule Violation; APF – Adverse Passport Finding; APMU – Athlete Passport Management Unit; IF – International Federation; WADA – World Anti-Doping Agency.

Source: Dragcevic *et al.* (2024)

evidence-based adjudication, especially for substances or methods that are otherwise difficult to detect (Hassanpour and Salybekov, 2024). The ABP operates through a structured workflow involving data collection, analysis, and evaluation by anti-doping organisations, athlete passport management units, and international federations under WADA oversight, as shown in Figure 10.

and reference range, thus improving interpretative accuracy and reducing false positives due to natural variability.

MODULES OF THE ABP

The ABP currently encompasses three main modules. The haematological module detects blood manipulation through variables such as haemoglobin concentration, reticulocyte percentage, and OFF-score. The steroidal module monitors urinary steroid profiles particularly the testosterone/epitestosterone (T/E) ratio using gas chromatography-mass spectrometry and isotope-ratio mass spectrometry (IRMS) for exogenous source confirmation (Putz et al., 2019; WADA, 2024). The endocrine module, introduced more recently, evaluates growth hormone (GH) and insulin-like growth factor-1 (IGF-1) dynamics to uncover peptide-hormone-related

CONCEPT AND PRINCIPLE OF ABP

The ABP concept is grounded in the principle that doping leaves measurable physiological “footprints” even after the substance is metabolised or cleared. Instead of seeking banned drugs directly, it tracks changes in biological markers that respond predictably to doping practices such as erythropoietic stimulation or anabolic manipulation (Dragčević et al., 2024). These markers are recorded longitudinally to establish each athlete’s unique baseline doping (International Testing Agency, 2024). Collectively, these modules provide a multivariate approach to anti-doping surveillance. The parameters measured within each module reflect specific physiological markers associated with potential doping activity, integrating both direct measurements and calculated indices to improve sensitivity and interpretive accuracy. Table 7 summarises the principal measured and derived variables across the ABP modules

*Pending implementation. Abbreviations: 5 α -/5 β -diol – 5 α -androstanediol/5 β -androstanediol ratio; A/Etio – androsterone/etiocholanolone ratio; A/T – androsterone/testosterone ratio; ABPS – abnormal blood profile score; GH-2000 – composite score from IGF-1 and P-III-NP concentrations adjusted for sex and age; Hct – haematocrit; HGB – haemoglobin; IGF-1 – insulin-like growth factor 1; IRF – immature reticulocyte fraction; MCH – mean corpuscular

Table 7. Measured and calculated parameters in athlete biological passport (ABP) modules

ABP module	Measured parameters	Calculated parameters
Haematological	HGB	OFF-score
	Ret%	OFF-score = [HGB] [g/l] - 60√Ret%
	Hct	
	RBC#	ABPS
	Ret#	Hct, HGB, RBC, Ret%, MCV, MCH, and MCHC
	IRF	
	MCV	
	MCH	
	MCHC	
	RDW	
	WBC	
	PLT	

Steroids	testosterone	T/E
	epitestosterone	A/T
	etiocholanolone	A/Etio
	5 α -androstanediol	5a-diol/5b-diol
	5 β -androstandiol	
Endocrinological*	IGF-1	GH-2000
	P-III-NP	

haemoglobin; MCHC – mean corpuscular haemoglobin concentration; MCV – mean corpuscular volume; P-III-NP – procollagen III peptide; PLT – platelet count; RBC – red blood cell count; RDW – red cell distribution width; Ret% – reticulocyte percentage; Ret# – absolute reticulocyte count; T/E – testosterone/epitestosterone ratio; WBC – white blood cell count.

MECHANISMS OF OPERATION AND STATISTICAL MODELLING

The Athlete Biological Passport (ABP) operates through the Anti-Doping Administration and Management System (ADAMS), which centralises athlete data, applies statistical algorithms, and flags atypical longitudinal profiles for expert review (Deliu and Liseo, 2024). Sophisticated

Bayesian models quantify the probability that observed fluctuations are consistent with doping rather than normal physiological variation (Leuenberger et al., 2010). The structure and application of these Bayesian models in evaluating longitudinal biomarker data are illustrated in Figure 11. The combination of expert interpretation and probabilistic modelling enhances the evidentiary strength and scientific reliability of ABP-based determinations.

INTEGRATION WITH ARTIFICIAL INTELLIGENCE AND BIOINFORMATICS

Recent research explores the integration of machine learning (ML) and bioinformatics to enhance the ABP's analytical capacity. Artificial intelligence (AI) algorithms can identify non-linear patterns and subtle correlations

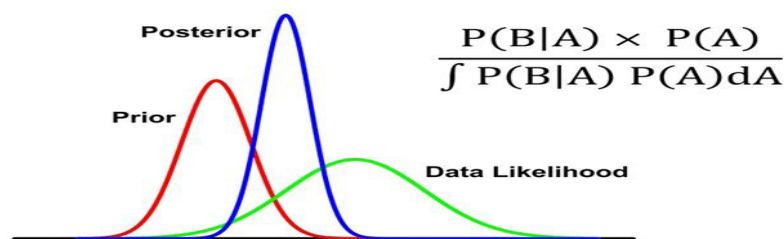


Figure 11. *The Bayesian models. This diagram illustrates a probabilistic framework used in anti-doping analysis to assess variations in athletes' biological markers over time. By incorporating prior information and new data, these models estimate the likelihood of abnormal results, improving the accuracy and reliability of detecting doping-related anomalies.*

Source: <https://vsni.co.uk/bayesian-analysis-what-is-it/>

across multi-omics datasets such as metabolomic, proteomic, and performance metrics improving anomaly detection (AbuHaweeleh et al., 2025). Studies such as Ryoo et al. (2024) demonstrated that AI-driven “athlete performance passports” can predict suspicious patterns with significant accuracy. Bioinformatics frameworks also allow data fusion from various biomarker domains, facilitating comprehensive systems-level interpretation of doping effects.

