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A critical review of workplace drug testing methods for old and new psychoactive substances: Gaps, advances, and perspectives

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ABSTRACT

Workplace drug testing (WDT) is essential to prevent drug abuse disorders among the workforce because it can impair work performance and safety. However, WDT is limited by many challenges, such as urine adulteration, specimen selection, and new psychoactive substances (NPS). This review examined the issues related to WDT. Various scientific databases were searched for articles on WDT for drug detection published between 1986 (when WDT started) and January 2024. The review discussed the history, importance, and challenges of WDT, such as time of specimen collection/testing, specimen adulteration, interference in drug testing, and detection of NPS. It evaluated the best methods to detect NPS in forensic laboratories. Moreover, it compared different techniques that can enhance WDT, such as immunoassays, targeted mass spectrometry, and nontargeted mass spectrometry. These techniques can be used to screen for known and unknown drugs and metabolites in biological samples. This review assessed the strengths and weaknesses of such techniques, such as their validation, identification, library search, and reference standards. Furthermore, this review contrasted the benefits and drawbacks of different specimens for WDT and discussed studies that have applied these techniques for WDT. WDT remains the best approach for preventing drug abuse in the workplace, despite the challenges posed by NPS and limitations of the screening methods. Nontargeted techniques using high-resolution liquid chromatography-mass spectrometry (MS)/gas chromatography–tandem MS can improve the detection and identification of drugs during WDT and provide useful information regarding the prevalence, trends, and toxicity of both traditional and NPS drugs. Finally, this review suggested that WDT can be improved by using a combination of techniques, multiple specimens, and online library searches in case of new NPS as well as by updating the methods and databases to include new NPS and metabolites as they emerge. To the best of the author's knowledge, this is the first review to address NPS as an issue in WDT and its application and propose the best methods to detect these substances in the workplace environment.

1. Introduction

Throughout its history, drug abuse analysis has been a necessary means of saving human lives and securing their abilities to perform well in their workplace. The side effects of illicit drug use can affect social life, workplace performance, and public safety; for example, the crime rates have increased because of the misuse of drugs. In addition to rising public concerns regarding the harmful effects and consequences of drug abuse and increases in relevant emergency department visits caused by overdoses (Kim et al., 2019; Els et al., 2020; Fu et al., 2019; White et al, 2023), well-trained personnel are at risk if they start using such drugs of abuse. This will markedly damage their productivity and may increase the incidence of accidents at the workplace. Therefore, companies and industries conduct workplace drug testing (WDT) to screen job applicants (pre-employment testing, random testing, and testing in cases of unjustified incidents at work), thereby achieving the growing demand for drug-free work environments in public and private sectors [5–8]. Conversely, the use of adulteration products to pass WDT examination has increased (Embers et al., 2019). The desire to manipulate WDT has threatened the reliability of drug testing, and in some cases, the applicants successful to pass the WDT (Matriciani et al., 2018; Franz et al., 2022; The Committee Clinical Toxicology, 2005). The accuracy of drug testing protocols has often been questioned, because various chemicals can be used to manipulate urine samples and cause false-negative results in WDT (Mikkelsen and Ash, 1988; Fu, 2019)[13]. This study examined the different viewpoints obtained through these analyses. The

