IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Review Of Various Forensic Analytical Methods For The Detection Of Doping In Sportspersons

[1]Shrihari Sanap, [2]Preet Kumar, [3]Deepika Bhandari

[1] Assistant Professor, Department of Forensic Chemistry, Institute of Forensic Science, Mumbai-400032

[2] Institute of Forensic Science, Mumbai

3 Head & Assistant Professor, Department of Forensic Science, Institute of Forensic Science, Mumbai-400032

[1] Department of Forensic Chemistry,

[1] Institute of Forensic Science, Mumbai-400032

Abstract : The act of doping in sports refers to the violation of any guidelines against doping set forth by the World Anti-Doping Agency (WADA). This can encompass the use of illegal or unauthorized substances to enhance performance, or engaging in other illicit practices. Scientific research has demonstrated that doping can have severe negative effects on health, such as elevated blood pressure, increased instances of cardiovascular diseases, liver damage, and psychological addiction. Furthermore, it diminishes the integrity, reputation, and value of sports. Additionally, it has been associated with premature deaths in mainstream sports. Consequently, addressing the global issue of doping in sports has become a significant priority. Health authorities are increasingly recognizing this problem, leading to the development of various new methods and guidelines to combat doping. Many literature reviews and editorials addressing this issue have been publicly released. However, a large portion of these publications focus specifically on advances in anti-doping analytical methods related to specific categories of drugs. Advancements in the field of analytical chemistry are crucial for anti-doping research. Therefore, a variety of experimental approaches and techniques based on analytical methods such as mass spectrometry and gas chromatography are routinely used to detect doping in athletes. This review aims to comprehensively and critically evaluate academic research related to significant advancements in anti-doping analytical methods made over the past decade. It also provides a broader perspective on different classes and subclasses of prohibited drugs and substances, outlining their chemical, structural, biological, and analytical characteristics. This information is presented in accordance with the official guidelines provided by the World Anti-Doping Agency (WADA).

Keywords **– Doping, Forensic Science, WADA, Drug Analysis**

I. INTRODUCTION

Across history, human beings have displayed an inherent drive to engage in competitive sports and emerge victorious. Since ancient times, athletes have sought every possible advantage over their opponents, leading to the use of performance-enhancing substances in various forms such as food, supplements, and other concoctions. Civilizations including the Romans, Africans, and Greeks have documented the use of stimulating herbs to combat fatigue and injuries. The term "doping" itself stems from the Dutch word "dop," referencing an alcoholic beverage made from grape skins believed to enhance the strength of South African Zulu warriors[1]. However, despite this extensive historical context of performance-enhancing substances in sports, doping remains a highly contentious and widely debated issue in contemporary athletic competitions. This problem transcends all sports, regardless of technological advancements, popularity, or historical significance [1, 2]. By 1933, "doping" had become a part of the English lexicon [3]. The escalating use of performance-enhancing substances for doping purposes came to a tragic climax in the 1960 Olympic Games

in Rome, leading to the unfortunate death of a cyclist. This event prompted action, and in 1962, The Council of Europe published the initial list of banned substances, encompassing narcotics, stimulants, alkaloids, respiratory tonics, and specific hormones. In response, the International Olympic Committee (IOC) established a Medical Commission in 1967, entrusted with preventing doping by maintaining a catalog of prohibited substances and methods. Systematic testing of athletes for performance-enhancing drugs commenced in 1968, initially focusing on stimulants like amphetamines during the Summer Olympic Games in Mexico [2].

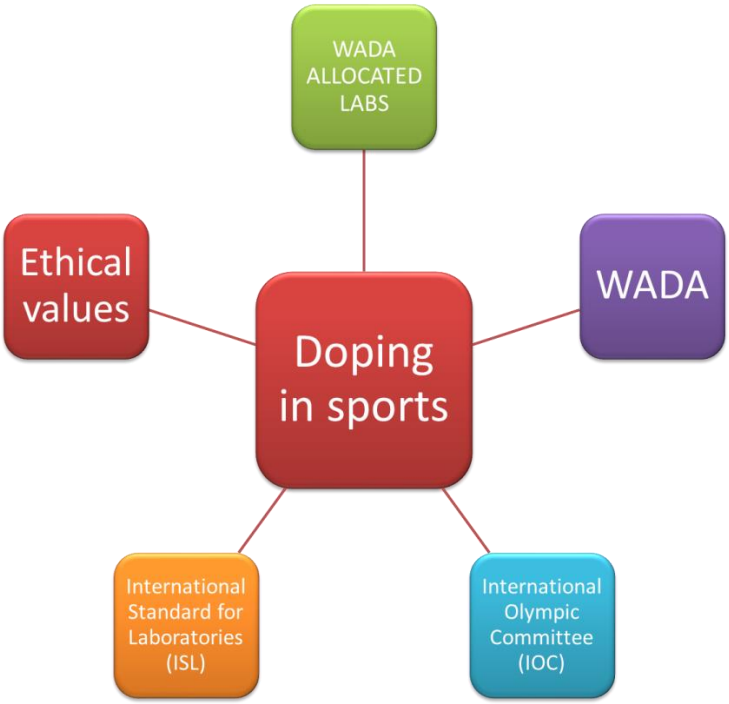

Fig.1 : Doping Ethical Agencies

Although these early tests primarily targeted stimulants, the need arose to detect other compounds. Advances in organic chemistry during the 1950s and 1960s yielded a wide range of pharmacologically active substances, including diuretics, beta-blockers, corticosteroids, and anabolic steroids. Athletes extensively used these substances throughout the 1970s. Tests conducted during competitions proved ineffective in controlling long-acting agents such as anabolic substances because athletes could cease usage before competitions yet still benefit from enhanced performance after the substance had been excreted. Consequently, it became evident that sample collections outside of competitions were indispensable. Despite initial resistance, these out-of-competition sample collections were introduced and now constitute a fundamental aspect of doping control. Pressure to standardize anti-doping methods led to the requirement that laboratories should be accredited according to the International Organization for Standardization [4].

By this time, the use of doping substances had become a global phenomenon in numerous sports, both professional and amateur, and had transformed into a significant public health concern. This global concern in 1999 culminated in a unique collaboration between sports organizations and governments, resulting in the formation of the World Anti-Doping Agency (WADA) [5].

The International Olympic Commission (IOC) stands as a pivotal institution founded in Lausanne, Switzerland, on June 23, 1984, by Pierre de Coubertin and Demetrios Vikelas. Serving as the ultimate authority of the Olympic Movement, the IOC ensures the consistent organization of the Olympic Games. It extends support to all affiliated member organizations within the Olympic Movement and actively advocates for the fundamental principles of the Olympics. Its membership comprises 204 National Olympic Committees [6].

As outlined in the Olympic Charter, the roles of the IOC encompass several significant domains:

- Promotion of Ethical Values and Fair Play: The IOC is committed to fostering ethical conduct in sports, promoting youth education through sports, and upholding the spirit of fair play while condemning violence.
- Organization and Advancement of Sports: The IOC works diligently to foster, organize, advance, and coordinate sports and sporting events on a global scale.
- Collaboration for Global Peace: The organization collaborates with pertinent public or private entities, leveraging sports in the pursuit of humanity's welfare and advocating for peace.

- Preservation of Unity and Independence: The IOC takes proactive measures to strengthen unity and safeguard the independence of the Olympic Movement.

- Advancement of Gender Equality: The IOC actively promotes and supports the participation of women in sports at all levels, striving for gender equality.

- Combatting Doping: The IOC leads the charge against doping in sports, ensuring that competitions remain clean and fair.

- Protection of Athlete Health: The IOC supports initiatives aimed at ensuring the health and well-being of athletes.

- Prevention of Abuse and Athlete Well-being: The IOC opposes any political or commercial misuse of sports and athletes, advocating for measures that secure athletes' social and professional futures.

- Promotion of Inclusive Sports: The IOC actively encourages and supports the development of sports that are accessible to everyone, promoting inclusivity.

- Environmental Stewardship: The organization advocates for environmental awareness, endorsing sustainable development in sports and ensuring that the Olympic Games adhere to eco-friendly practices.

- Legacy Building: The IOC aims to create a positive and enduring impact from the Olympic Games on host cities and countries, leaving behind a legacy of benefit.

- Integration of Culture and Education: The IOC fervently supports initiatives integrating sports with culture and education, fostering a holistic approach to learning.

Furthermore, the IOC actively endorses and supports the endeavors of institutions like the International Olympic Academy (IOA) and other entities dedicated to the cause of Olympic education.

The World Anti-Doping Agency (WADA), established in 1999, operates as an independent international entity devoted to advocating, coordinating, and overseeing the battle against doping across all aspects of sports. Several key factors spurred the formation of WADA:

- Standardizing Anti-Doping Rules: The absence of uniform anti-doping regulations worldwide necessitated cohesive global standards.

- Proliferation of Doping Substances: The widespread use of performance-enhancing substances among both amateurs and athletes posed a serious public health concern.

- Promoting Research: WADA aimed to stay abreast of advancements in the pharmaceutical sector, necessitating continuous research initiatives.

- Centralized Collaboration: Streamlining cooperation between national and international anti-doping initiatives was imperative.

In 2002, WADA introduced its comprehensive World Anti-Doping Program, comprising six crucial documents. Among these, three are pertinent to accredited laboratories: The World Anti-Doping Code, the List of Prohibited Substances and Methods, and the International Standard for Laboratories (ISL). WADA's core activities encompass scientific research, educational initiatives, enhancing anti-doping capabilities, and overseeing compliance with the World Anti-Doping Code.

Headquartered in Montreal, Canada, WADA operates as a Swiss law foundation based in Lausanne, Switzerland. Its stakeholders include diverse entities such as athletes, National Anti-Doping Organizations, major event organizers, governments, and anti-doping laboratories. WADA released its first list of banned substances in sports in 2004, subsequently updating it to align with evolving knowledge.

The organization actively administers and supervises doping control programs globally through its governing committee and international federations. Stringent guidelines, including the World Anti-Doping Code, ISL, and anti-doping educational initiatives, are employed to implement anti-doping programs. Education plays a pivotal role, raising awareness about the detrimental effects of drugs. Simultaneously, drug testing occurs, with urine or blood samples collected from athletes during competitions or training periods. These samples undergo rigorous analysis in WADA-accredited analytical laboratories to detect prohibited substances.

The World Anti-Doping Code serves as the fundamental cornerstone of the World Anti-Doping Program. It delineates anti-doping rules and principles, elucidates the roles and responsibilities of diverse stakeholders, and provides guidelines for implementation, modification, and compliance. The Code's primary objectives are to safeguard athletes' right to participate in doping-free sports and promote equitable competition globally by ensuring consistent detection, deterrence, and prevention of doping activities. Doping encompasses various rule violations, extending beyond the mere presence of prohibited substances or their byproducts. It encompasses attempts to use banned methods, refusal or failure to provide samples, unavailability for out-of-competition testing, tampering with any aspect of doping control, trafficking prohibited substances or methods, and administering such substances or methods to athletes.

The practice of drug testing in sports commenced in 1968, initially cataloguing around 30-40 prohibited substances by the International Olympic Committee (IOC). However, a pivotal moment occurred during the 1972 Munich Olympic Games, marking the inaugural use of chromatography-mass spectrometry for doping control at a major sporting event. Subsequently, advancements have been made in testing methods, adapting to the expanding array of drugs available globally. Initially, stimulants, anabolic steroids, beta-blockers, and narcotics were analyzed using Gas Chromatography-Nitrogen Phosphorous Detector (GCNPD) and Gas Chromatography-Mass Spectrometric Detector (GCMSD).

During the 2004 Olympic Summer Games in Athens, Liquid Chromatography interfaced with tandem mass spectrometry (LC-MS/MS) was introduced to identify diverse drug classes, including anabolic agents, corticosteroids, narcotics, and β-2 agonists. Since the 1972 Olympics, substantial progress has been made in doping testing techniques through enhanced extraction methods and advanced equipment. Novel mass spectrometric approaches, such as High-Resolution Mass Spectrometry (introduced at the 1996 Atlanta Olympics), Isotope Ratio Mass Spectrometry (utilized at the Special Olympic Winter Games in 1998), and Liquid Chromatography Mass Spectrometry (implemented at the Athens Olympic Games in 2004), have significantly improved doping tests. Furthermore, the introduction of Time of Flight (TOF) Mass Spectrometry in 2008 and Ultra Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry (UPLC-HRMS) at the London Olympics in 2012 has further refined the doping control process. Each laboratory employs specific testing protocols and equipment, including GC, GC/NPD/ECD, GC/MS, HRMS, LC-MS/MS, and IRMS. When combined with Gas/Liquid Chromatography and various ionization methods such as Chemical Ionization, Electro Spray Ionization, Atmospheric Chemical Ionization, Atmospheric Pressure Photo Ionization, MALDI, numerous applications have been established, enabling sensitive and selective detection of drugs in biological matrices.

The analysis of stimulants and narcotics currently relies on GC-NPD and GC-MSD, which are standard techniques due to their robustness, sensitivity, selectivity for non-polar and volatile substances, and high level of standardization. However, drugs that are thermally labile and highly polar pose challenges for GC-MS analysis because of their non-volatility and high temperature requirements. The introduction of liquid chromatography coupled with tandem mass spectrometry (LC/MS) in doping analysis has addressed this issue, particularly for detecting polar metabolic products excreted in urine. In most major drug testing laboratories, three core technologies are utilized: gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS).

The fundamental aspects of contemporary doping analysis can be delineated as follows:

1. Analytical Approaches: Doping control laboratories utilize diverse analytical methodologies, including mass spectrometry (MS), gas chromatography (GC), and liquid chromatography (LC), to identify banned substances in athletes' specimens. The selection of the biological matrix—be it blood, urine, or another medium—depends on the substance's properties and its elimination rate from the athlete's system.

2. Prohibited Substances Catalogue: The World Anti-Doping Agency (WADA) maintains an updated list of prohibited substances and methods, continually informed by ongoing scientific research and evaluation. This comprehensive list encompasses various categories such as anabolic agents, peptide hormones, stimulants, narcotics, and more.

3. Athlete Screening Protocols: Athletes undergo both in-competition and out-of-competition testing, which can be conducted randomly or based on intelligence-driven information. Skilled specialists collect urine, blood, or saliva samples, adhering to stringent protocols to ensure the accuracy and integrity of the results.

4. Doping Consequences: Athletes found positive for banned substances may face penalties, ranging from disqualification and suspension to expulsion from competitions. Moreover, they may endure substantial damage to their reputation and missed career opportunities due to the adverse publicity associated with doping allegations [2,3,5,6].

In summary, the core principles of contemporary doping analysis are designed to maintain the fairness and integrity of sports. This is accomplished through the detection and prevention of banned substance and method usage among athletes, utilizing advanced analytical techniques, a comprehensive catalog of prohibited substances, and regular athlete testing initiatives. These measures lead to appropriate sanctions against offenders, ensuring the credibility of athletic competitions. In contemporary times, ensuring the fairness and integrity of sports has led to doping control analysis becoming a standard practice [3,7]. Conventional doping detection primarily relies on two chromatography methods [8]: GC-MS and LC-MS. These methods are complementary, covering all low- to medium-molecular-weight drugs of abuse mandated by WADA regulations. For instance, certain compounds challenging to detect with LC-MS, such as oxymesterone due to its low ionization efficiency, necessitate the use of GC-MS [7].