ETHICAL AND LEGAL CONSIDERATIONS IN THE BIOCHEMICAL DETECTION OF DRUG ABUSE

Advances in biochemical testing and data analytics have transformed anti-doping science but have also generated complex ethical, legal, and operational challenges. As analytical precision, longitudinal profiling, and artificial-intelligence-assisted evaluations expand, concerns about privacy, data ownership, and procedural fairness intensify

(Lea, 2009). A responsible anti-doping framework must therefore reconcile scientific progress with respect for athlete autonomy and integrity.

ETHICAL DIMENSIONS OF BIOMARKER SURVEILLANCE

Long-term biological monitoring such as the Athlete Biological Passport (ABP) requires repeated sampling and detailed personal data collection. These processes raise ethical issues concerning informed consent, confidentiality, and secondary data use (Kayser et al., 2007). The emergence of genomic, metabolomic, and AI-derived biomarkers adds layers of sensitivity, as they may reveal medical or genetic information unrelated to doping control. Ethical governance now prioritizes transparency, data minimization, and independent oversight to preserve athlete trust.

LEGAL AND REGULATORY CONTEXT

The World Anti-Doping Code provides the legal backbone for biochemical detection, yet applying probabilistic or AI-derived evidence introduces interpretive complexity. Courts and disciplinary panels increasingly scrutinize the statistical validity of biomarker evidence and the balance between scientific inference and legal certainty. Algorithmic decision-support systems also raise questions about accountability, bias, and due process under data-protection laws. Achieving global consistency demands harmonized laboratory standards and transparent validation of digital tools (WADA, 2024).

CHALLENGES AND FUTURE DIRECTIONS

The Athlete Biological Passport (ABP) and advanced biochemical testing have revolutionized anti-doping science by shifting from direct substance detection to monitoring physiological patterns over time. Despite these achievements, practical and analytical challenges remain. Variations in sampling conditions, laboratory capabilities, and athlete physiology can introduce data inconsistencies that complicate interpretation (Oliveira et al., 2024). Maintaining sample integrity and reproducibility across laboratories also demands strict adherence to validated protocols and quality control systems (Putz et al., 2019).

High costs of advanced techniques such as isotope ratio mass spectrometry (IRMS) and multi-omics profiling limit widespread adoption, especially in regions with constrained resources. Integrating artificial intelligence (AI) for data interpretation offers improved pattern recognition and predictive accuracy, yet it raises

demands for secure data infrastructure and transparent model validation (AbuHaweeleh et al., 2025). Balancing technological innovation with fairness remains crucial. Disparities in laboratory resources and analytical expertise can lead to uneven detection sensitivity across countries. Transparent communication of test limitations and algorithmic uncertainty is vital to maintaining athlete trust and system credibility.

Looking ahead, the future of anti-doping lies in integrating molecular and computational sciences. Emerging biomarkers such as microRNAs and epigenetic signatures promise higher sensitivity and longer detection windows (Hassanpour and Salybekov, 2024). Portable biosensors and dried-blood-spot testing may reduce costs and improve accessibility. Strengthening global harmonisation and continuous model refinement will ensure the ABP remains both scientifically rigorous and ethically sound (WADA, 2024). Together, these developments signal a future where technology enhances fairness, transparency, and athlete protection upholding integrity as the foundation of competitive sport.

CONCLUSION

The evolution of biochemical marker research and the integration of advanced analytical technologies have transformed the landscape of anti-doping science. From early substance-based testing to the sophisticated use of indirect biomarkers and longitudinal biological monitoring, the field continues to move toward precision, transparency, and fairness. The Athlete Biological Passport exemplifies this progress, combining scientific innovation with ethical responsibility to ensure a cleaner and more equitable sporting environment.

However, the dynamic nature of doping practices demands continuous adaptation. Emerging technologies, such as artificial intelligence, bioinformatics, and multi-omics profiling, are redefining detection strategies while simultaneously introducing new challenges related to validation, cost, and data governance. Sustained collaboration among scientists, policymakers, and sporting authorities will be essential to balance innovation with fairness and to safeguard the integrity of athletic competition. Ultimately, the future of anti-doping lies in harmonizing scientific excellence with human values. A truly effective system will not only deter and detect drug misuse but also uphold the principles of honesty, respect, and health that underpin the spirit of sport.

Acknowledgement

We thank all the researchers who contributed to the success of this research work.

Conflict of Interest

The authors declared that there are no conflicts of interest.

Funding

No funding was received for this research work.

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Cite this article: Moses Adondua Abah et.al. *Biochemical Markers of Drug abuse in Athletics. International Journal of Research in Biological Sciences (IJRBS)* 2026; 3(1): 08-23.

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