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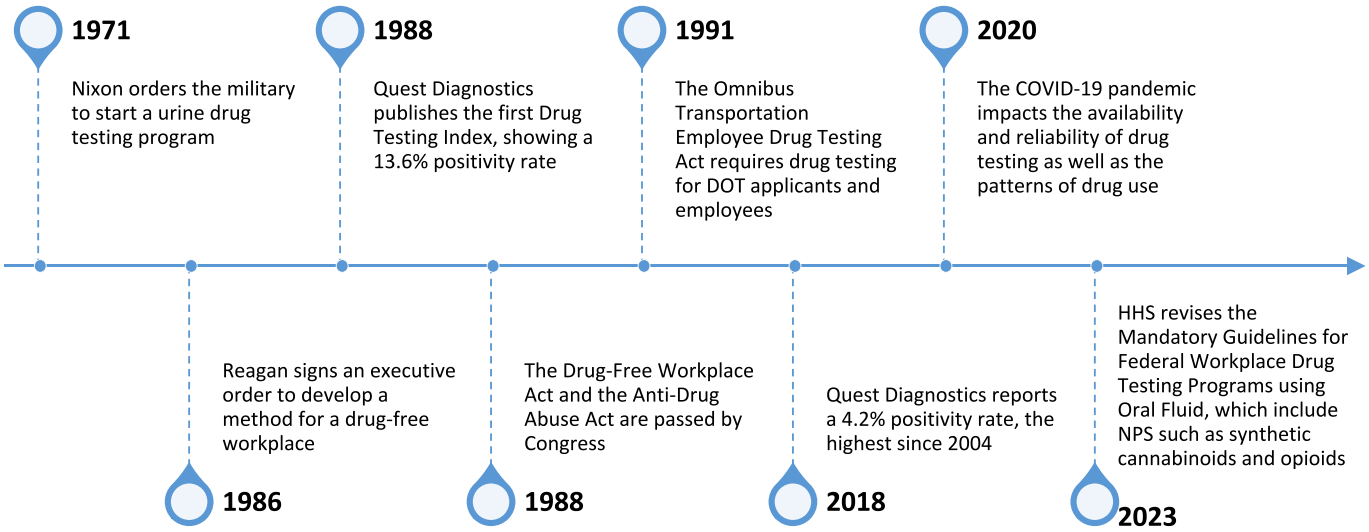

Fig. 1. Historical Overview of Workplace Drug Testing.

approaches that have been used to detect adulteration were evaluated, and some solutions were suggested. The argument in favor of suitable detection methods to avoid false-negative results is persuasive.

Currently, drug abuse testing has become mandatory before securing certain job positions, depending on specific roles or industries. For example, this requirement applies to positions in the army and pilot roles. Drug misuse is a major factor contributing to the increase in crime rates, violence, and accidents. The National Survey on Drug Use and Health, conducted in the USA in 2015, concluded that 70 % of adults self-reporting that they have used drugs of abuse were employees, with ~ 20 % of them being classified as having drug abuse disorder (Els et al., 2020). In the USA, in 2018, the annual rate of positive drug results among employees soared to the highest level (4.4 %) among the > 9 million samples tested (Secaucus, 2019). Therefore, testing for illicit drugs has a significant impact on the prevention of problems associated with drug abuse disorder in workplace communities for various reasons. One of them is that a growing number of employers demand the creation of a drug-free work environment, especially when the applicants are about to secure a job and when an employee is suspected of illegal drug use because of a change in behavior or after an unusual accident. Therefore, WDT is a necessary step toward achieving the goal of a drug-free work environment (Dasgupta et al., 2004). Many companies have experienced a drop in productivity associated with the absenteeism of their workers because of the use of illicit drugs. Although WDT can help keep the workplace free of drugs, it remains controversial whether it can always provide accurate results about the status of the applicants. One of these uncertainties is the time of drug testing. If the applicants know the time of testing, they may use adulteration products to yield a negative WDT result. The selection of specimens can be challenging, with urine samples being the gold-standard specimen in WDT; however, in some cases, the applicants cannot provide such samples because of a so-called shy bladder. Another issue is that some drugs can interfere with immunoassay reagents, which increases the workload of WDT. In some applications, urine analysis using confirmation techniques, i.e., chromatography systems coupled with mass spectrometry (MS), is offered, but this also would increase the time, labor, and cost of the analysis. Nevertheless, the way WDT deals with emerging new psychoactive substances (NPS) using primary and confirmation techniques remains the most challenging issue (Fu et al., 2019; Salomone et al., 2020).