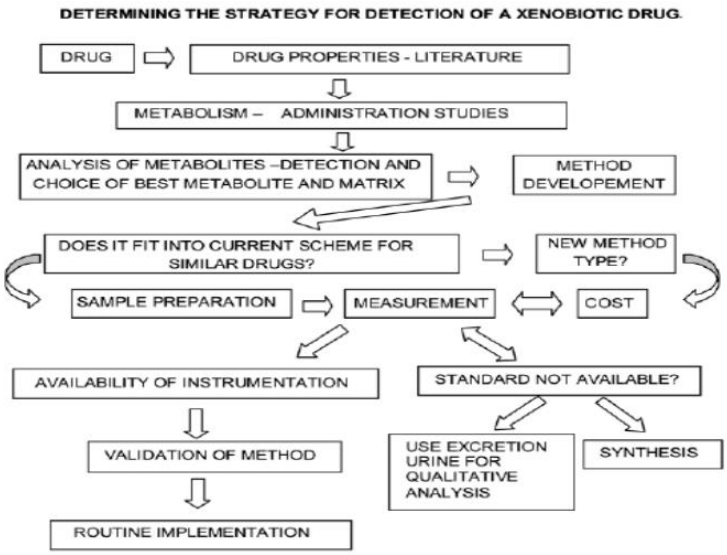

Fig.2 : Strategy determination for any xenobiotic drug

However, as detection technology advances, new methods like various fluorescence [9], colorimetric [10], electrochemical [11], and biosensor techniques [12] have emerged for doping detection. Additionally, PCR technology is now applied in emerging doping methods [13]. Anti-doping agencies employ qualitative or quantitative analysis on biological samples (e.g., urine or blood) collected during and outside of competitions to determine the presence of prohibited substances or metabolites, enabling them to assess athletes' potential use of doping [14,15,16,17].

Doping methods can be broadly categorized into two types based on how prohibited substances enter athletes: drug doping [11,18] and gene doping [13,19]. Drug doping encompasses organic drugs explicitly prohibited by WADA [1]. Depending on whether it occurs during competition and specific sports, prohibited substances and methods are grouped into three categories: substances and methods prohibited at all times (S0–S5, M1–M3); substances and methods prohibited in competition (S6–S9); and substances prohibited in particular sports (P1) **(Table 1).**

Table 1: S0 to P1 Listing by WADA

Category of the Doping method involved	**Relevant Analyte**	**Recommended Detection Methods**
S0 Non-approved substances		GC-MS/MS; LC-MS/MS; HILIC-HRMS
S1 Anabolic agents	Anabolic androgenic steroids (AAS)	GC-MS/MS; LC-MS/MS; GC-C/IRMS; LC–IM–Q/TOF; LC–HRMS/MS; GC–HRMS/MS; LC-Ag+CIS/MS/MS
S2 Peptide hormones, growth factors, related substances, and mimetics	Erythropoietin (EPO); Growth hormone (GH)	LC-MS/MS; ELISA; Transcriptomics; Proteomics; SAGE; SELDI-TOF MS; LC-MS/MS; LC-HRMS/MS; Immunoassay
S3 Beta-2 agonists	Salmeterol; Tretoquinol	LC-MS/MS; UHPLC-HRMS; LC-HRMS/MS
S4 Hormone and metabolic modulators	Aromatase inhibitors	GC-MS/MS; GC-C/IRMS; LC-MS/MS; Hyperpolarized NMR based metabolomics
S5 Diuretics and masking agents	Desmopressin; Probenecid; Acetazolamide	GC-MS/MS; LC-MS/MS
S6 Stimulants	Cocaine; Strychnine	GC-MS/MS; LC-MS/MS; ESI-MS/MS; LC-HRMS/MS;
S7 Narcotics	Morphine; Pentazocine	LC-MS/MS
S8 Cannabinoids	Cannabinoids	GC-MS/MS; LC-MS/MS
S9 Glucocorticoids	Cortisone; Dexamethasone	LC-MS/MS
M1 Manipulation of blood and	Blood doping	LC-MS/MS; Proteomics; Transcriptomics

blood components		
M2 Chemical and physical manipulation	Sample substitution and/or adulteration	Vigilance
M3 Gene and cell doping	Gene editing; Gene silencing; Gene transfer technologies	Polymerase chain reaction (PCR) (WADA-approved); NGS; WGR; HPLC-MS; CRISPR-Cas based systems
P1 Beta-blockers	Bunolol; Propranolol	LC-MS/MS

As scientific and technological progress continues, genetic modification technologies have become increasingly sophisticated. Consequently, the landscape of doping has grown more complex, encompassing the latest form known as gene doping [20]. Gene doping refers to the use of substances or methods that involve introducing foreign genes or cells into athletes for nontherapeutic purposes, enhancing athletic performance through improper means [19,21,22]. By incorporating specific genes into the human body to trigger the expression of proteins like insulin-like growth factor, vascular endothelial growth factor, and endorphins, athletes' muscle recovery is accelerated, illicitly enhancing their performance. Detecting gene doping poses substantial challenges compared to conventional drug doping [19]. The difficulty arises from the striking resemblance between the exogenous genes introduced through gene doping and the body's natural DNA, making identification a formidable task using non-invasive direct detection methods. Indirect assays attempting to identify gene vectors often struggle to differentiate natural immune responses (e.g., to an adenovirus or other vectors) from those triggered by artificially introduced viruses.

The existence of a wide range of prohibited substances (approximately 250 different compounds) [23] has placed a significant burden on doping detection efforts [24]. To meet the growing demand for qualitative and quantitative analysis of hundreds of substances with diverse chemical and biological properties, the requirements for doping control methods continue to rise, leading to rapid advancements in detection methods [25,26,27]. This review aims to provide a comprehensive and critical analysis of the scientific literature related to significant progress in analytical anti-doping methodologies over the past decade. The strengths and limitations of each analysis method have been assessed, and potential future directions for doping detection methods have also been explored.

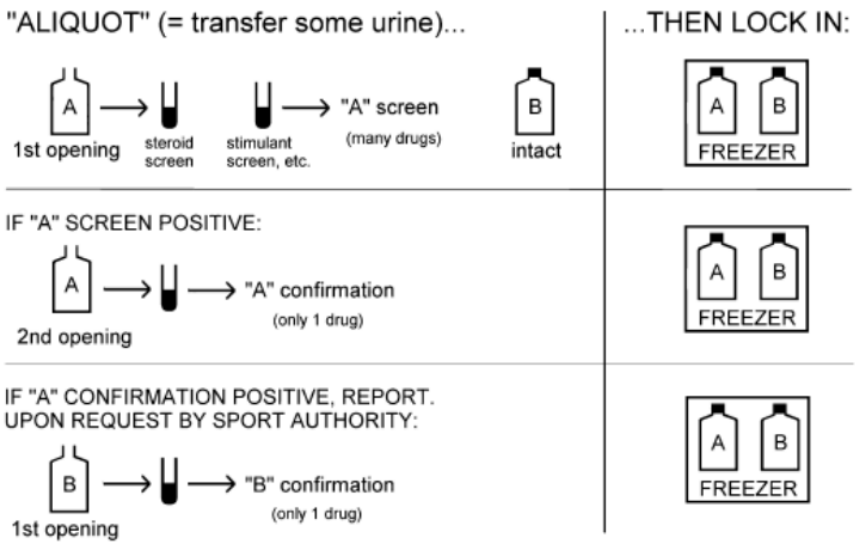

Fig.3 : Doping screening process followed by WADA

Next, the player or the doping control officer pours the urine sample into the bottles. Initially, 30mL is poured into bottle B, and then the remaining 60mL is poured into bottle A. A small amount of urine is retained in the beaker for specific gravity testing. Bottles A and B are then sealed. The corresponding numbers are noted on form O-2, which is signed by the doping control officer, the player, and the accompanying person. The form is further completed by the doping control officer, who records the competition details, match number, venue, date, code numbers for samples A and B, specific gravity of the urine sample, and then signs the form. Following the competition, all collected samples, along with the blue copy of the O-2 form, are sent to the laboratory for analysis.

a) Therapeutic Use Exemption: In the realm of sports, a participant might face a medical issue requiring medication. In such instances, the player must complete the TUE form and submit it to the authorities before the competition begins. A review committee can grant an exemption. To qualify for a TUE exemption, the following conditions must be met:

1. The TUE form must be submitted within the specified timeframe.
2. The player requires the prohibited substance or method to treat a severe or on-going medical condition, and withholding it would significantly jeopardize the player's health.
3. The prohibited substance or method won't enhance the player's performance.
4. No viable alternative to the prohibited substance or method exists. In such situations, the player may receive an exemption to use the banned substance or method.

Table 2: Number of GHRFs Urine Tests performed by WADA

Year	GHRFs Urine Tests	AAFs Total	# of Sports	# of TAs
2021	65,170	7	104	222
2020	46,341	13	102	199
2019	66,990	26	126	234
2018	60,964	21	124	231
2017	57,869	19	119	218
2016	42,730	15	111	207
2015	21,654	14	88	145

Table 3: Number of GH Isoforms and Biomarkers Tests performed by WADA

Year	GH lsoforms Tests	GH Biomarkers Tests	AAFs Total	# of Sports	# of TAs
2021	14,734	4,720	7*	84	130
2020	7,509	2,855	1	82	114
2019	17,393	6,790	6	103	156
2018	15,487	8755	2	99	137
2017	13,474	7008	0	90	124
2016	11,555	5983	6	68	111
2015	11,082	2182	4	74	103

Table 4: Number of ESAs Urine and Blood Tests performed by WADA

Year	ESAs Urine Tests	ESAs Blood Tests	AAFs Urine	AAFs Blood	AAFs Total	# of Sports	# of TAs
2021	50,940	4,953	52	14	66	100	217
2020	35,963	1,845	29	3	32	102	197
2019	51,929	3,757	78	14	92	120	243
2018	47,955	4792	61	16	77	118	229
2017	44,322	4531	56	29	85	116	220
2016	43,246	3464	44	22	66	108	212
2015	32,999	3219	45	1	46	94	183

II. Drug Doping Detection

For decades, detecting drug doping has been a crucial aspect of doping control efforts [28]. The evolution of modern analytical instruments has significantly enhanced the ability to identify doping agents. Alongside a range of chromatography and mass spectrometry-based separation techniques, numerous fluorescence, electrochemical, and colorimetric methods, as well as various biosensors, have demonstrated their effectiveness in doping control analyses. However, with the continuous design of new drug molecules possessing doping effects, doping control labs face the pressure to enhance and refine their testing methods and strategies. This evolution is essential to provide the World Anti-Doping Agency (WADA) with

comprehensive information supporting anti-doping decisions. Consequently, these methods for drug doping detection have been consistently upgraded, enabling quicker and more precise identification of pertinent doping substances in recent years [29].

Sample preparation stands as a pivotal stage owing to the significant disparities in the physicochemical characteristics of analytes and the intricate nature of matrices containing salts, lipids, and proteins. Ensuring reliable sensitivity and selectivity to the analytical method is crucial, necessitating precautions against contamination, column clogging, and potential ion suppression using mass spectrometric detection. Various sample preparation techniques are employed in doping control, ranging from rapid and straightforward methods like dilute and shoot and protein precipitation to multistep procedures such as solid-phase extraction (SPE), liquid-liquid extraction (LLE), or supported-liquid extraction (SLE). These techniques serve to achieve acceptable recovery for most analytes during the screening phase.

Fig. 4: Various techniques of Doping analysis

Utilizing selective and sensitive mass spectrometric detection simplifies the selection of appropriate sample preparation methods since screening a wide range of compounds does not demand highly selective extraction techniques. However, matrix effects introduced by urine or blood samples can compromise method selectivity and sensitivity, particularly in rapid and simple procedures like dilute-and-shoot. Managing these matrix effects typically involves employing an isotope-labeled internal standard (IL-ISTD), ensuring the attainment of accurate and reproducible results, especially in the quantitative determination of threshold substances.

Table 5: Drug Classes as per WADA and their Examples

S.No.	Class	Representative examples
S0.	Non-approved substances	Any pharmacologically active substance under clinical/pre-clinical trial, discontinued drug, veterinary drug, designer substance
S1.	Anabolic agents 1. anabolic androgenic steroids (AAS)	1-androstenediol,bolandiol, drostanolone, methandienone, testosterone, 5-androstenedione, stanozolol, 19-norandrosterone
	2. other anabolic agents	Clenbuterol, selective androgen receptor modulators (SARMs), tibolone, zeranol, zilpaterol
S2.	Peptide hormones, growth factors and related substances	erythropoietin (EPO), Chorionic Gonadotrophin (CG) and Luteinizing Hormone (LH) in males , Growth Hormone (GH), Insulin-like Growth Factor-1 (IGF-1)
S3.	Beta-2 agonists	All beta-2 agonists (both d & l isomers) except inhaled salbutamol (cut off 100 ng/ml), inhaled formoterol (cut off 40 ng/ml) and inhaled salmeterol
S4.	Hormone and metabolic modulators	Aromatase inhibitors: aminoglutethimide, anastrozole, exemestane, formestane, letrozole, testolactone Selective estrogen receptor modulators (SERMs):

		raloxifene, tamoxifen, toremifene Other anti-estrogenic substances : clomiphene, cyclofenil, fulvestrant Metabolic modulators:a) Insulins b) Peroxisome Proliferator Activated Receptor δ (PPARδ) agonists (e.g. GW 1516),myostatin inhibitors
S5.	Diuretics and other masking agents	Plasma expanders: glycerol, dextran, hydroxyethyl starch, desmopressin, mannitol Diuretics: Acetazolamide, amiloride, furosemide, indapamide, metolazone, spironolactone, , triamterene;
M1.	Manipulation of blood and blood components	Autologous, homologous or heterologous blood or red blood cell transfusion Enhancement of oxygen uptake: perfluorochemicals, efaproxiral
M2.	Chemical and physical manipulation	Tampering of sample during Doping Control: urine substitution and/or adulteration (e.g. proteases); Intravenous infusions and/or injections of more than 50 mL per 6 hour period
M3.	Gene doping	1. The transfer of polymers of nucleic acids or nucleic acid analogues; 2. The use of normal or genetically modified cells
S6.	Stimulants	Specified: Cathine; ephedrine; l-metamfetamine; methylhexaneamine;; selegiline; sibutramine; strychnine;etc
		Non-specified: Amphetamine; benfluorex; bromantan; cocaine; fenetylline; fenfluramine; mephentermine,etc
S7.	Narcotics	Buprenorphine, heroin, fentanyl, methadone, morphine, pentazocine, etc
S8.	Cannabinoids	Natural (e.g. cannabis, hashish, marijuana) or synthetic delta 9- tetrahydrocannabinol (THC) and cannabimimetics (e.g. “Spice”, JWH018, JWH073, HU-210)
S9.	Glucocorticosteroids	All glucocorticosteroids are prohibited when administered by oral, intravenous, intramuscular or rectal routes
P1.	Alcohol	Prohibited in particular sports at cut oof of 0.10 g/L in blood
P2.	Beta-blockers	Acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, carteolol, carvedilol, metipranolol, nadolol, oxprenolol, pindolol, propranolol, sotalol, timolol

III. EXTRACTION TECHNIQUES

Liquid-Liquid Extraction (LLE)

To concentrate substances present in biological fluids at trace levels (e.g., AAS), a pre-concentration step is necessary. LLE was historically a primary pre-concentration technique in doping control, predating the widespread use of LC-MS or gas chromatography/mass spectrometry (GC-MS). LLE achieves analyte extraction by partitioning between two immiscible solvents differentially. Despite its simplicity and cost-effectiveness, LLE isn't suitable for polar compounds and demands large sample and solvent volumes. Typically, LLE involves parallel extractions at basic and acidic pH, facilitating simultaneous extraction of acidic and basic substances during the screening stage [11].