1.1. History of WDT

Fig. 1 provides a historical overview of WDT since the time when President Nixon launched a war on drugs in 1971 and ordered the military to test its service members for drug abuse, especially heroin. This led to the establishment of the first urine drug testing laboratory and the development of forensic drug testing standards and procedures (Gannon, 2014). In 1986, WDT was implemented when President Ronald Reagan gave the executive order to develop a method for keeping the workplace free of drug abuse (Bush, 2008; Willette et al., 1988). At that time, the establishment of urine drug testing laboratories was needed. Therefore, many institutes were created to design quality assurance programs for drug testing and provide suitable models that must be met by all laboratories performing WDT. The Substance Abuse and Mental Health Administration (SAMHSA) has designed a suitable standard for performing WDT (Bush, 2008; White et al., 2023; D. of H. and H.S, 2017; DHHS, 1988). This program has covered many aspects of WDT and its consequences, including the nature of the drug of abuse to be screened as well as the preparation of a proper employee education program on drug abuse disorder. This process should be conducted under supervision to achieve a workplace free of drug abuse, followed by the introduction of a special program to help employees who have drug abuse disorder and identify employees who can be classified as illegal drug users. These cases should then be subjected to suitable approaches for drug abuse testing and monitoring (Bush, 2008).

1.2. WDT structure

The SAMHSA standard and other guidelines prepared by different countries, such as the European Workplace Drug Testing Society (Taskinen et al., 2017), have always considered three stages in the processing of each sample: collection, analysis, and interpretation. First, assessment of sample integrity is a crucial step that is conducted at the site of collection. This involves many essential tests, such as measurement of temperature, color, creatinine level, pH, smell, specific gravity, and nitrate level. Moreover, it is difficult to distinguish a genuine urine sample from an adulterated one (Kim et al., 2019; Embers et al., 2019; Vikingsson et al., 2022; Kyle and Kaur, 2020; Goggin et al., 2017). For example, Kim et al. (2019) revealed that the substitution of urine samples with synthetic urine that is currently used in forensic toxicology laboratories for preparing quality control samples can beat WDT, without being able to differentiate gunning from synthetic samples. Consequently, direct supervision and assessment of sample integrity, especially during illicit drug testing, are becoming increasingly necessary to identify adulterated samples.

The analytical stage consists of two steps in the laboratory: first, a primary screening is performed using immunoassays, followed using chromatography instruments (e.g., gas chromatography coupled with

Table 1
Common adulteration methods and their detection during workplace drug testing.

Adulteration Method	Description	Detection	Example	Reference
Synthetic urine	Artificial urine that mimics the appearance and composition of human urine	Can be detected by checking the temperature, pH, specific gravity, creatinine level, and other parameters of the urine sample	A product known as Quick Fix Synthetic Urine has been claimed to be laboratory-grade urine that can pass any drug test	(Fu, 2019; Quick Fix Synthetic Urine, 2023)
Substituted urine	Urine from another person or animal used instead of patient's own urine	Can be detected by checking the temperature and DNA of the urine sample as well as the sex of the patient	A man in Ohio was caught using his girlfriend's urine for a drug test, but he failed to do so when the urine tested positive for pregnancy	(Bengel, 2019)
Diluted urine	Urine diluted with water or other fluids to lower the concentration of drug metabolites	Can be detected by checking the creatinine level, specific gravity, and color of the urine sample	A study found that 2.8% of urine samples collected from federal employees were diluted, indicating an attempt to mask drug use	(Nuclear Regulatory Commission, 2022)
In vitro urinary adulterants	Chemicals or substances that are added to the urine sample to interfere with the drug testing process	Can be detected by checking the pH, specific gravity, oxidants, and other parameters of the urine sample	A product known as Urine Luck contains pyridinium chlorochromate, which can oxidize drug metabolites and produce false-negative results	(Pham et al., 2013; Wu et al., 1999)

MS [GC–MS]) to confirm whether the samples are positive or negative. At this time, the confirmatory step cannot be performed directly because the chromatography machines are sensitive; thus, the specimens need to be extracted before injection into the chromatography machines. In addition to hydrolysis, derivatization procedures may be needed when using GC–MS (Wu et al., 1999; Fu, 2019), but not when using liquid chromatography (LC)-MS. In the latter case, the urine sample can be injected directly without any sample pretreatment, or the sample can be diluted and injected, including extremely polar glucuronide metabolites of interest (Fu, 2019; Gallardo et al., 2009). The selection of the best protocol for analysis remains controversial. Each country considers its own drug of interest to be tested during WDT, as immunoassays have an advantage of easy handling by laboratory personnel, whereas high-resolution MS techniques require an expert to interpret the results. Although the samples can be processed rapidly, the interpretation of the results may take several days, which increases the turnaround time (TAT).

Sample collection and analysis are important to aid in the decision-making process; however, the interpretation of both positive and negative results is comprehensive and crucial for finalizing the outcome of the analysis; for example, determining how to interpret any positive or negative result obtained for suspected adulterated samples (Liu, 1992). WDT is an application of forensic toxicology that depends on the testing of metabolites, rather than that of the parent drugs, as urine is the specimen of choice. Some drugs are prodrugs that are converted into extremely polar metabolites, such as glucuronide or sulfate conjugates, which lead to a decrease in the concentration of the parent drug in some cases to a level below the confirmatory cutoff value. Moreover, such drugs require the use of sufficient hydrolysis or a direct method for the detection of drugs and their metabolites. These two approaches are neither time consuming nor expensive, especially for routine testing of a high number of samples (Fu, 2016). In addition, the interpretation of positive or negative results of WDT can be complicated by similarities between different drugs with comparable chemical structures, especially when some of these drugs are illicit and others are legal drugs. Furthermore, the suspected drugs can be metabolites of legal drugs and vice versa; for example, amphetamine is a prescribed drug in the USA and a metabolite of methamphetamine (Al-Asmari, 2021). In addition, *l*-methamphetamine is a metabolite of selegiline, which is a drug used for treating Parkinson's disease (Shin et al., 2021). Because *d*-methamphetamine is an illicit drug and a highly addictive psychostimulant, special chromatographic separation technique should be performed to differentiate these isomers, which is often not available at all WDT laboratories (Shin et al., 2021; West et al., 2013). In addition to the challenges of distinguishing drug classes with similar chemical structures and identifying source analytes, especially when two drugs share similar metabolic pathways, unintentional ingestion of NPS or illicit drugs may be encountered, which are known as fake drugs or counterfeit drugs in which NPS act as adulterants (Liu, 1992; Salomone et al., 2020; Salomone and Palamar., 2021; Oliver et al., 2019). In the USA, despite NPS being an ingredient, "Molly" is sold as MDMA (Moeller et al., 2008; Palamar et al., 2016).

1.3. Adulteration

Urine manipulation is a significant problem in drug testing. It is common in various sectors, including WDT, addiction treatment programs, and forensic investigations. The rate of sample manipulation varies from 2 % to 50 % depending on the context (The Committee Clinical Toxicology, 2005). Despite these challenges, comprehensive testing for manipulation should become a standard to ensure the integrity of the drug testing procedures.

Although the SAMHSA standard has been used for WDT and assessment of sample integrity to fully ensure the accuracy of the drug testing results, the reliability of drug testing has often been questioned (Cody, 1990). In fact, it is easy to manipulate urine samples using various substances, which is termed adulteration, to produce false-negative results. Simple adulteration using products such as soap, detergent, and household cleaner has a long history (Cody and Valtier, 2001). The products that are used for adulteration have become more sophisticated, because they have been deliberately designed to cause interference with the immunoassay system and yield a false-negative result (Liu, 1992). The use of adulteration products has increased sharply because employees who are being tested already know that the result would be positive (Table 1). In these cases, the only conceivable approach to pass WDT is to use adulteration products to produce a false-negative result (Fu, 2019). Another reason is the increasing number of drivers who are under the influence of illicit drugs and wish to pass drug tests (Wissenbach and Steuer, 2023; The Committee Clinical Toxicology, 2005). In many laboratories, a procedure to detect sample adulteration has not yet been implemented (Fu, 2019). Moreover, adulterants have become available on the market, and methods to adulterate specimens have been described by companies that sell adulterants on their websites; anyone can adulterate a drug test specimen by simply surfing internet to obtain detailed information about adulteration (The Committee Clinical Toxicology, 2005).