Solid-Liquid Extraction (SLE)

In SLE, biological samples are adsorbed onto a diatomaceous earth stationary phase with a high surface area, housed in a cartridge or well plate. Analyte elution occurs when an immiscible solvent is applied to the cartridge. SLE can be seen as a simplified and automated version of LLE. Unlike LLE, SLE's sample preparation is quicker, without concerns related to phase separation like emulsion formation. SLE techniques also offer high throughput through the use of 96-well plates and exhibit superior recovery rates compared to LLE [62]. However, there isn't sufficient evidence of SLE's applicability for multiclass screening of prohibited substances, including both acidic and basic compounds. Therefore, SLE plays a valuable role in confirming specific substances, especially low-polarity compounds like steroids and glucocorticoids [11].

Solid-Phase Extraction (SPE)

SPE stands as a principal sample preparation method in doping analysis, given its suitability for various substances such as anabolic agents, β2-agonists, hormone antagonists and modulators, diuretics, stimulants, narcotics, glucocorticoids, and β-blockers. It surpasses LLE and SLE in terms of solvent consumption and high-throughput capabilities (using 96- or 384-well plates). SPE enables simultaneous clean-up and pre-concentration and offers versatility through a wide range of sorbents: normal phase (for polar analytes from non-polar organic solvents), reversed phase (for hydrophobic or polar organic analytes from aqueous matrices), ion exchange (for charged analytes from aqueous or non-polar organic samples), and mixed mode. Polymeric C18 sorbents are effective for extracting many prohibited substances during screening. Mixed-mode cartridges, utilizing C18 sorbents bonded with ion exchange groups, have demonstrated promise by enhancing analyte retention. These features enhance their applicability in screening scenarios involving a large number of compounds [64, 65]. Polymeric sorbents containing both polar and non-polar groups prove highly suitable for sample preparation in doping analysis due to their compatibility with the diverse physicochemical properties of prohibited substances.

Microextraction by packed sorbent (MEPS)

Microextraction by packed sorbent (MEPS) is a scaled-down version of solid-phase extraction (SPE), comprising millilitre to microliter packed sorbent volumes [66]. It seamlessly connects to GC and/or LC without additional modifications [67, 68]. Successfully applied to diverse biological matrices like urine, plasma, saliva, and blood, MEPS employs a syringe (100–250 µL) filled with about 1–2 mg of sorbent as a plug between the barrel and needle, functioning as a cartridge or barrel insert (BINs) within the eVol device by Trajan scientific and medical [71]. Various separation sorbents like reversed phases, normal phases, mixed mode, and ion exchange sorbents can be utilized in MEPS [69, 70, 72]. Reversed phase sorbents (C18, C8, and C2), normal phase (silica), restricted access material (RAM), hydrophilic interaction liquid chromatography (HILIC), carbon, polystyrene-divinylbenzene copolymer (PS-DVB), molecular imprinted polymers (MIPs), strong cation exchange (SCX), and mixed-mode (C8/SCX) chemistries are applicable [70].

MEPS proves invaluable for sample purification and preconcentration, especially with limited sample volumes (10 µL to 1000 µL), common in biological or environmental samples [73]. Remarkably, the packed sorbent can be reused more than 100 times [70, 73], unlike conventional SPE columns, which are disposable after single use. Analyte elution from sorbent beds can be achieved with minimal volumes of organic solvents like methanol or other mobile phases, ensuring an eco-friendly sample preparation approach while maintaining high analyte yield. MEPS emerges as a promising alternative to conventional SPE due to its speed, ease of use, potential for full automation in online procedures, and reduced usage of organic solvents and sample volumes.

Alternative Samples

There's a rising trend favouring alternative samples over blood, plasma, and urine for drug detection in clinical and forensic applications [74]. These alternatives include hair [75], sweat [76], breath [77], and saliva [78]. Saliva, unlike blood and urine, offers swift and non-invasive sampling. Unlike blood collection, which demands skilled personnel, saliva sampling requires no specialized expertise. In supervised sports competitions, saliva collection ensures privacy without direct observation of private functions, unlike urine samples [79]. Challenges in saliva sampling arise due to insufficient fluid caused by physiological factors or drug use [80, 81]. Techniques stimulating saliva production can influence drug concentrations. Limited drugs are clinically monitored in saliva due to the lack of correlation between saliva and plasma concentrations for many substances [82]. Unlike urine, which measures accumulated analyte concentrations,

plasma or serum samples reflect actual circulating analyte concentrations [79]. Saliva only contains the free (unbound) fraction of drugs, offering a better representation of the pharmacologically active drug fraction compared to plasma [82, 84]. Consequently, saliva is pivotal for therapeutic drug monitoring and serves as a diagnostic medium for measuring endogenous markers [85-92].

IV. DETECTION METHODS

MS-Based Methods

Historically, mass spectrometry (MS) methods have been the gold standard in doping detection due to their unparalleled accuracy, sensitivity, speed, and throughput. Classic techniques like gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) have been fundamental in doping screening, covering a wide range of low- to medium-molecular-weight drugs of abuse mandated by the World Anti-Doping Agency (WADA) regulations [7,15,18,30,31,32]. These methods complement each other effectively. For example, in the 2010s, triple quadrupole mass spectrometry emerged as a potent analytical tool, with Voelcker's team pioneering rapid detection of anabolic and narcotic doping agents in saliva and urine using nanostructured silicon-based surface-assisted laser desorption/ionization MS. Their assay enables high-throughput analysis of hundreds of biological samples, opening the door for real-time MS analysis during sporting events [8]. Crucially, MS and MS-based chromatographic techniques provide intricate structural insights, facilitating both qualitative and quantitative analyses at trace levels across diverse sample types [33,34]. Recent advancements in micro/nanofluidics have led to the integration and miniaturization of MS instruments. Coppieters' group engineered a nanoflow LC-MS device that automates filtration and detection of doping-relevant small peptide hormones in urine samples. This innovative approach combines nano-liquid chromatography with electrospray ionization MS (ESI-MS), reducing the required sample volume and enhancing detection sensitivity by transitioning from analytical-scale LC instruments to micro/nano LC scale systems [35]. Furthermore, as technology advances, MS methods have evolved from single MS molecules to dual MS coupled with liquid chromatography-tandem MS (LC-MS/MS). This technique merges the separation capabilities of high-performance liquid chromatography (HPLC) with the sensitive and selective detection abilities of MS/MS. In doping detection, HPLC-MS/TS excels not only in sensitivity, enabling the detection of minute traces of doping substances, but also in selectivity, uniting the separation potency of HPLC with the specific identification capabilities of MS/MS. Certainly, advancements in doping detection techniques have been remarkable. For example, Lu's research team made significant strides in identifying and characterizing higenamine metabolites in human urine, employing cutting-edge LC-MS/MS technology [5]. Meanwhile, Ponzetto's group utilized the UHPLC-MS/MS method to simultaneously quantify endogenous steroids and their phase II metabolites in serum, showcasing the method's robustness [36]. The integration of multiplexed mass spectrometers has ushered in an era of detailed and precise analysis of trace doping molecules within complex biological samples. These technological innovations have drastically improved the accuracy and sensitivity of doping detection.

In recent years, the advent of ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) has revolutionized the field of doping detection. Its exceptional sensitivity, selectivity, and speed have transformed the landscape of doping analysis. UHPLC-MS/MS has significantly enhanced separation efficiency, peak capacity, and analysis speed, enabling the rapid examination of a vast number of samples. This capability is particularly valuable for anti-doping laboratories dealing with substantial sample volumes.

Researchers have also made remarkable strides in developing specialized techniques. Lian's team pioneered an online purification and high-throughput platform, enabling the swift screening of 39 glucocorticoids in animal-derived foods like pork, chicken, milk, and eggs. This innovative approach involved online solid-phase extraction technology coupled with liquid chromatography-tandem high-resolution mass spectrometry (LC-HRMS) [37]. Additionally, Ai's group devised a rapid, sensitive, and confirmatory method, validated for detecting 14 diuretics in various animal-derived foods using UHPLC-MS/MS [6]. This high-throughput methodology not only enhances the analytical efficiency of the MS method but also enables a comprehensive analysis of potential stimulants within the tested samples, ensuring the utmost precision in the results.

However, while MS-based techniques boast essential qualities like selectivity, sensitivity, and stability, they are not without their challenges. These drawbacks, including the high cost of instruments, complex operation, demanding technical requirements, and intricate sample preparations, cannot be overlooked [38,39]. Additionally, the hidden conformation of the target doping molecules and the growing complexity of sample matrices necessitate detection systems with unparalleled resolution. Surprisingly, the integration

of high-resolution technology with LC/GC has not yet materialized as a comprehensive LC-MS/GC-MS screening method for doping control analyses. Existing high-resolution instruments in LC/GC often lack the necessary sensitivity for numerous compounds or exhibit insufficient linearity to meet the stringent criteria set by WADA. As a result, there has been no equivalent method using GC high-resolution MS that could replace the current MS-based screening techniques, making them indispensable [7]. These limitations emphasize the need for innovative doping detection methods, particularly in developing countries. The future lies in developing LC-MS or GC-MS methods that are not only sensitive enough, especially for exogenous compounds, but also economically feasible. This strategic approach is crucial for the ongoing evolution of MS-based methods in drug doping detection [7].

In the realm of doping detection, Mass Spectrometry (MS) is poised for significant advancements along several key pathways:

1. Elevated Sensitivity and Precision: MS techniques are on an unwavering quest to refine their sensitivity and precision. The objective is clear: to enhance detection limits while minimizing the potential for both false positives and negatives.
2. Synergy with Sample Preparation: Efforts are underway to seamlessly integrate MS with streamlined sample preparation methods. This strategic fusion aims to simplify the intricacies and time-consuming aspects of the doping detection process, making it more efficient and accessible.
3. Miniaturization and Mobility: A paradigm shift is occurring toward the development of miniaturized and portable MS devices. These innovations are designed to facilitate on-the-spot, real-time doping tests. Such advancements hold immense promise, enabling instant detection and intervention in doping cases during sporting events.
4. Exploring Metabolite and Biomarker Analysis: The spotlight is on developing methodologies for in-depth analysis of metabolites and biomarkers associated with doping. This research avenue not only aids in identifying novel doping agents but also elevates the accuracy and reliability of detection techniques.
5. Non-Targeted Comprehensive Analysis: MS-based non-targeted analysis is being extensively explored. This sophisticated approach allows for the simultaneous identification of a wide array of compounds, spanning known and unknown doping substances. This panoramic view of doping in athletes is invaluable, providing a holistic perspective.
6. Harnessing Advanced Data Analytics: The landscape of data processing and interpretation is evolving. Harnessing the power of cutting-edge technologies, including artificial intelligence and machine learning algorithms, enables the comprehensive analysis of intricate data sets. These advanced tools enhance the overall reliability and efficiency of doping detection, ensuring fairness and integrity in sports competitions.

Fluorescence Methods

In recent years, numerous cutting-edge fluorescence detection techniques have emerged, capitalizing on the intricate interactions between doping agents and fluorescent probes to create intricate fluorescent emission complexes. These methods, characterized by their high sensitivity, exceptional selectivity, and effortless portability, have gained prominence in the realm of doping analysis, outshining traditional methods like LC-MS and GC-MS [27,40,41].

For instance, Cheng's team pioneered a novel approach employing fluorescent covalent polymers (CPs) as signal carriers, revolutionizing the detection of methamphetamine hydrochloride doping. By exploiting the inherent interactions between target molecules and signal probes, CPs exhibited precise fluorescence quenching, showcasing the method's unparalleled efficiency [42]. Another breakthrough came from Yan's research group, which engineered a unique cucurbit[7]uril-anchored bis-functionalized metal-organic framework hybrid as a signal probe. Through strategic interactions with amphetamine-type stimulants, this innovative design led to fluorescence quenching, enabling the creation of a highly sensitive signal-off-type fluorescent sensor for detecting these stimulants [43]

However, sensors employing the "signal-off" mechanism have their limitations, especially when confronted with diverse unknown media and interferences. To counter these challenges, researchers have shifted their focus towards developing advanced "signal-on" fluorescent sensors for doping detection. In a pioneering effort, Hof's team employed a parallel synthesis-driven approach, crafting self-assembling dimeric supramolecular chemosensors. These ingenious dimer-dyes, acting as functional units, emitted fluorescence when exposed to micromolar concentrations of illicit drugs in water and saliva. This revolutionary technique enabled the creation of a "signal-on" fluorescent detection system for drug doping [44]

Yet, despite the remarkable progress, these techniques are rooted in the interactions between target molecules and fluorophores, leaving crucial gaps in our understanding of the intricate microstructures of doping agents and fluorescent substances [45]. This knowledge gap poses a significant challenge, especially when distinguishing between structurally similar doping agents or their metabolites using fluorescent

sensors. Additionally, the complexity of biological matrices demands heightened anti-interference capabilities from these sensors.
It's worth noting that the majority of existing fluorescence sensing methods rely on fluorescence quenching triggered by photo-induced charge transfers, rendering them susceptible to various interferences and mediums. Hence, the imperative lies in developing a highly specific fluorescence "turn-on" sensor. Such a sensor, boasting exceptional sensitivity, unmatched selectivity, and rapid responsiveness, is vital for accurate and reliable detection of drug doping, marking a pivotal advancement in the field. The future direction of fluorescence detection in doping analysis encompasses several key areas of development:
1. Increased Sensitivity: Efforts will be dedicated to enhancing the sensitivity of fluorescence detection methods in doping analysis. This will involve the creation of more sensitive fluorophores and the refinement of detection systems to achieve lower detection limits.
2. Advanced Multiplexing: Future initiatives will concentrate on expanding the multiplexing capabilities of fluorescence detection techniques in doping analysis. This expansion will enable the simultaneous identification of multiple doping substances or their metabolites, facilitating a more comprehensive and efficient analysis.
3. High-Throughput Screening: Significant focus will be placed on devising high-throughput screening methods employing fluorescence detection. This r rapid analysis of numerous samples is particularly vital for anti-doping agencies during major sports events.
4. Miniaturization and Portability: Progress will be made in downsizing fluorescence detection devices, making them more portable and user-friendly. This advancement will enable on-site doping testing, reducing the time and costs associated with transporting samples to centralized laboratories.
5. Integration with Sample Preparation Techniques: Exploration will center on integrating efficient sample preparation techniques with fluorescence detection methods. This integration will streamline the doping analysis process, enhancing the reliability and accuracy of results.
6. Utilization of Nanotechnology: Nanotechnology will play a pivotal role in the future of fluorescence detection in doping analysis. Functionalized nanoparticles can act as selective probes for doping agents, enhancing sensitivity and specificity in detection.
7. Advanced Data Analysis Algorithms: The application of sophisticated data analysis algorithms, such as machine learning and pattern recognition, will be pivotal. These algorithms will decipher complex fluorescence datasets, aiding in the identification of doping agents and patterns, thus improving the efficiency of doping detection.
These future endeavors aim to elevate the capabilities of fluorescence detection in doping analysis, contributing to the ongoing mission of ensuring fair and unblemished sports competitions.