Adulteration can be divided into two types: in vivo adulteration and *in vitro* adulteration (Fu, 2019). *In vivo* adulteration refers to the ingestion of adulterants by drinking a liquid or performing actions to excrete or eliminate these drugs or their metabolites from the body or to dilute the concentration of the drug (Fu, 2019; Fu, 2016). Another possibility is to mask these substances to produce a false-negative result via the immunoassay. For example, *in vivo* adulteration approaches often alter the pH value and drug concentration in the urine sample through the intake of a large amount of water. Diuretics decrease the drug concentration to skew the primary immunoassay drug screening toward a

Table 2
Effect of adulterants on different immunoassay methods for drug detection in urine samples.

Method	Principle	Interfering Adulterants	Effect	Example	Reference
Cloned Enzyme Donor Immunoassay (CEDIA)	Cloned enzyme-donor fragments recombine with enzyme-acceptor molecules in the presence of the drug or its metabolite	Oxidizing agents (bleach, peroxide, and pyridinium chlorochromate), glutaraldehyde, detergent, and nitrite	False-negative or −positive results caused by enzyme or drug destruction, enzyme activity, or color development interference	Cocaine metabolites (benzoylecgonine), methadone metabolites (EDDP), and buprenorphine metabolites (norbuprenorphine) can be degraded by bleach or peroxide, yielding false-negative results	(Wu et al., 1995)
Enzyme Multiplied Immunoassay Technique (EMIT)	Enzyme-labeled drug competes with the drug or its metabolite in urine samples for antibody-binding sites	Oxidizing agents (bleach, peroxide, and pyridinium chlorochromate), glutaraldehyde, detergent, and nitrite	False-negative or −positive results caused by enzyme or drug inactivation, enzyme activity, or antibody-binding interference	Cannabis metabolites (THC-COOH), opiates (morphine, codeine, and heroin), and benzodiazepines (diazepam, oxazepam, and temazepam) can be inactivated by pyridinium chlorochromate, yielding false-negative results	(Mikkelsen and Ash, 1988)
Fluorescence Polarization Immunoassay (FPIA)	Fluorescent-labeled drug competes with the drug or its metabolite in urine samples for antibody-binding sites	Oxidizing agents (bleach, peroxide, and pyridinium chlorochromate), glutaraldehyde, detergent, and nitrite	False-negative or −positive results caused by fluorescent labeling, drug destruction, fluorescence, or antibody-binding interference	Opiates (morphine, codeine, and heroin), amphetamines (amphetamine, methamphetamine, and MDMA), and barbiturates (phenobarbital, secobarbital, and pentobarbital) can be destroyed by bleach or peroxide, yielding false-negative results	(Chou and Giang, 2007)
Radioimmunoassay (RIA)	Radioactive-labeled drug competes with the drug or its metabolite in urine samples for antibody-binding sites	Oxidizing agents (bleach, peroxide, and pyridinium chlorochromate), glutaraldehyde, detergent, and nitrite	False-negative or −positive results caused by radioactive labeling or drug destruction, radioactivity, or antibody-binding interference	Amphetamine, methadone, and phencyclidine (PCP) can be destroyed by bleach or peroxide, yielding false-negative results	(Bronner et al., 1990)
Biochip	A panel of up to 44 related tests on a single biochip using competitive chemiluminescent immunoassays	Oxidizing agents (bleach, peroxide, and pyridinium chlorochromate), glutaraldehyde, detergent, and nitrite	False-negative or −positive results caused by oxidant or drug destruction, light signaling, or antibody-binding interference	Methamphetamine, ketamine, and tramadol can be destroyed by pyridinium chlorochromate, yielding false-negative results	(Alwaeel et al., 2022)
Gas Chromatography-Mass Spectrometry (GC–MS)	Gas chromatography separates the components of the urine sample, and mass spectrometry identifies and quantifies the drug or its metabolite	Derivatization agents (acetic anhydride, trifluoroacetic anhydride, and heptafluorobutyric anhydride), interfering compounds (endogenous or exogenous substances with similar mass spectra), and matrix effects (ion suppression or enhancement)	False-negative results caused by derivatization failure, misidentification, or inaccurate quantification	Cocaine, cannabis, and fentanyl can be altered by acetic anhydride, resulting in inaccurate quantification	(Liu et al., 2007)
Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	Liquid chromatography separates the components of the urine sample, and tandem mass spectrometry identifies and quantifies the drug or its metabolite using multiple-reaction monitoring	Ionization agents (formic acid, acetic acid, and ammonium acetate), interfering compounds (endogenous or exogenous substances with similar mass transitions), and matrix effects (ion suppression or enhancement)	False-negative results caused by ionization failure, misidentification, or inaccurate quantification	Cannabis, synthetic cannabinoids, and synthetic cathinones can be degraded by formic acid, resulting in inaccurate quantification	(Luong et al., 2014)

negative result (Fu, 2019; Fu, 2016; Smith and Bluth, 2016). Another study reported that the most complicated method is to dilute a urine sample using diuretics combined with vitamins and creatine because normal creatinine can be stimulated by creatine to pass the creatine test. In addition, positive drugs can be masked by vitamins owing to their color (The Committee Clinical Toxicology, 2005). The effects of adulterants on various screening and confirmation methods used for detecting drugs in urine samples are summarized in Table 2.

The first type of adulteration aims to alter sample integrity. The second type of adulteration is employed to interfere with the immunoassay reagent, thus preventing the reaction between antibodies in the immunoassay reagent and the drug metabolites of the sample, which leads to negative results (The Committee Clinical Toxicology, 2005). Some adulteration techniques have been designed to affect a specific immunoassay reagent; for example, detergents have a strong effect on the Enzyme multiplied immunoassay technique (EMIT) reagent but do not affect radioimmunoassay (RIA) results. The third type of adulteration can affect a particular class of drugs; for example, potassium nitrite can prevent a positive result of cannabinoid metabolites. Moreover, many substances have been used as adulterants, such as alcohols, ammonia, ascorbic acid, blood, bleach, detergent, lemon juice, peroxide, golden seal root, and vinegar (The Committee Clinical Toxicology, 2005; Cody, 1990).

False-negative results have also become a problem, and researchers have proposed many approaches to address this issue (Fu, 2019). They argued that the best way to overcome this issue is to ensure that WDT yields no false-negative results, which seems challenging. A study claimed that urine and blood samples are suitable candidates for drug testing; however, the collection site must be improved using a collection device to ensure that there is no possibility of specimen manipulation among the suspects who are being tested (Rajšić et al., 2020). In the late 80 s, they presented the Franklin collector as a model for use in this context. This container hampers the adulteration of liquids because it takes 1–2 min for adjusting the temperature. In addition, Warmer stated that some adulterants, such as sodium hypochlorite, can be easily smelled, and solid adulterations are detected by observing a residue in

Table 3
Advantages and disadvantages of different sample types for workplace drug testing.

Sample Type	Advantages	Disadvantages	Detection Methods	Workplace Drug Testing	Reference
Urine	Widely used and accepted, easy to collect and analyze	Noninvasive, short detection window, and prone to adulteration and substitution	Immunoassay, and confirmatory test	Yes	(Bush, 2008; Taskinen et al., 2017; DHHS, 1988)
Blood or Serum	Accurate, reliable, and can be used to detect drug concentration and impairment	Invasive, expensive, requires trained personnel, short detection window, and is prone to adulteration and substitution	Immunoassay and confirmatory test	Rarely	(Huppertz et al., 2014; Partridge et al., 2018)
Hair	Noninvasive, long detection window, difficult to manipulate, and suitable for detecting chronic drug use	Low concentration; requires sensitive and selective techniques; may vary according to hair color, growth rate, and environmental factors; and cannot detect recent drug ingestion	Analytical techniques, immunoassay, and confirmatory test	Yes	(Salomone et al., 2016)
Saliva or Oral Fluid	Noninvasive, easy to collect, and suitable for detecting recent drug ingestion	Low concentration, requires sensitive and selective techniques, short detection window, and may be affected by oral hygiene and food intake	Analytical techniques, immunoassay, and confirmatory test	Yes	(Brcak et al., 2018; Rodrigues et al., 2013; Tsanaclis et al., 2012)

the device (Warner, 1989). Another step is that the person who is responsible for collecting urine samples from job applicants for WDT should ask them to empty their pockets and wash their hands. This individual should also ensure that the job applicants do not have a powder or any substance under their fingernails, and the tap water should be turned off in the restroom to prevent its use for dilution of specimens (Cody, 1990). Warner believes that pre-analysis at the collection site is important. Pre-analysis can reveal whether the sample contains an adulterant when the temperature is < 37°C; this would indicate that the sample has been switched. Finally, pH assessment would help the chemist detect the presence of adulterants (Warner, 1989).