Electroanalytical Methods
Similar to fluorescence methods, electroanalytical methods offer high sensitivity, making them valuable tools in the anti-doping field, particularly for doping agents or related metabolites exhibiting electrochemical activities [11]. These methods, known for their simplicity, speed, cost-effectiveness, and user-friendly nature, have gained prominence. They come with minimal matrix effects, and there's potential for miniaturization and use in portable electrochemical devices [46]. Electrochemical analytical methods are recognized for their sensitivity, precision, and accuracy, positioning them at the forefront of doping control analysis [47]. In the electrochemical detection process, doping agent detection modes can be broadly categorized into two groups: characteristic redox potential electrochemical sensors [48,49] and molecularly imprinted electrochemical sensors [46,50]. For instance, Tarley’s group developed a reliable voltammetric method to detect the designer drug, 1-(3-chlorophenyl)piperazine, using a boron-doped diamond electrode. These electrochemical sensors exhibited exceptional interference resistance, attributed to the auxiliary reagent sodium dodecyl sulfate. The method's accuracy was validated through comparison with liquid chromatography using a diode array detector as the reference method [48].
Over the past decade, molecularly imprinted electrochemical sensors have been extensively explored to enhance the anti-interference capabilities of electrochemical methods in biological sample detection. These sensors achieve specific target doping detection through polymer-mediated molecular imprinting on the electrode surface [50]. The molecularly imprinted polymer, specifically recognizing a target molecule, is created by including the target doping as an imprinted molecule during the polymerization process and then eluting it after polymerization is complete [46]. For instance, Han’s group developed an electrochemical-surface plasmon resonance sensor for detecting amphetamine-type doping based on molecular imprinting. Utilizing 3,4-methylenedioxyphenethylamine as the template and dopamine as the functional monomer, a molecularly imprinted polymer with specific recognition of amphetamine-type stimulants was applied to a

surface plasmon resonance chip using a one-step electrochemical polymerization method. This sensor exhibited low detection limits for 3,4-methylenedioxeamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) and resistance to interference from various illicit drugs and substances due to hydrogen bonding between the target and template molecules [50]

Additionally, π-π stacking between target and template molecules can facilitate doping recognition. Alizadeh's group developed an efficient voltammetric method for trace-level monitoring of methamphetamine (MTM) in human urine and serum samples. This method employed fast Fourier transform square wave voltammetry (FFT-SWV) to determine MTM at a molecularly imprinted polymer/multi-walled carbon nanotube-modified carbon paste electrode. The sensor, utilizing π-π stacking interactions, exhibited exceptional resistance to interference and demonstrated a linear response range and low detection limit, ensuring reliable results for real samples [51]

While electroanalytical methods have demonstrated excellent sensitivity in detecting doping agents, it's crucial to recognize their limitations. Electrochemical sensors often lack stability, impacting the reliability of results. Additionally, relying solely on characteristic redox peaks to distinguish between different doping agents doesn't account for potential interference from coexisting substances in complex biological samples. In modern analytical chemistry, electrochemical analysis serves primarily as a signal detection tool, frequently combined with specific bio-recognition units to create electrochemical detection-based biosensors, a topic we'll delve into later. It's essential to note that electrochemical assays, although highly sensitive, demand skilled techniques and are susceptible to measurement conditions. The preparation of chemically modified electrodes is often time-consuming and costly due to intricate modification processes, involving complex steps like incorporating modifiers onto the electrode surface and synthesizing polymeric matrices and composites. These complexities can lead to higher background currents and inconsistent outcomes, primarily due to the non-reproducibility of the preparation of the specific chemically modified substance.

The future direction of electroanalytical detection in the context of doping involves several key areas:

1. Device Miniaturization and Portability: The trend is moving towards developing compact, portable electroanalytical devices for doping detection. These advancements enable on-the-spot analysis and real-time monitoring, simplifying the detection process in sporting events and anti-doping efforts.
2. Enhanced Sensitivity and Specificity: Ongoing efforts are focused on increasing the sensitivity and specificity of doping detection methods. Researchers are exploring innovative sensing materials such as nanomaterials, molecularly imprinted polymers, and biomimetic receptors. These materials substantially improve detection limits and reduce the occurrence of false positive and negative results.
3. Simultaneous Detection of Multiple Compounds: The ability to detect multiple doping substances simultaneously is becoming increasingly crucial. Electroanalytical methods capable of identifying a wide range of prohibited substances at once enhance the efficiency and accuracy of doping control measures.
4. Integration with Diverse Analytical Techniques: Integrating electroanalytical methods with other analytical techniques like mass spectrometry and chromatography offers complementary insights, bolstering the reliability of doping detection. Researchers are exploring platforms that combine various techniques for comprehensive doping analysis.
5. Automation and Streamlined Analysis: Automation and high-throughput analysis are essential for efficient doping testing, particularly in large-scale sports events. Advancements in robotic systems and sophisticated data processing algorithms facilitate quicker and more accurate doping detection procedures.
6. Advances in Data Interpretation: Given the complexity of doping substances and the increasing volume of data, sophisticated data analysis techniques such as chemometrics, machine learning, and artificial intelligence are pivotal. These methods enhance the interpretation and reliability of doping test results.
7. Alignment with Anti-Doping Regulations: Progress in electroanalytical detection methods must align closely with evolving anti-doping regulations and policies. Sustained collaboration among scientists, sports authorities, and regulatory agencies is fundamental for the effective integration of electroanalytical techniques into anti-doping initiatives.

Crucially, it is imperative that advancements in doping detection technologies remain ethically sound and uphold the principles of fairness in sports.

Colorimetric Methods

When compared to electrochemical analysis methods, colorimetric assays may have a slightly lower sensitivity, but they come with a significant advantage: the ability to visually identify targets. This feature enables rapid screening and real-time detection of doping substances in the field [38]. Recently, colorimetric techniques have gained considerable attention in the field of doping detection due to their favorable characteristics, including simplicity, real-time analysis, cost-effectiveness, and applicability in on-site scenarios [10,26,38,39]. These methods exploit the interaction forces between stimulants and identification probes, causing color changes in the reaction system, allowing for quick and straightforward visual detection of drug doping. For example, Lim and colleagues achieved swift colorimetric detection of amphetamine-type stimulants through hydrogen bonding and π–π interactions between the drug doping agents and sensing materials [26] Kim's team developed a sensitive and rapid colorimetric detection method for amphetamine by forming donor-receptor (doping and probe) adducts. Activated furan-based probes (compound 1 and compound 3) created colored donor–acceptor Stenhouse adducts (compound 2 and compound 4) upon binding to the target doping molecule [38] Additionally, the distinctive optical properties of gold nanoparticles (Au NPs) enable the transformation of molecular interactions into detectable colorimetric signals visible to the naked eye. Wu's group utilized cysteine-modified Au NPs as signal probes for rapid colorimetric detection of clenbuterol, utilizing interactions between target molecules and the surface groups of gold nanoparticles [39]. Notably, the sensor developed in this study exhibited robust anti-interference capabilities and successfully detected clenbuterol in real blood samples.

Certainly, it's important to note that these colorimetric methods can be integrated into colorimetric test strips, allowing for immediate on-site detections. However, it's crucial to acknowledge that while colorimetric analysis is extensively used in doping control, its limited sensitivity poses challenges in detecting low concentrations of doping agents. The method's reduced sensitivity, especially in the presence of low levels of the target analogue, could lead to false-negative results. Furthermore, complex substances co-existing in biological samples might interfere with the sensors' detection outcomes. Additionally, the handling of color spot reagents raises health concerns due to the highly corrosive and toxic nature of many of these chemicals. Consequently, there is a growing interest in developing color spot tests that are exceptionally selective, highly sensitive, rapid, and safe for accurate on-site testing [10,26,39].

The future direction of colorimetric detection methods for doping encompasses several crucial aspects:

1. Increased Sensitivity: Efforts will focus on improving the sensitivity of colorimetric detection techniques through the exploration of new materials and meticulous experimental optimization. This will enable the detection of doping agents at even lower concentrations, ensuring the method's reliability and practicality.
2. Multiplex Detection: Ongoing research aims to develop colorimetric methods capable of simultaneously identifying multiple doping agents within a single test. This multiplexing capability offers a more comprehensive analysis, saving both time and resources compared to traditional methods.
3. Portable and Rapid Devices: The growing demand for on-site, real-time detection of doping agents, especially in sports events, is driving the development of portable and rapid detection devices. These devices will be user-friendly, sensitive, and capable of delivering swift results.
4. Integration of Emerging Technologies: Colorimetric detection methods are set to benefit from the integration of cutting-edge technologies like nanomaterials, microfluidics, and biosensors. These innovations have the potential to enhance the accuracy, sensitivity, and reliability of colorimetric doping detection.
5. Standardization and Validation: To ensure the widespread adoption of colorimetric detection methods for doping, establishing standardized protocols and rigorously validating method performance are essential. This approach guarantees consistent and dependable results across diverse laboratories, facilitating the acceptance of colorimetric detection in both research and practical applications.

Biosensors

Biosensors are analytical devices created by combining various bioactive materials with physical/chemical signal transducers. They offer significant advantages such as high sensitivity, user-friendly operation, excellent selectivity, and minimal sample consumption. This versatility allows biosensors to accurately and swiftly detect various analytes, including complex doping substances. Traditional biosensors rely on specific antibody-antigen immunoreaction interactions. These biosensors involve immobilizing antibodies on specific substrates to capture antigen molecules. Subsequent utilization of appropriate optical or electrochemical methods helps extract and amplify the weak immunorecognition signals. In the realm of doping detection, many immunosensors, capitalizing on these principles, have played crucial roles. For instance, Dignan’s group harnessed the specificity of biological antibodies. They achieved precise, cost-

effective, and semi-quantitative detection of morphine using a centrifugal microfluidic colorimetric enzyme-linked immunosorbent assay [14]. Yuan and colleagues successfully developed a hydrophilic C60-based nanomaterial and designed an immunosensor for detecting erythropoietin, utilizing the inner redox activity of fullerene (depicted in This immunosensor demonstrated a broad linear range and a relatively low detection limit for erythropoietin [4]. However, it's crucial to recognize that the limited stability of biological antibodies and the high cost involved in their preparation have hindered their widespread application. Furthermore, immunoassays face challenges, including the potential for false negatives or positives due to detection ambiguities (such as faint signals), degradation of antibodies, and cross-reactivity with other substances. Several studies using different commercially available assays have indicated a detection accuracy of 70% for false positives and sometimes 50% for false negatives [53]. Consequently, immunoassays are primarily employed as initial on-site screening methods, followed by confirmation using chromatographic techniques to validate the results.

The systematic exploration of aptamers using the in vitro SELEX technique for various bioassay applications has substantially widened the scope for enhancing biosensor performance [54]. Aptamer-based biosensors, distinguished from immunosensors, offer advantages such as reduced detection costs, heightened biostability, and superior interference resilience, making them highly adaptable in doping detection scenarios. Acting as adaptable biorecognition components, aptamers exhibit precise binding to a diverse array of target molecules, encompassing doping agents. Over time, biosensors employing aptamers as recognition units have gained significant traction in doping detection endeavors [16,55]. A case in point is the work by Sun's research group, which utilized the aptamer sequence specific to methamphetamine as a recognition element, leading to the development of an electrochemical sensor for methamphetamine detection via square wave voltammetry. This sensor showcased remarkable sensitivity, detecting methamphetamine well below the clinical threshold, making it highly suitable for widespread deployment [16].

Given the distinct recognition benefits conferred by aptamers [56], biosensors incorporating aptamer technology stand poised to become the predominant method for doping detection in the future. While substantial progress has been made in immune-based sensors, their broader application in doping detection entails the development of high-throughput immunoassays capable of concurrently detecting multiple types of doping agents. Furthermore, the evolution of biochips holds significant promise in the domain of doping detection. Consequently, the future trajectory of biosensors reliant on immune recognition and aptamer recognition will focus on engineering faster and more sensitive biosensors with enhanced detection capabilities.

The future trajectory of biosensors in doping detection could encompass several key facets:

(1) Enhanced Sensitivity: Elevating biosensors' sensitivity will empower the detection of minute traces of doping agents within biological samples, thereby enhancing the precision and reliability of detection outcomes.

(2) Multi-Substance Detection: Pioneering biosensors that can simultaneously identify multiple banned substances will be pivotal in countering athletes' use of diverse illicit compounds. Achieving this entails integrating diverse recognition elements or leveraging cutting-edge nanotechnology.

(3) Miniaturization and Portability: Downsizing biosensor devices will enable real-time, on-site monitoring of doping, fostering convenience for authorities conducting tests during sports events. Portable biosensors will also facilitate doping detection within athletes' training environments.

(4) Non-Invasive Sampling: Advancements in non-invasive sampling methods, like saliva or sweat-based detection, can minimize athletes' discomfort during sample collection. Biosensors tailored to detect doping agents from these alternative sources may gain traction.

(5) Improved Selectivity: Enhancing biosensors' selectivity will mitigate erroneous positive and negative results in doping detection. This could involve developing precise recognition elements or integrating sophisticated signal processing algorithms.

(6) Integration with Data Analysis: Merging biosensors with data analysis tools, such as machine learning algorithms, can enrich the interpretation of detection outcomes. This integration offers valuable insights into doping-related patterns, trends, and individual athlete profiles.

(7) Enhanced Affordability: Rendering biosensor technology more cost-effective will encourage broader adoption, not only in professional sports but also in grassroots and amateur-level competitions. Streamlining manufacturing processes and embracing scalable production methods can drive cost reductions.

Crucially, the future progress of biosensors in doping detection necessitates ongoing collaboration among researchers, sports authorities, and regulatory bodies. This collaboration is vital to address emerging doping techniques and proactively confront the challenges posed by the evolving landscape of doping substances.

Gene Doping Detection

gene doping denotes the utilization of genetic engineering methods to amplify athletic abilities. There are diverse approaches within gene doping, each with distinct merits and demerits.

(1) Gene Therapy: This method entails inserting a healthy version of a gene into a person's cells to rectify a genetic flaw or augment a specific trait. This process often employs modified viruses to transport the desired gene. In human applications, gene therapy has been employed to address specific genetic disorders, like cystic fibrosis and muscular dystrophy.

(2) Epigenetic Modification: This technique involves altering gene expression without changing the actual DNA sequence. By modifying chemical tags, such as methyl groups, attached to DNA, genes can be activated or deactivated, influencing athletic performance.

(3) Gene Editing: Gene editing employs molecular tools like CRISPR/Cas9 to precisely modify an organism's DNA sequence. This method could boost athletic prowess by introducing advantageous mutations or eliminating detrimental ones.

(4) Gene Transfer: This method involves transferring genes from one organism to another. For instance, transferring genes responsible for augmented muscle growth from a bull to a human might enhance athletic abilities.

Gene doping raises ethical and safety concerns, leading numerous sports organizations to prohibit its usage in competitions. Despite these bans, the technology continues to progress, necessitating regulatory bodies to stay abreast of advancements in the field to prevent its misuse.

Gene doping targets genes controlling physical traits, such as muscle growth, oxygen metabolism, and endurance, with the aim of enhancing athletic abilities beyond natural limits [60,61]. The approach involves introducing or modifying these specific genes. Experiment design for gene doping necessitates careful selection of relevant genetic markers or sequences, crucial for detecting the presence or absence of the altered gene [22,62].

For instance, if the objective is to introduce a gene promoting increased muscle growth, the assay might involve scrutinizing changes in gene expression levels of markers like myostatin or follistatin. Additionally, the assay could encompass genome sequencing or analyzing the structure of the modified gene to confirm accurate insertion. Obtaining results from gene doping experiments poses challenges, requiring precise measurement of changes in an organism's physiological or physical traits. This often involves complex assays like muscle biopsies, assessments of oxygen uptake, or evaluations of physical performance. Moreover, results may need prolonged observation to assess the lasting impact of the gene modification. It's crucial to note that gene doping is universally prohibited by most sports organizations due to safety and ethical concerns. While advancements in gene editing and delivery technologies hold future potential, their responsible application under stringent oversight by regulatory bodies is essential to ensure athlete safety and well-being [21,63].