1.4. Alternative samples

Other researchers have presented different views; they have suggested that, instead of urine and blood, alternative samples such as hair, saliva, and oral fluid samples should be used, which may be the best approach to avoid false-negative results (Salomone et al., 2016; Gallardo et al., 2009; Tsanaclis et al., 2012; Brcak et al., 2018). SAMHSA and other authorities have published guidelines for the analysis of oral fluid (Brcak et al., 2018; D. of H. and H.S, 2020a) and hair (Salomone et al., 2016; D. of H. and H.S, 2020b) samples for WDT. These samples can be easily collected, and the analytes of interest can be easily detected because the corresponding analytical techniques have been improved over the years and are extremely sensitive and accurate. Moreover, these specimens are difficult to manipulate (Inoue and Seta 1992), and their method of collection is less invasive than that used for urine and blood (Huestis et al., 1999).

It remains unclear which of the following two approaches ensure the validity of the results: collecting samples under tight scrutiny or using unconventional samples instead of urine and blood. Unconventional samples offer many advantages compared with urine and blood samples; in particular, long-term detection using hair samples and considerably short-term detection using oral and saliva samples. In contrast, these samples have limitations, such as extremely low concentrations that require very sensitive and selective testing (e.g., hair testing). Furthermore, some of these unconventional samples have high drug stability, whereas many drugs and their metabolites cannot be detected in urine and blood few days after drug use (Inoue and Seta 1992; Huestis et al., 1999). Adulteration can be detected using simple laboratory tests, such as measurement of temperature, pH, and specific gravity as well as smell detection; however, laboratories should set up a standard procedure to detect adulteration. Therefore, the use of these unconventional specimens is less attractive than that of urine samples. Moreover, stealth adulteration at the collection site is challenging, although it is feasible via many techniques. Stealth adulteration could be detected using a simple specimen-check reagent (The Committee Clinical Toxicology, 2005; Cody, 1990; Inoue and Seta 1992).

Although both suggestions have led to the detection of false-negative results during drug testing, the complexity of the problem remains obvious (Edwards et al., 1993). Nonetheless, using alternative subjects is an attractive idea that can provide greater reliability of drug testing in the context of long-term use and recent drug ingestion. However, several simple tests can detect various adulterations. Unfortunately, many adulterations are difficult to detect (Fu, 2019). The use of unconventional samples as complementary specimens to urine and blood samples is recommended. Finally, we must cooperatively fight against adulteration companies to ensure safety. The different sample types used for WDT, and their advantages and disadvantages are summarized in Table 3.

1.5. NPS and WDT

NPS are a diverse group of synthetic compounds that mimic the effects of traditional drugs of abuse, such as stimulants, hallucinogens, opioids, and cannabinoids. NPS pose a challenge for drug testing because they are often not covered by routine methods and can have unpredictable pharmacological and toxicological effects. The application of the current SAMHSA standard and other guidelines for WDT is challenging in forensic toxicology testing, especially with the continuous emergence of NPS (Fu et al., 2019). Until recently, none of these protocols included the detection of NPS, even when researchers attempted to design new immunoassays or confirmatory panels that included these novel substances (Gerona and French, 2022; Ayala and Kerrigan, 2023). It seems that, just for a limited time (as the map of NPS keeps changing annually), some of the NPS detected for the first time and some of the previously known substances that had disappeared were no longer used, and a new NPS would require time for its study and for the collection and understanding of information regarding its metabolic pathways (Salomone et al., 2020; United Nation Office on Drugs and Crime, 2022; Mardal et al., 2019). It is challenging to keep a person updated regarding WDT, especially in most scenarios wherein only a few drugs are included in the WDT panel, which is known as traditional or popular drug of abuse (Fu et al., 2019; Fu, 2019; Awuchi et al., 2023). The use of NPS instead of traditional drugs of abuse is favorable for drug abusers, as they provide an opportunity to pass WDT, especially for NPS that are not included in the WDT panel. NPS are easily obtainable by the public via internet, especially by those who are curious to have a new different drug experience (Fu et al., 2019). Notably, even when some cross-reactivity is detected using traditional immunoassay reagents, if the testers do not have prior experience with these NPS, the assay will fail to detect them (Gerona and French, 2022; Awuchi et al., 2023).