Gene doping stands as a clear violation of anti-doping regulations within the sports sector, and its utilization for enhancing athletic performance carries significant legal ramifications. In China, the oversight of gene doping in sports falls under the jurisdiction of the General Administration of Sport and affiliated sports governing bodies, bolstered by national legal frameworks. As stipulated in the regulations governing the supervision of sports events, athletes or their teams found employing gene doping methods will be subject to a range of penalties, including suspension, nullification of awards, and financial penalties, among others. Furthermore, if these actions are deemed to constitute criminal offenses, those involved may face arrest, legal proceedings, and consequent sentencing [61]. Fundamentally, gene doping constitutes an unethical practice in the realm of sports, leading to profound legal consequences and leaving lasting impacts on those engaging in such activities. Consequently, its eradication is essential to uphold fair competition and safeguard the fundamental values of sportsmanship [13,64].

Gene doping detection techniques are broadly categorized into direct and indirect methods. Direct methods involve detecting prohibited substances or their byproducts directly in physiological samples, akin to conventional drug doping detection. Indirect methods, on the other hand, focus on monitoring the body's responses resulting from the introduction and expression of transgenes or gene doping agents. Indirect methods can be viable if the detected gene doping matches endogenous expression products or rapidly metabolized substances. However, factors like age, gender, ethnicity, and even immune responses induced by natural viral infections can complicate result interpretation and elevate the risk of false positives [65]. Consequently, direct methods remain popular for developing effective gene doping detection techniques. This section further classifies direct methods into PCR-based and PCR-free methods, providing a brief overview of their recent advancements in gene doping detection.

Typical PCR Methods

In contrast to natural genetic material, gene doping typically involves the use of complementary DNA (cDNA). Consequently, their unique exon/exon junctions serve as distinctive target regions, allowing them to be distinguished from intrinsic genes [62]. PCR detection technology, renowned for its precision and reproducibility, stands as the benchmark in gene detection and has been extensively explored. Not surprisingly, it has gained widespread application in the domain of gene doping detection in recent years. Recognizing its significance, WADA has issued specific guidelines on employing PCR technology in gene doping detection. These guidelines advocate using whole blood as the source for DNA extraction, acting as a template for transgene detection through quantitative PCR employing target-specific primers and hydrolysis probes [66]. Several gene doping detection methods based on quantitative PCR have been developed, utilizing diverse starting materials like whole blood, plasma, and urine, and employing different PCR systems including real-time PCR and digital PCR [19,22,62,67].

Certainly, Ryder's research group effectively identified gene doping in horses using quantitative real-time PCR (qPCR) techniques [68]. They employed two innovative approaches: attaching sequence-ready adapters to qPCR products and utilizing tailed primers in qPCR assays. These methods enabled the direct analysis of amplified qPCR products from five candidate genes through next-generation sequencing, eliminating the need for additional amplification steps. Another significant study by Tozaki et al. focused on assessing the robustness, specifically the specificity and sensitivity, of digital PCR and real-time PCR in transgene detection. Through the strategic use of modified primers and probes that partially matched the target template, they achieved low-copy transgene detection through nested digital PCR [19]. These advancements have gradually elevated PCR technology into a reliable method for detecting gene doping. Despite their potential, these methods have not yet found application in accredited anti-doping laboratories due to a lack of validation [65]. Moreover, most of these protocols involve multiple stages, increasing the risk of contamination and demanding substantial expertise, time, and expensive equipment. Hence, exploring simpler and more rapid sample manipulation techniques becomes imperative. Notably, PCR methods utilizing SYBR fluorescent dyes as signal probes prove unsuitable for detecting gene doping in real samples. The reason lies in their utilization of a non-specific fluorescent dye, rendering them incapable of distinguishing specific double-stranded DNA and highly susceptible to generating false positive results when analyzing biological samples.

Certainly, the future trajectory of PCR-based methods in doping detection can be outlined as follows:

1. Enhanced Sensitivity: PCR techniques will undergo continuous advancements to heighten their sensitivity, enabling the detection of minuscule amounts of doping agents. This progression will involve the refinement of amplification methods and the incorporation of cutting-edge detection technologies.
2. Multiplexing Capabilities: The demand for simultaneous detection of multiple doping agents is growing. Future developments will concentrate on enhancing the multiplexing capabilities of PCR methods, allowing the identification of several target genes or molecules within a single analysis.
3. Accelerated Testing: Initiatives will focus on reducing the turnaround time for doping detection using PCR methods. Optimization of protocols, simplification of sample preparation steps, and the introduction of swifter amplification and detection technologies will be pivotal in achieving rapid results.
4. Biomarker Exploration: Ongoing research will seek novel and specific biomarkers serving as doping indicators. PCR methods will be instrumental in amplifying and identifying these biomarkers, facilitating their validation and integration into doping detection protocols.
5. Non-Invasive Sampling: Non-invasive sampling methods, such as saliva or urine testing, are gaining prominence in doping control. Future developments will concentrate on PCR-based techniques capable of amplifying and detecting doping biomarkers from non-invasive samples.
6. Integration with Advanced Technologies: PCR methods will be synergized with cutting-edge technologies like microfluidics, nanotechnology, and high-throughput sequencing to augment doping detection. This integration will enhance sensitivity, increase throughput, and reduce sample volume requirements.
7. Standardization and Quality Assurance: Standardizing PCR-based doping detection methods is crucial for ensuring dependable and consistent outcomes. International standards, guidelines, and quality assurance programs will be established, ensuring the precision and reproducibility of PCR-based doping tests.

These future advancements in PCR techniques aim to enhance the efficacy, expediency, and dependability of doping detection, ultimately upholding the principles of fairness and integrity in sports.

Sequencing-Based Methods

In comparison to traditional PCR methods, loop-mediated isothermal amplification (LAMP) stands out as an advanced technique for nucleic acid amplification. It operates efficiently, specifically, and rapidly under constant temperature conditions. LAMP offers distinct advantages over conventional PCR approaches, including simplified reaction setups and quicker amplification processes. Typically conducted at a stable temperature range of 60–65 °C, LAMP achieves a substantial 109-fold amplification within a mere hour [70]. Despite its simpler operational requirements compared to PCR, the underlying amplification mechanism of LAMP is more intricate. Essential to this process are primers that form unique foldback structures and their subsequent extension by a strand-displacing DNA polymerase [65,69,70]. Through this DNA polymerase-driven amplification, the target gene sequence undergoes cascade amplification, enabling the accurate detection of gene doping. Its straightforward methodology, exceptional specificity, and robustness make LAMP an attractive option for nucleic acid detection, especially in settings with limited resources. For instance, researchers like Leuenberger et al. have harnessed LAMP as a substitute for PCR in their innovative gene doping detection approach. By designing primers meticulously to initiate LAMP reactions with exceptional specificity and efficiency, this method allows for a simple, swift, and precise detection of gene doping, observable without sophisticated equipment [65]. Beyond the scope of LAMP and PCR techniques that target specific exon/exon sequences in intron-less transgenes, assays based on next-generation sequencing (NGS) provide enhanced capabilities by offering increased throughput and covering a broader range of potential gene doping areas [71,72]. NGS enables the rapid and simultaneous sequencing of millions of DNA fragments, furnishing intricate insights into the genetic composition of an individual or a sample. In gene analysis, NGS holds a pivotal role in the identification, characterization, and comprehension of genetic variations linked to diverse diseases and conditions. This technology provides a comprehensive overview of the entire genome, encompassing both coding and non-coding regions, facilitating the identification of disease-causing mutations, structural variations, and patterns of gene expression.

Furthermore, in genomics research, whole-genome resequencing (WGR) is a methodology employed to obtain the complete DNA sequence of an individual's genome. This approach involves comparing the individual's DNA sequence with a reference genome to pinpoint variations, encompassing single nucleotide polymorphisms (SNPs), insertions, deletions, and structural variances [73]. Both Next-Generation Sequencing (NGS) and WGR stand out as potent tools in genomics studies for DNA sequence analysis. Despite their similarities, there are distinctive features that set them apart. NGS encompasses a suite of high-throughput sequencing technologies enabling the simultaneous sequencing of millions of DNA fragments. This process involves breaking down the DNA sample into smaller fragments, attaching adapters to these fragments, and then amplifying them through multiple cycles of sequencing. In contrast, WGR involves sequencing an organism's entire genome, covering both coding and non-coding regions, to identify genetic variances in comparison to a reference genome. In WGR, an individual's or a population's DNA is either sequenced from scratch or compared to a known reference genome.

WGR offers several advantages for SNP analysis. Firstly, it provides comprehensive coverage, enabling an in-depth analysis of the entire genome and offering a holistic perspective on all potential SNPs. Secondly, it facilitates the discovery of rare and novel variants by scrutinizing the complete genome, allowing the identification of SNPs that might be missed by other methods [73]. On the other hand, NGS offers distinct advantages for SNP analysis. Firstly, it boasts high-throughput capabilities, allowing the simultaneous analysis of millions to billions of DNA fragments within a single sequencing run. Secondly, its accuracy is enhanced through the implementation of various error correction algorithms during data processing, minimizing sequencing errors and ensuring more dependable SNP calling [71,72]. It is crucial to emphasize that various DNA enzymes hold the potential to revolutionize gene doping detection, potentially replacing the intricate PCR detection technique [65]. Recent advancements in DNA enzyme technology offer a promising alternative for identifying gene doping. DNA enzymes, also known as DNAzymes or catalytic DNA, are synthetic single-stranded DNA molecules endowed with enzymatic activity. For instance, polymerases [74] can catalyze the synthesis of polymers, specifically nucleic acids like DNA or RNA. Widely utilized in PCR-based detection and DNA sequencing methods such as Sanger sequencing and next-generation sequencing (NGS), these enzymes incorporate modified nucleotides, halting DNA synthesis at specific points. Analyzing this termination pattern provides insights into the original DNA sequence. Endonucleases [75] can cleave phosphodiester bonds within DNA or RNA molecules. They play a crucial role in various applications related to DNA and RNA analysis, recognizing specific DNA sequences and cutting DNA at or near these sites. This capability facilitates the analysis of DNA fragments of specific sizes and simplifies the isolation of genes or specific DNA regions of interest. Exonucleases [76] aid in the degradation of nucleic acids by removing nucleotides from the ends of DNA or RNA molecules. They

recognize and cleave specific DNA or RNA sequences, serving essential functions in molecular biology research. Metal ion-dependent DNAzymes [77], artificial DNA enzymes, exhibit catalytic activity in the presence of specific metal ions. These DNA molecules fold into precise three-dimensional structures, allowing them to bind metal ions and catalyze reactions. By designing DNAzymes requiring specific metal ions, assays detecting target gene doping can be developed. G-quadruplex-based catalytic nucleic acids [78] encompass DNA or RNA molecules possessing both G-quadruplex structures and catalytic activity. Their unique structure and catalytic abilities make them valuable tools for sensitive, specific, and versatile genetic analysis and gene expression regulation.

As a whole, these DNA enzymes have the ability to identify specific genetic sequences and catalyze targeted reactions. The key advantage of using DNA enzymes in gene doping detection is their direct amplification and identification of modified genes, bypassing the need for complex PCR processes. This eliminates the complexity and time constraints associated with PCR amplification, streamlining the entire detection process. Additionally, DNA enzymes offer high specificity and sensitivity, ensuring precise detection of gene doping. Moreover, they are more stable and cost-effective than traditional PCR methods, requiring no sophisticated lab equipment or specialized techniques for analysis. This accessibility makes them practical for widespread use in anti-doping efforts. In summary, employing various DNA enzymes for gene doping detection holds significant promise in replacing the intricate PCR method. These enzymes offer simplicity, accuracy, exceptional specificity, and cost-effectiveness. Continuous research and development in this area can greatly enhance anti-doping measures in the sports community.

CRISPR Methods

Aside from the widely utilized DNAzymes, the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats) technique, renowned for its gene-editing capabilities, has displayed significant potential in the domains of biosensing and bioassays in recent years [57,79,80,81]. Due to the DNase/RNase attributes of the CRISPR/Cas system, its fusion with various nucleic acid recognition and amplification reactions enables the creation of a diverse array of potent methods. These methods offer highly efficient detection of numerous target analytes, including gene doping. In comparison to the conventional DNAase-based assays employed in qPCR, LAMP, and NGS, the CRISPR detection approach provides enhanced sensitivity, rapidity, specificity, and adaptability in identifying specific DNA sequences. CRISPR technology, widely employed in genome editing and genetic engineering, constitutes a natural immune system found in bacteria and archaea. This system can pinpoint and cleave DNA, facilitating the repair, replacement, or deletion of specific gene sequences. The fundamental principles of CRISPR technology involve three key elements: single guide RNA (sgRNA), CRISPR RNA (crRNA), and a fluorescence reporter gene. Firstly, sgRNA is produced from two constituents: a guiding RNA (tracrRNA) and a specific sequence. The guiding RNA segment binds to the Cas protein, aiding in the precise location of target DNA by the Cas enzyme. The specific sequence is employed for recognizing the target DNA, forming a "signal sequence" upon pairing with a specific sequence on the target DNA. This allows the Cas enzyme to accurately identify and bind to the target DNA. Secondly, crRNA, an RNA molecule, is custom-designed to specifically pair with a particular sequence in the target DNA. In CRISPR genome editing, crRNA is engineered to guide the Cas enzyme's activity, ensuring the precise cleavage of the target DNA sequence. Thirdly, fluorescent reporter genes serve as common labels in CRISPR technology. By merging a fluorescent gene with the target gene, it becomes possible to observe whether the target gene has been successfully altered.

Recently, our research team developed an innovative CRISPR/Cas-based approach for assessing gene doping. This method, incorporating CRISPR/Cas12a and multiplexed recombinase polymerase amplification (RPA), demonstrated remarkable specificity and sensitivity for swift, robust, and on-site gene doping detection. Moreover, it effectively identified transgenes in a cellular model and integrated a four-plexed microfluidic chip for simultaneous detection of three transgenes [82]. Additionally, Sung's group achieved the development of an in vitro CRISPR-Cas9 cleavage system to analyze the site-specific exogenous gene doping of human erythropoietin [69]. The exceptional capabilities of CRISPR-Cas9 have enabled straightforward, precise, and sensitive assays. Moreover, a variant called CRISPR-deadCas9 has been utilized for genetic doping detection. Sung’s research team successfully detected exogenous human erythropoietin gene doping using CRISPR/deadCas9. Under optimal conditions, this method achieved highly sensitive detection of gene doping in whole blood samples, detecting concentrations as low as 12.3 fM (7.41 × 105 copies) and up to 10 nM (6.07 × 1011 copies) within just one hour [57]. Notably, as far as our knowledge extends, neither CRISPR-Cas13 nor CRISPR-Cas13a has been employed in gene doping

detection. This might be attributed to the fact that CRISPR-Cas13 and CRISPR-Cas13a are primarily utilized for RNA cleavage.

MS-Based Methods

In addition to nucleic acid-based detection methods, traditional drug doping detection techniques utilizing mass spectrometry (MS) have been successfully adapted for gene doping detection. Thevis's research group, for instance, has pioneered MS-based gene doping detection methods, applying conventional analytical doping methods to identify new dopants. For instance, they utilized HPLC-HRMS/MS to detect the presence of the exogenous protein Cas9 from Streptococcus pyogenes in athlete serum samples. By monitoring the misuse of the CRISPR/Cas system, this approach helps assess gene doping in athletes. Moreover, biosensors, particularly various aptasensors traditionally used for drug doping detection, can also be employed for gene doping detection.

In comparison to conventional PCR-based gene doping detection methods, PCR-free gene doping detection techniques offer distinct advantages in terms of practicality, detection costs, and equipment requirements. We foresee a future trend where PCR-free gene doping detection methods will gradually replace PCR methods and become the primary approach for gene doping detection.

The future trajectory of PCR-free methods for detecting doping involves several key aspects:

1. Enhancing Sensitivity: It is crucial to boost the sensitivity of PCR-free methods to detect doping substances at even lower concentrations. This can be achieved through advanced sample preparation techniques, optimized detection platforms, and innovative signal amplification strategies.
2. Multiplexing Capabilities: Developing PCR-free methods capable of simultaneously detecting multiple doping substances will significantly enhance their efficiency. This can be accomplished by designing specific probes or primers targeting different doping targets and utilizing advanced multiplexing detection technologies.
3. Portable and Point-of-Care Devices: Creating portable and user-friendly devices for PCR-free doping detection enables rapid on-site analysis. These devices should be easy to operate and provide accurate results promptly.
4. Integration with Other Analytical Techniques: Integrating PCR-free methods with other techniques like MS or immunoassays can enhance selectivity and accuracy. This integration offers complementary information, improving overall detection capabilities.
5. Standardization and Validation: Establishing standard protocols and validation procedures is essential for widespread adoption. Defining performance criteria, optimizing sample preparation, and conducting rigorous validation experiments using reference materials are necessary steps.
6. Data Analysis and Interpretation: Developing advanced algorithms for data analysis and interpretation tailored to PCR-free doping detection is crucial. These algorithms ensure precise identification of doping substances based on specific signals generated by the detection method.

Table 6: Some Quick Screening in Doping Analysis: Identifying and detecting substances used for doping through mass spectrometric analysis conducted with liquid and gas chromatography.

Quick Screening in Doping Analysis: Identifying and detecting substances used for doping through mass spectrometric analysis conducted with liquid and gas chromatography.										Ref.
S. No	**Name**	Formula e	M W	CAS	RO A	Human Metabol ism	Excretion	Analytical approach		
								Extract ion	Techn ique	
1	3,4-Methylenedioxya mphetamine	C10H13 NO2	179 .22	4764 -17-4	O, SL, IN	Hepatic	Renal	Enzy. hydrol ysis LLE/S PE	GC-MS-NPD/ LC-MS/ MS/G C-MS/ MS/L	[2]

									C-HRMS	
2	4-Methylamphetamine	C10H15N	149.23	22683-78-9	O, SL, IN	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/ LC-MS/ MS/GC-MS/ MS/LC-HRMS	[32]
3	Adrafinil	C15H15NO3S	289.35	63547-13-7	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/ LC-MS/ MS/GC-MS/ MS/LC-HRMS	[34]
4	Amfepramone	C13H19NO	205.3	134-80-5	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/ LC-MS/ MS/GC-MS/ MS/LC-HRMS	[53]
5	Amiphenazole	C9H9N3S	191.25	490-55-1	O	Hepatic	Renal	Direct LLE/SPE	LC-MS/ MS/GC-MS/ MS/LC-HRMS	[43]
6	Amphetamine	C9H13N	135.2	300-62-9	O, IV, IN, IS, R, SL	Hepatic,	Renal	Enzy. hydrolysis LLE/SPE	GC-MS-NPD/ LC-MS/ MS/GC-MS/	[32]

									MS/LC-HRMS	
7	Amphetaminil	C17H18N2	250.33	17590-01-01	O, SL, IN	Hepatic,	Renal	Direct LLE/SPE	Direct LLE/SPE	[6]
8	Modafinil	C15H15NO2S	273.35	112111-43-0	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-	[37]
9	Benzphetamine	C17H21N	239.35	0156-08-01	O, SL, IN	Hepatic,	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-	[54]
10	Benzylpiperazine	C11H16N2	176.25	2759-28-6	O, IV, IS	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[31]
11	Bromantane	C16H20BrN	306.24	87913-26-6	O, SL, IN	Hepatic,	Renal	Enzy. hydrolysis LLE/SPE	GC-MS/GC-MS/MS/LC-HRMS	[54]
12	Cathine	C9H13NO	151.2	492-39-7	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-	[39]

									MS/MS/LC-HRMS	
13	Cocaine	C17H21NO4	303.35	50-36-2	T, O, IS, IV	Hepatic	Renal Benzoylecgonine ecgonine methyl ester, ecgonine 1% unchanged norcocaine	Direct/Enz. Hy LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[67]
14	Cyclazodone	C12H12N2O2	216.09	14461-91-7	O	Hepatic	Renal	Direct/Enz. Hy LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[98]
15	Dimethylamphetamine	C11H17N	163.25	4075-96-1	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[56]
16	Ephedrine	C10H15NO	165.23	299-42-3	O, IV, IM, SC	Minimal Hepatic	Renal (22-99%)	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[96]
17	L-adrenaline	C9H13NO3	183.2	51-43-4	IV, IM, ET, IC	Adrenergic synapse (MAO and	Renal			[102]

						COMT)				
18	Etamivan	C12H17NO3	223.26	304-84-7	O	Hepatic	Renal	Direct/Enz. Hy, LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[106]
19	Ethylamfetamine	C11H17N	163.25	457-87-4	O, SL, IS, IN, IV, R	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[90]
20	Etilefrine	C10H15NO2	181.23	709-55-7	O	Hepatic	Renal	Enzy. hydrolysis LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[98]
21	Fenbutrazate	C23H29NO3	367.48	4378-36-3	O	Hepatic	Renal	Direct/Enz. Hy LLE/SPE	LC-MS/MS/GC-MS/MS/LC-HRMS	[77]
22	Fencamfamine	C15H21N	215.33	2240-14-4	O	Hepatic	Renal	Direct/Enz. Hy LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[87]

23	Fencamine	C20H28N6O2	384.48	28947-50-4	O	Hepatic	Renal	Direct /Enz. Hy LLE/SPE	LC-MS/MS/GC-MS/MS/LC-HRMS	[44]
24	Fenethylline	C18H23N5O2	341.4	3736-08-01	O	Hepatic	Renal Amphetamine (24.5% of dose) Theophylline (13.7% of dose)	Direct /Enz. Hy LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[55]
25	Fenfluramine	C12H16F3N	231.26	458-24-2	O	Hepatic Dealkylation	Renal Norfenfluramine	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[32]
26	Fenproporex	C12H16N2	188.26	16397-28-7	O	Hepatic Converted to amphetamine	Renal, Unchanged (5-9%)Amphetamine(30 to 60%)	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[43]
27	Furfenorex	C15H19NO	229.31	13445-60-8	O	Hepatic	Urine	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRM	[65]

									S	
28	Heptaminol	C8H19NO	145.24	372-66-7	O, IV, IM	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/ LC-MS/ MS/GC-MS/ MS/LC-HRMS	[34]
29	Parahydroxyamfetamine	C9H13NO	151.2	1518-86-1	O	Hepatic	Renal	Enzy./ acid hydro LLE/SPE	GC-MS/LC-MS/ MS/GC-MS/ MS/LC-HRMS	[96]
30	Isometheptene	C9H19N	141.25	0503-01-05	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/ LC-MS/ MS/GC-MS/ MS/LC-HRMS	[57]
31	Levomethamphetamine	C10H15N	149.2	33817-09-03	IN	Hepatic	Renal	Direct /Enz. Hy LLE/SPE	GC-MS-NPD/ LC-MS/ MS/GC-MS/ MS/LC-HRMS	[76]
32.	Meclofenoxate	C12H16ClN O3	257.71	51-68-3	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/ LC-MS/ MS/GC-MS/	[55]

									MS/LC-HRMS	
33.	Mefenorex	C12H18ClN	211.73	17243-57-1	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[32]
34.	Mephentermine	C11H17N	163.25	100-92-5	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[49]
35	Mesocarb	C18H18N4 O2	322.36	34262-84-5	O	Hepatic	Renal Hydroxymesocarb	Enzy./acid hydro LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[85]
36	Methamphetamine	C10H15N	149.23	537-46-2	P,O, IV, IS, IN, S	Hepatic	Renal Unchanged (30-54%) Amphetamine (active 10-23%) 4-hydroxymethamphetamine 4-hydroxyamphetamine,norephedrine, 4-	Direct /Enz. Hy LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[56]

							hydroxynorephedrine			
37	Methylphenidate	C14H19NO2	233.3	113-45-1	O, TD	Hepatic (80%)	Renal Ritalinic acid	Direct/Enz. Hy LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[75]
38	Modafinil	C15H15NO2S	273.35	68693-11-8	O	Hepatic	Renal Unchanged (0% to 18.7%) Modafinilic acid Modafinilsulfone (inactive)	Direct/Enz. Hy LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[85]
39	Methylephedrine	C11H17NO	179.26	552-79-4	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[32]
40	Norfenefrine	C8H11NO2	153.17	536-21-0	O	Hepatic	Renal	Direct/Enz. Hy LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[21]
41	Norfenfluramine	C10H12F3N	203.2	19036-73-8	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/L	[22]

									C-HRMS	
42	Octopamine	C8H11NO2	153.17	104-14-3	O	Hepatic	Renal	Enzy./acid hydro LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[34]
43	Ortetamine/ methylamphetamine	C10H15N	149.23	5580-32-5	O	Hepatic	Renal	Direct/Enz. Hy LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[53]
44	Oxilofrine	C10H15NO 2	181.23	365-26-4	O	Hepatic	Renal	Enzy./acid hydro LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[33]
45	Pemoline	C9H8N2O2	176.17	2152-34-3	O	Hepatic	Renal	Enzy.hydro LLE/SPE	GC-MS//GC-MS/MS/LC-HRMS	[21]
46	Pentylenetetrazol	C6H10N4	138.17	54-95-5	O	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[20]

47	Phendimetrazine	C12H17NO	191.27	0634-03-07	O	Hepatic	Renal phenmetrazine (~30%)	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[11]
48	Phenmetrazine	C11H15NO	177.24	134-49-6	O, IV, IN, IS, S	Hepatic	Renal Unchanged (19%) 70% dose eliminated within 24 h	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[100]
49	Phentermine	C10H15N	149.23	0122-09-08	O, IS, IV	Hepatic	Renal	Direct LLE/SPE	GC-MS-NPD/LC-MS/MS/GC-MS/MS/LC-HRMS	[40]
50	Prenylamine	C24H27N	329.48	390-64-7	O	Hepatic	Renal Amphetamine	Direct /Enz. Hy LLE/SPE	GC-MS/LC-MS/MS/GC-MS/MS/LC-HRMS	[42]

V. DISCUSSION

Comparison of the Reported Assays

Doping detection methods can be broadly categorized into drug doping detection and gene doping detection. A comparison of these methods, along with commonly used doping detection assays, is presented in Table 3. Among the widely employed techniques, GC-MS and LC-MS/MS, both based on mass spectrometry (MS), are predominant. These methods are highly effective, enabling the identification of minute quantities and metabolites of banned substances in biological samples. While fluorescence, electrochemical, and colorimetric methods offer simplicity and cost advantages over MS-based techniques, they lack the same level of accuracy and sensitivity. In the realm of gene doping detection, two primary assays are currently utilized: PCR and PCR-free methods, each with distinct strengths and weaknesses. PCR exhibits superior sensitivity, capable of detecting even a single copy of target DNA, and boasts high specificity due to the use of specific primers binding to the target sequence. On the other hand, PCR-free methods excel in terms of detection time, technological simplicity, and instrument requirements. In summary, each assay possesses its unique advantages and limitations, guiding their usage based on specific needs and contexts. The choice of assay depends on factors such as the substances under examination and the required levels of accuracy, sensitivity, and specificity in the detection process.

Table 6: Comparison of the methods of analysis, along with commonly used doping detection assays

Method for Analysis	Detection Time	Cost of Detection	Target-Analyte	Required Sampling	Range of LOD	Range of LOQ	Reference
MS related methods	<15 min	High	pharmaceuticals active compounds	Fish sampling points	5–50 ng/g	2.0 ng/g	[33]
Fluorescence methods	<15 min	Low	amphetamine-type stimulants	2 mL saliva	10−3–10−9 M	0.72 µM	[43]
Electroanalytical methods	< 5 min	Low	3,4-Methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine	10 µL urine	0.05–7.5 µM and 0.1–7.5 µM	37 nM and 54 nM	[50]
Colorimetric methods	< 10 min	Low	amphetamine-type stimulants	20 µL urine	0–50 µg/mL	0.66 µg/mL	[38]
Biosensors methods	> 120 min	High	methamphetamine	saliva, serum and urine,	0.02–20 µM	20 nM	[16]
PCR-based methods (Conventional)	>120 min	High	myostatin gene	2.2 µL horse plasmid solution	No mention	No mention	[19]
PCR-free based methods (Modern)	< 40 min	Medium	Human EPO gene	10 µL human plasmid solution	10−11–10−8 M	01:00	[82]

VI. CONCLUSION:

In recent years, the integrity of competitive sports has been significantly compromised by the widespread use of doping, posing a threat to fairness and justice in the athletic arena. However, stringent measures implemented by regulatory bodies and advancements in doping detection technologies have successfully curbed the illicit use of prohibited substances by athletes. This review focuses on the latest developments in methods for detecting doping, encompassing both organic drug doping and gene doping, over the past decade.

Significant progress has been made in mass spectrometry (MS), enabling the identification of trace amounts of banned substances or their metabolites in biological samples. This progress has paved the way for the development of complementary technologies such as fluorescence methods, electrochemical methods, colorimetric methods, and various biosensors. A thorough comparison of the advantages and disadvantages of each detection method is presented. Additionally, the review explores future directions in detecting these forms of doping.

We suggests three key areas for the future of doping detection: firstly, the advancement of high-throughput detection sensors capable of identifying multiple categories of doping substances simultaneously; secondly, the integration of nucleic acid amplification, nanomaterial amplification, and other innovative signal amplification technologies to achieve highly sensitive detection of doping molecules in complex samples; and thirdly, the creation of integrated and miniaturized sensors to meet the demands of on-site detection of doping substances. It is important to acknowledge that the development of new prohibited substances designed to enhance athletic performance continues, posing a constant challenge to monitoring efforts. Consequently, the pursuit of doping detection will persist, requiring ongoing updates and innovations in detection methodologies.

REFERENCES:

[1]. Nicoli, R.; Guillarme, D.; Leuenberger, N.; Baume, N.; Robinson, N.; Saugy, M.; Veuthey, J.L. Analytical Strategies for Doping Control Purposes: Needs, Challenges, and Perspectives. *Anal. Chem.* **2016**, *88*, 508–523.

[2]. Judak, P.; Esposito, S.; Coppieters, G.; Van Eenoo, P.; Deventer, K. Doping control analysis of small peptides: A decade of progress. *J. Chromatogr. B* **2021**, *1173*, 122551.

[3]. Malekzad, H.; Zangabad, P.S.; Mohammadi, H.; Sadroddini, M.; Jafari, Z.; Mahlooji, N.; Abbaspour, S.; Gholami, S.; Ghanbarpoor, M.; Pashazadeh, R.; et al. Noble metal nanostructures in optical biosensors: Basics, and their introduction to anti-doping detection. *Trends Anal. Chem.* **2018**, *100*, 116–135.

[4]. Han, J.; Zhuo, Y.; Chai, Y.Q.; Xiang, Y.; Yuan, R. New type of redox nanoprobe: C60-based nanomaterial and its application in electrochemical immunoassay for doping detection. *Anal. Chem.* **2015**, *87*, 1669–1675.

[5]. Zhao, X.; Yuan, Y.; Wei, H.; Fei, Q.; Luan, Z.; Wang, X.; Xu, Y.; Lu, J. Identification and characterization of higenamine metabolites in human urine by quadrupole-orbitrap LC-MS/MS for doping control. *J. Pharm. Biomed. Anal.* **2022**, *214*, 114732.

[6]. Yan, Y.; Lian, K.; Zhang, H.; An, J.; Zhang, Y.; Kang, W.; Ai, L. Doping-control analysis of 14 diuretics in animal-derived foods using ultra-high-performance liquid chromatography-tandem mass spectrometry. *Microchem. J.* **2022**, *174*, 106948.

[7]. Polet, M.; Van Gansbeke, W.; Van Eenoo, P. Development and validation of an open screening method for doping substances in urine by gas chromatography quadrupole time-of-flight mass spectrometry. *Anal. Chim. Acta* **2018**, *1042*, 52–59.

[8]. Minhas, R.S.; Rudd, D.A.; Al Hmoud, H.Z.; Guinan, T.M.; Kirkbride, K.P.; Voelcker, N.H. Rapid Detection of Anabolic and Narcotic Doping Agents in Saliva and Urine By Means of Nanostructured Silicon SALDI Mass Spectrometry. *ACS Appl. Mater. Interfaces* **2020**, *12*, 31195–31204.

[9]. Hu, Q.; Wei, Q.; Zhang, P.; Li, S.; Xue, L.; Yang, R.; Wang, C.; Zhou, L. An up-converting phosphor technology-based lateral flow assay for point-of-collection detection of morphine and methamphetamine in saliva. *Analyst* **2018**, *143*, 4646–4654.

[10]. Adegoke, O.; Zolotovskaya, S.; Abdolvand, A.; Daeid, N.N. Biomimetic graphene oxide-cationic multi-shaped gold nanoparticle-hemin hybrid nanozyme: Tuning enhanced catalytic activity for the rapid colorimetric apta-biosensing of amphetamine-type stimulants. *Talanta* **2020**, *216*, 120990.

[11]. dos Santos, W.T.P.; Compton, R.G. A simple method to detect the stimulant modafinil in authentic saliva using a carbon-nanotube screen-printed electrode with adsorptive stripping voltammetry. *Sens. Actuators B Chem.* **2019**, *285*, 137–144.

[12]. Morita, I.; Kiguchi, Y.; Oyama, H.; Takeuchi, A.; Tode, C.; Tanaka, R.; Ogata, J.; Kikura-Hanajiri, R.; Kobayashi, N. Derivatization-assisted enzyme-linked immunosorbent assay for identifying hallucinogenic mushrooms with enhanced sensitivity. *Anal. Methods* **2021**, *13*, 3954–3962.

[13]. Scarano, S.; Ermini, M.L.; Spiriti, M.M.; Mascini, M.; Bogani, P.; Minunni, M. Simultaneous detection of transgenic DNA by surface plasmon resonance imaging with potential application to gene doping detection. *Anal. Chem.* **2011**, *83*, 6245–6253.

[14]. Dignan, L.M.; Woolf, M.S.; Ross, J.A.; Baehr, C.; Holstege, C.P.; Pravetoni, M.; Landers, J.P. A Membrane-Modulated Centrifugal Microdevice for Enzyme-Linked Immunosorbent Assay-Based Detection of Illicit and Misused Drugs. *Anal. Chem.* **2021**, *93*, 16213–16221.

[15]. Alsenedi, K.A.; Morrison, C. Determination of amphetamine-type stimulants (ATSs) and synthetic cathinones in urine using solid phase micro-extraction fibre tips and gas chromatography-mass spectrometry. *Anal. Methods* **2018**, *10*, 1431–1440.

[16]. Xie, Y.; Wu, S.; Chen, Z.; Jiang, J.; Sun, J. Rapid nanomolar detection of methamphetamine in biofluids via a reagentless electrochemical aptamer-based biosensor. *Anal. Chim. Acta* **2022**, *1207*, 339742.

[17]. Raro, M.; Ibanez, M.; Gil, R.; Fabregat, A.; Tudela, E.; Deventer, K.; Ventura, R.; Segura, J.; Marcos, J.; Kotronoulas, A.; et al. Untargeted metabolomics in doping control: Detection of new markers of testosterone misuse by ultrahigh performance liquid chromatography coupled to high-resolution mass spectrometry. *Anal. Chem.* **2015**, *87*, 8373–8380.

[18]. Bade, R.; Abdelaziz, A.; Nguyen, L.; Pandopulos, A.J.; White, J.M.; Gerber, C. Determination of 21 synthetic cathinones, phenethylamines, amphetamines and opioids in influent wastewater using liquid chromatography coupled to tandem mass spectrometry. *Talanta* **2020**, *208*, 120479.

[19]. Tozaki, T.; Ohnuma, A.; Iwai, S.; Kikuchi, M.; Ishige, T.; Kakoi, H.; Hirota, K.; Kusano, K.; Nagata, S. Robustness of Digital PCR and Real-Time PCR in Transgene Detection for Gene-Doping Control. *Anal. Chem.* **2021**, *93*, 7133–7139.

[20]. Passreiter, A.; Thomas, A.; Grogna, N.; Delahaut, P.; Thevis, M. First Steps toward Uncovering Gene Doping with CRISPR/Cas by Identifying SpCas9 in Plasma via HPLC-HRMS/MS. *Anal. Chem.* **2020**, *92*, 16322–16328.

[21]. Cheung, H.W.; Wong, K.S.; Lin, V.Y.C.; Wan, T.S.M.; Ho, E.N.M. A duplex qPCR assay for human erythropoietin (EPO) transgene to control gene doping in horses. *Drug Test. Anal.* **2021**, *13*, 113–121.

[22]. Tozaki, T.; Ohnuma, A.; Kikuchi, M.; Ishige, T.; Kakoi, H.; Hirota, K.I.; Kusano, K.; Nagata, S.I. Robustness of digital PCR and real-time PCR against inhibitors in transgene detection for gene doping control in equestrian sports. *Drug Test. Anal.* **2021**, *13*, 1768–1775.

[23]. Neuberger, E.W.; Jurkiewicz, M.; Moser, D.A.; Simon, P. Detection of EPO gene doping in blood. *Drug Test. Anal.* **2012**, *4*, 859–869.

[24]. Sorribes-Soriano, A.; Esteve-Turrillas, F.A.; Armenta, S.; Amoros, P.; Herrero-Martinez, J.M. Amphetamine-type stimulants analysis in oral fluid based on molecularly imprinting extraction. *Anal. Chim. Acta* **2019**, *1052*, 73–83.

[25]. Geng, P.; Sun, S.; Wang, X.; Ma, L.; Guo, C.; Li, J.; Guan, M. Rapid and sensitive detection of amphetamine by SERS-based competitive immunoassay coupled with magnetic separation. *Anal. Methods* **2022**, *14*, 2608–2615.

[26]. Jang, S.; Son, S.U.; Kang, B.; Kim, J.; Lim, J.; Seo, S.; Kang, T.; Jung, J.; Lee, K.S.; Kim, H.; et al. Electrospun Nanofibrous Membrane-Based Colorimetric Device for Rapid and Simple Screening of Amphetamine-Type Stimulants in Drinks. *Anal. Chem.* **2022**, *94*, 3535–3542.

[27]. He, C.; He, Q.; Deng, C.; Shi, L.; Fu, Y.; Cao, H.; Cheng, J. Determination of Methamphetamine Hydrochloride by highly fluorescent polyfluorene with NH2-terminated side chains. *Synth. Met.* **2011**, *161*, 293–297.

[28]. Guan, F.; You, Y.; Fay, S.; Li, X.; Robinson, M.A. Novel Algorithms for Comprehensive Untargeted Detection of Doping Agents in Biological Samples. *Anal. Chem.* **2021**, *93*, 7746–7753.

[29]. Thevis, M.; Walpurgis, K.; Thomas, A. Analytical Approaches in Human Sports Drug Testing: Recent Advances, Challenges, and Solutions. *Anal. Chem.* **2020**, *92*, 506–523.

[30]. Smith, C.R.; Swortwood, M.J. Analysis of methylphenidate, ethylphenidate, lisdexamfetamine, and amphetamine in oral fluid by liquid chromatography-tandem mass spectrometry. *J. Forensic Sci.* **2022**, *67*, 669–675.

[31]. Czerwinska, J.; Jang, M.; Costa, C.; Parkin, M.C.; George, C.; Kicman, A.T.; Bailey, M.J.; Dargan, P.I.; Abbate, V. Detection of mephedrone and its metabolites in fingerprints from a controlled human administration study by liquid chromatography-tandem mass spectrometry and paper spray-mass spectrometry. *Analyst* **2020**, *145*, 3038–3048.

[32]. Wang, S.; Wang, W.; Li, H.; Xing, Y.; Hou, K.; Li, H. Rapid On-Site Detection of Illegal Drugs in Complex Matrix by Thermal Desorption Acetone-Assisted Photoionization Miniature Ion Trap Mass Spectrometer. *Anal. Chem.* **2019**, *91*, 3845–3851.

[33]. Pena-Herrera, J.M.; Montemurro, N.; Barcelo, D.; Perez, S. Combining quantitative and qualitative approaches using Sequential Window Acquisition of All Theoretical Fragment-Ion methodology for the detection of pharmaceuticals and related compounds in river fish extracted using a sample miniaturized method. *J. Chromatogr. A* **2020**, *1620*, 461009.

[34]. Lopez-Garcia, E.; Mastroianni, N.; Postigo, C.; Barcelo, D.; Lopez de Alda, M. A fully automated approach for the analysis of 37 psychoactive substances in raw wastewater based on on-line solid phase extraction-liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2018**, *1576*, 80–89.

[35]. Coppieters, G.; Deventer, K.; Van Eenoo, P.; Judak, P. Combining direct urinary injection with automated filtration and nanoflow LC-MS for the confirmatory analysis of doping-relevant small peptide hormones. *J. Chromatogr. B* **2021**, *1179*, 122842.

[36]. Ponzetto, F.; Parasiliti-Caprino, M.; Gesmundo, I.; Marinelli, L.; Nonnato, A.; Nicoli, R.; Kuuranne, T.; Mengozzi, G.; Ghigo, E.; Settanni, F. Single-run UHPLC-MS/MS method for simultaneous quantification of endogenous steroids and their phase II metabolites in serum for anti-doping purposes. *Talanta* **2023**, *255*, 124218.

[37]. Yan, Y.; Ai, L.; Zhang, H.; Kang, W.; Zhang, Y.; Lian, K. Development an automated and high-throughput analytical platform for screening 39 glucocorticoids in animal-derived food for doping control. *Microchem. J.* **2021**, *165*, 106142.

[38]. Cho, S.; Kim, Y. Donor–acceptor Stenhouse adduct formation for the simple and rapid colorimetric detection of amphetamine-type stimulants. *Sens. Actuators B Chem.* **2022**, *355*, 131274.

[39]. Kang, J.; Zhang, Y.; Li, X.; Miao, L.; Wu, A. A Rapid Colorimetric Sensor of Clenbuterol Based on Cysteamine-Modified Gold Nanoparticles. *ACS Appl. Mater. Interfaces* **2016**, *8*, 1–5.

[40]. Rouhani, S.; Haghgoo, S. A novel fluorescence nanosensor based on 1,8-naphthalimide-thiophene doped silica nanoparticles, and its application to the determination of methamphetamine. *Sens. Actuat. B Chem.* **2015**, *209*, 957–965.

[41]. Liszt, K.I.; Eder, R.; Wendelin, S.; Somoza, V. Identification of Catechin, Syringic Acid, and Procyanidin B2 in Wine as Stimulants of Gastric Acid Secretion. *J. Agric. Food Chem.* **2015**, *63*, 7775–7783.

[42]. Fu, Y.; Shi, L.; Zhu, D.; He, C.; Wen, D.; He, Q.; Cao, H.; Cheng, J. Fluorene–thiophene-based thin-film fluorescent chemosensor for methamphetamine vapor by thiophene–amine interaction. *Sens. Actuators B Chem.* **2013**, *180*, 2–7.

[43]. Zhang, Y.; Yan, B. A novel cucurbit[7]uril anchored bis-functionalized metal-organic framework hybrid and its potential use in fluorescent analysis of illegal stimulants in saliva. *Sens. Actuators B Chem.* **2020**, *324*, 128656.

[44]. Beatty, M.A.; Selinger, A.J.; Li, Y.; Hof, F. Parallel Synthesis and Screening of Supramolecular Chemosensors that Achieve Fluorescent Turn-on Detection of Drugs in Saliva. *J. Am. Chem. Soc.* **2019**, *141*, 16763–16771.

[45]. Li, D.; Xi, H.; Han, S.; Zhao, S. A turn-on fluorescent probe based on N-(rhodamine-B)-thiolactam-2-n-butane with ionic liquids for selective and sensitive detection of mustard gas stimulant. *Anal. Methods* **2021**, *13*, 484–490.

[46]. Li, C.; Han, D.; Wu, Z.; Liang, Z.; Han, F.; Chen, K.; Fu, W.; Han, D.; Wang, Y.; Niu, L. Polydopamine-based molecularly imprinted electrochemical sensor for the highly selective determination of ecstasy components. *Analyst* **2022**, *147*, 3291–3297.

[47]. Hasso, M.; Sarakhman, O.; Stankovic, D.M.; Svorc, L. A new voltammetric platform for reliable determination of the sport performance-enhancing stimulant synephrine in dietary supplements using a boron-doped diamond electrode. *Anal. Methods* **2020**, *12*, 4749–4758.

[48]. Rocha, L.R.; de Cássica Mendonça, J.; Boareto Capelari, T.; Antigo Medeiros, R.; Teixeira Tarley, C.R. Development of a reliable and selective voltammetric method for determination of designer drug 1-(3-chlorophenyl)piperazine (mCPP) using boron-doped diamond electrode and exploiting surfactant-mediated measurements. *Sens. Actuators B Chem.* **2020**, *310*, 127812.

[49]. Takahashi, F.; Nitta, S.; Shimizu, R.; Shoji, T.; Tatsumi, H.; Jin, J. Sensitive screening of methamphetamine stimulant using potential-modulated electrochemiluminescence. *Anal. Chim. Acta* **2022**, *1191*, 339229.

[50]. Li, C.; Han, D.; Liang, Z.; Han, F.; Fu, W.; Wang, W.; Han, D.; Wang, Y.; Niu, L. Novel electrochemical-surface plasmon resonance (EC-SPR) sensor for amphetamine-type stimulants detection based on molecularly imprinted strategy. *Sens. Actuators B Chem.* **2022**, *369*, 132258.

[51]. Akhoundian, M.; Alizadeh, T.; Ganjali, M.R.; Norouzi, P. Ultra-trace detection of methamphetamine in biological samples using FFT-square wave voltammetry and nano-sized imprinted polymer/MWCNTs -modified electrode. *Talanta* **2019**, *200*, 115–123.

[52]. Frasconi, M.; Mazzarino, M.; Botre, F.; Mazzei, F. Surface plasmon resonance immunosensor for cortisol and cortisone determination. *Anal. Bioanal. Chem.* **2009**, *394*, 2151–2159.

[53]. Saar-Reismaa, P.; Erme, E.; Vaher, M.; Kulp, M.; Kaljurand, M.; Mazina-Sinkar, J. In Situ Determination of Illegal Drugs in Oral Fluid by Portable Capillary Electrophoresis with Deep UV Excited Fluorescence Detection. *Anal. Chem.* **2018**, *90*, 6253–6258.

[54]. Huang, W.; Zhou, Y.; Zhan, D.; Lai, G. Homogeneous biorecognition reaction-induced assembly of DNA nanostructures for ultrasensitive electrochemical detection of kanamycin antibiotic. *Anal. Chim. Acta* **2021**, *1154*, 338317.

[55]. Alijanianzadeh, M.; Qadami, F.; Molaeirad, A. Detection of methamphetamine using aptamer-based biosensor chip and cyclic voltammetry technique. *J. Indian Chem. Soc.* **2021**, *98*, 100189.

[56]. Gu, C.; Ren, P.; Zhang, F.; Zhao, G.; Shen, J.; Zhao, B. Detection of Six beta-Agonists by Three Multiresidue Immunosensors Based on an Anti-bovine Serum Albumin-Ractopamine-Clenbuterol-Salbutamol Antibody. *ACS Omega* **2020**, *5*, 5548–5555.

[57]. Yi, J.Y.; Kim, M.; Ahn, J.H.; Kim, B.G.; Son, J.; Sung, C. CRISPR/deadCas9-based high-throughput gene doping analysis (HiGDA): A proof of concept for exogenous human erythropoietin gene doping detection. *Talanta* **2023**, *258*, 124455.

[58]. Wei Hu, S.; Ding, T.; Tang, H.; Guo, H.; Cui, W.; Shu, Y. Nanobiomaterial vectors for improving gene editing and gene therapy. *Mater. Today* **2023**, *66*, 114–136.

[59]. Azzazy, H.M.; Mansour, M.M.; Christenson, R.H. Gene doping: Of mice and men. *Clin. Biochem.* **2009**, *42*, 435–441.

[60]. Borchers, A.; Pieler, T. Programming pluripotent precursor cells derived from Xenopus embryos to generate specific tissues and organs. *Genes* **2010**, *1*, 413–426.

[61]. Tozaki, T.; Ohnuma, A.; Kikuchi, M.; Ishige, T.; Kakoi, H.; Hirota, K.I.; Kusano, K.; Nagata, S.I. Identification of processed pseudogenes in the genome of Thoroughbred horses: Possibility of gene-doping detection considering the presence of pseudogenes. *Anim. Genet.* **2022**, *53*, 183–192.

[62]. Zhang, J.J.; Xu, J.F.; Shen, Y.W.; Ma, S.J.; Zhang, T.T.; Meng, Q.L.; Lan, W.J.; Zhang, C.; Liu, X.M. Detection of exogenous gene doping of IGF-I by a real-time quantitative PCR assay. *Biotechnol. Appl. Biochem.* **2017**, *64*, 549–554.

[63]. Sugasawa, T.; Aoki, K.; Yanazawa, K.; Takekoshi, K. Detection of Multiple Transgene Fragments in a Mouse Model of Gene Doping Based on Plasmid Vector Using TaqMan-qPCR Assay. *Genes* **2020**, *11*, 750.

[64]. Minunni, M.; Scarano, S.; Mascini, M. Affinity-based biosensors as promising tools for gene doping detection. *Trends Biotechnol.* **2008**, *26*, 236–243.

[65]. Salamin, O.; Kuuranne, T.; Saugy, M.; Leuenberger, N. Loop-mediated isothermal amplification (LAMP) as an alternative to PCR: A rapid on-site detection of gene doping. *Drug Test. Anal.* **2017**, *9*, 1731–1737.

[66]. Tozaki, T.; Ohnuma, A.; Hamilton, N.A.; Kikuchi, M.; Ishige, T.; Kakoi, H.; Hirota, K.I.; Kusano, K.; Nagata, S.I. Low-copy transgene detection using nested digital polymerase chain reaction for gene-doping control. *Drug Test. Anal.* **2022**, *14*, 382–387.

[67]. Jiang, Z.; Haughan, J.; Moss, K.L.; Stefanovski, D.; Ortved, K.F.; Robinson, M.A. A quantitative PCR screening method for adeno-associated viral vector 2-mediated gene doping. *Drug Test. Anal.* **2022**, *14*, 963–972.

[68]. Maniego, J.; Pesko, B.; Hincks, P.; Taylor, P.; Stewart, G.; Proudman, C.; Scarth, J.; Ryder, E. Direct sequence confirmation of qPCR products for gene doping assay validation in horses. *Drug Test. Anal.* **2022**, *14*, 1017–1025.

[69]. Yi, J.Y.; Kim, M.; Min, H.; Kim, B.G.; Son, J.; Kwon, O.S.; Sung, C. New application of the CRISPR-Cas9 system for site-specific exogenous gene doping analysis. *Drug Test. Anal.* **2021**, *13*, 871–875.

[70]. Cai, S.; Jung, C.; Bhadra, S.; Ellington, A.D. Phosphorothioated Primers Lead to Loop-Mediated Isothermal Amplification at Low Temperatures. *Anal. Chem.* **2018**, *90*, 8290–8294.

[71]. Maniego, J.; Pesko, B.; Habershon-Butcher, J.; Huggett, J.; Taylor, P.; Scarth, J.; Ryder, E. Screening for gene doping transgenes in horses via the use of massively parallel sequencing. *Gene Ther.* **2022**, *29*, 236–246

[72]. de Boer, E.N.; van der Wouden, P.E.; Johansson, L.F.; van Diemen, C.C.; Haisma, H.J. A next-generation sequencing method for gene doping detection that distinguishes low levels of plasmid DNA against a background of genomic DNA. *Gene Ther.* **2019**, *26*, 338–346.

[73]. Tozaki, T.; Ohnuma, A.; Takasu, M.; Nakamura, K.; Kikuchi, M.; Ishige, T.; Kakoi, H.; Hirora, K.; Tamura, N.; Kusano, K.; et al. Detection of non-targeted transgenes by whole-genome resequencing for gene-doping control. *Gene Ther.* **2021**, *28*, 199–205.

[74]. Zhang, K.; Pinto, A.; Cheng, L.Y.; Song, P.; Dai, P.; Wang, M.; Rodriguez, L.; Weller, C.; Zhang, D.Y. Hairpin Structure Facilitates Multiplex High-Fidelity DNA Amplification in Real-Time Polymerase Chain Reaction. *Anal. Chem.* **2022**, *94*, 9586–9594.

[75]. Zhou, X.M.; Zhuo, Y.; Tu, T.T.; Yuan, R.; Chai, Y.Q. Construction of Fast-Walking Tetrahedral DNA Walker with Four Arms for Sensitive Detection and Intracellular Imaging of Apurinic/Apyrimidinic Endonuclease. *Anal. Chem.* **2022**, *94*, 8732–8739.

[76]. Zhao, X.; Yuan, Y.; Liu, X.; Mao, F.; Xu, G.; Liu, Q. A Versatile Platform for Sensitive and Label-Free Identification of Biomarkers through an Exo-III-Assisted Cascade Signal Amplification Strategy. *Anal. Chem.* **2022**, *94*, 2298–2304.

[77]. Xie, Y.; Niu, F.; Yu, A.; Lai, G. Proximity Binding-Triggered Assembly of Two MNAzymes for Catalyzed Release of G-Quadruplex DNAzymes and an Ultrasensitive Homogeneous Bioassay of Platelet-Derived Growth Factor. *Anal. Chem.* **2019**, *92*, 593–598.

[78]. Fu, Y.; Du, C.; Zhang, Q.; Xiao, K.; Zhang, X.; Chen, J. Colorimetric and Photocurrent-Polarity-Switching Photoelectrochemical Dual-Mode Sensing Platform for Highly Selective Detection of Mercury Ions Based on the Split G-Quadruplex-Hemin Complex. *Anal. Chem.* **2022**, *94*, 15040–15047.

[79]. Zhang, Q.; Zhao, S.; Tian, X.; Qiu, J.G.; Zhang, C.Y. Development of a CRISPR-Cas-Based Biosensor for Rapid and Sensitive Detection of 8-Oxoguanine DNA Glycosylase. *Anal. Chem.* **2022**, *94*, 2119–2125.

[80]. Li, X.; Li, R.; Yuan, Z.; Zhu, Z.; Xu, W.; Wang, Y.; Zhang, D.; Yang, L. One Versatile Cas9-Integrated Single-Tube Duplex Quantitative Real-Time PCR System for Rapid Analysis of CRISPR/Cas-Induced Mutants. *Anal. Chem.* **2022**, *94*, 10832–10840.

[81]. Passreiter, A.; Naumann, N.; Thomas, A.; Grogna, N.; Delahaut, P.; Thevis, M. How to detect CRISPR with CRISPR—Employing SHERLOCK for doping control purposes. *Analyst* **2022**, *147*, 5528–5536.

[82]. Yan, J.; Xu, Z.; Zhou, H.; Li, T.; Du, X.; Hu, R.; Jiang, Z.; Gaozhi, O.; Ying, L.; Yang, Y. Integration of CRISPR/Cas12a and multiplexed RPA for fast detection of gene doping. *Anal. Chem.* **2022**, *94*, 16481–16490

[83]. Thevis, M.; Geyer, H.; Thomas, A.; Schanzer, W. Trafficking of drug candidates relevant for sports drug testing: Detection of non-approved therapeutics categorized as anabolic and gene doping agents in products distributed via the Internet. *Drug Test. Anal.* **2011**, *3*, 331–336.

[84]. Thomas, A.; Walpurgis, K.; Delahaut, P.; Kohler, M.; Schanzer, W.; Thevis, M. Detection of small interfering RNA (siRNA) by mass spectrometry procedures in doping controls. *Drug Test. Anal.* **2013**, *5*, 853–860. [**Google Scholar**] [**CrossRef**] [**PubMed**]

[85]. Jing, J.; Yang, S.; Zhou, X.; He, C.; Zhang, L.; Xu, Y.; Xie, M.; Yan, Y.; Su, H.; Wu, M. Detection of doping with rhGH: Excretion study with WADA-approved kits. *Drug Test. Anal.* **2011**, *3*, 784–790.

[86]. Walker C.J., Cowan D.A., Taylor N.F., Kicman A.T., Determination of xenobiotic glucocorticoids
a. in urine by gas chromatography– mass spectrometry. Recent Advances in Doping Analysis, Sport und Strauß, 2006; 14: 35-44
[87]. Lloyd, H.A., Fales, H.M., Highet, P.F., et alSeparation of alkaloids by gas chromatography J.
a. Ameri. Chem. Soc.,1960; 82: 3791
[88]. Parker, K.D., Fontan, C.R., Kirk, P.L. Separation and identification of some sympathomimetic
a. amines by gas chromatography. Anal.Chem., 1962; 34: 1345 – 1346
[89]. Parker, K.D., Fontan, C.R., and Kirk, P.L. Rapid gas chromatographic method for screening of toxicological extracts for alkaloids, barbiturates, sympathomimetic amines, and tranquilizers. Anal. Chem. 1963; 35: 356 – 359
[90]. Thevis, M., Opfermann, G. and Schänzer, W. Liquid chromatography/ electrospray ionization tandem mass spectrometric screening and confirmation methods for β2-agonists in human or equine urine, J Mass Spectrom, 2003; 38: 1197–1206
[91]. Mareck, U., Thevis, M., Guddat, S., Gotzmann, A., Bredehöft, M., Geyer, H. and Schänzer, W. Comprehensive sample preparation for anabolic steroids, glucocorticosteroids, beta-receptor blocking agents, selected anabolic androgenic steroids and buprenorphine in human urine, Recent Advances in Doping Analysis, Sport und Strauß, 2004; 22: 65–68
[92]. Mazzarino, M., Turi, S. and Botrè, F. A screening method for the detection of synthetic glucocorticosteroids in human urine by liquid chromatography- mass spectrometry based on classcharacteristic fragmentation pathways, Anal Bioanal Chem ,2008; 390: 1389–1402
[93]. Reddy.I.M, Beotra. A, Jain. S, Ahi. S.A simple and rapid ESI-LC-MS/MS method for simultaneous screening of doping agents in urine samples Indi. J. Pharm. 2009; 41: 80-86
[94]. Dubey. S, Ahi.S,Reddy. I.M, Beotra. A, Kaur. T and Jain. S. Novel study of Mass Spectrometric fragmentation pattern of Modafinil, Adrafinil & their metabolite Modafinilic Acid under +ESILC-MS/MS & +EI-GC-MS ionization Ind. J. Pharm. 2009; 41: 278-283
[95]. Ahi. S, Beotra. A, Jain. S. Detection of mono-hydroxylated metabolites of Stanozolol by HPLCESI (+) MS/MS in Indian sports persons Drug Test. Anal., 2010, 1; 11-12: 538-54
[96]. Lu. J, Wanga. S, Donga. Y, Wanga. X, Yanga. S., Zhanga. J, Denga. J, Qina. Y, Xua. Y, Wua. M, Ouyangb. G. Simultaneous analysis of fourteen tertiary amine stimulants in human urine for doping control purposes by liquid chromatography–tandem mass spectrometry and gas chromatography–mass spectrometry Anal. Chim. Acta. 2010; 657:45–52
[97]. Kolmonen. M, Leinonen. A, Kuuranne. T, Pelander. A, Ojanpera. I. Hydrophilic interaction liquid chromatography and accurate mass measurement for quantification and confirmation of morphine codeine and their glucuronide conjugates in human urine, J. Chromatogr. B, 2010; 878: 2959– 2966
[98]. Touber. M.E., van Engelen M.C., Georgakopoulus C., Rhijn J.A. van, Nielen M.W.F., Multidetection of corticosteroids in sports doping and veterinary control using high-resolution liquid chromatography/time-of-flight mass spectrometry, Anal. Chim. Acta2007;586: 137–146
[99]. Badoud F., Grata E., Perrenoud L., Saugy M., Rudaz S., Veuthey J.L., Fast analysis of doping agents in urine by ultra-high pressure liquid chromatography–quadrupole time-of-flight mass spectrometry. II: confirmatory analysis, J. Chromatogr. A, 2010; 1217: 4109–4119
[100]. Mc Lafferty , F.W. Mass spectrometric analysis: Broad applicability to chemical research . Anal. Chem., 1956; 28 ; 306 – 316
[101]. Gohlke , R.S. , and Mc Lafferty , F.W. Mass spectrometric analysis: Aliphatic amines .Anal.Chem. , 1962; 34: 1281 – 1287
[102]. Mc Lafferty, F.W. , and Turecek , F. Interpretation of Mass Spectra.University Science Books , Mill Valley 1993
[103]. Reisch, J., Pagnucco , R. , Alfes , H. , et al. Mass spectra of derivatives of phenylalkylamines . J. Pharm. Pharma.; 1968: 20: 81 – 86
[104]. Barry, T., and Petzinger , G. “Structure and fragmentation mechanisms of some ions in the mass spectrum of ephedrine” . Biomed. Mass Spectrom., 1977; 4 , 129 – 133
[105]. Baba, S., and Kawai , K. “Studies on drug metabolism by use of isotopes. XI. Studies on synthesis and physicochemical features of 2 H - labeled l - ephedrine on benzene ring” Yakugaku zasshi. J. Pharmaceu. Soc. Japan, 1974; 94 :783 – 787

[106]. Valentine, J.L. , Middleton , R. "GC - MS identification of sympathomimetic amine drugs in urine: Rapid methodology applicable for emergency clinical toxicology" J. Anal. Toxico. , . 2000; 24: 211 – 222

[107]. Thevis, M., Sigmund , G. , Koch , A. , and Schänzer , W. "Determination of tuaminoheptane in doping control urine samples" Europ. J. Mass Spectrom. , 2007; 13 , 213 – 221

[108]. Rosano, T.G. , Hubbard , J.D. , Meola , J.M. , and Swift , T.A. "Fatal strychnine poisoning:Application of gas chromatography and tandem mass spectrometry" J. Anal. Toxico., 2000; 24 :642 – 647

[109]. Jackson, G. , and Diggle , G. "Strychnine - containing tonics". Brit. Med. Jour. , 1973; 2: 176 –177