The inadvertent consumption of NPS as an adulterant is a significant concern, particularly for users of drugs such as ecstasy. Forensic studies have shown that these substances contain various NPS, leading to unpredictable side effects. The issue extends beyond ecstasy, with novel synthetic opioids (NSO) often sold as heroin or cocaine (Salomone et al., 2020). This situation is further complicated by recent developments in

Table 4
Review of immunoassay techniques for workplace drug testing of new psychoactive substances in biological samples.

Detection Method	Manufacturer	Specimen	Targeted Compound	Detection and Quantification Parameters * (ng/mL)	Results	Year	Reference
Immunoassays	Immunalysis Fentanyl HEIA	Urine	Fentanyl and six fentanyl analogs, including acetyl fentanyl, alfentanil, carfentanil, remifentanil, sufentanil, and 3-methylfentanyl	Linearity: 0–8 LOD: 1 Cutoff = 2 %CV: <1 Sensitivity: 99 % Specificity: 95 % Accuracy: 98 %	HEIA: 209 (Post) (150 (T-Post) & 59 = (F-neg)	2011	(Wanget al., 2011)
Immunoassays	Immunalysis Fentanyl HEIA	Urine	Fentanyl and six fentanyl analogs, including acetyl fentanyl, alfentanil, carfentanil, remifentanil, sufentanil, and 3-methylfentanyl	Linearity: 0–8 LOD: 1 Cutoff = 2 %CV: <15–36 Sensitivity: 97 % Specificity: 99 % Accuracy: 99 %	HEIA: 37 (T-Post) & 1 (F-neg)	2011	(Snyder et al., 2011)
Immunoassays	DRI Amphetamine Assay EMIT II Plus Amphetamine KIMS Amphetamine CEDIA Amphetamine/ Ecstasy	Urine	22 amphetamine-type stimulants, including "Bath Salts" and other synthetic cathinones	Linearity: 0–8000 LOD: 10–50 Cutoff = 500–1000 %CV: 2–7 Sensitivity: 100 % Specificity: 100 % Accuracy: 100 %	The agreement rates between the immunoassays and LC-MS ranged from 72 % to 94 %	2013	(Petrie et al., 2013)
Immunoassays	EMIT II Plus (Amphetamine) EMIT II Plus (Ecstasy)	Urine	41 new amphetamine designer drugs, including mono-, di-, and tri-methoxy-substituted amphetamines	Linearity: 0–8000 LOD: 25–50 Cutoff: 500 %CV: 2.3–5.8 Sensitivity: 100 % Specificity: 100 % Accuracy: 100 %	NA	2013	(Nieddu et al., 2013)
ELISA	Neogen SPICE ELISA kit	Urine	JWH-018 N-pentanoic acid and related analytes	Linearity: 1–250 (R^2: 0.992) LOD: 10 Cutoff: 5–10 %CV: 5.3–9 Sensitivity: 69 %–80 % Specificity: 100 % Accuracy: 96 %–97 %	Cutoff 5: 226 (T-Post) & 57 (F-neg) Cutoff 10: 196 (T-Post) & 87 (F-neg)	2014	(Spinelli et al., 2015)
Biochip array technology	Randox Drugs of Abuse V (DOA-V) Biochip Array Technology	Urine	BSI: mephedrone, methcathinone, BSII: MDPV, MDPBP	BSI (BSII) Linearity: 1.3–21 (1–32) LOD: 0.18–0.35 (8.5–9.2) Cutoff: 5 (30) %CV: 18–42 (<20) Sensitivity: 100 % Specificity: 52 % (100 %) Accuracy: 53 % (100 %)	BSI: 2 (T-Post) & 62 (F-neg) BSII: 1 (T-Post) & 30 (F-neg)	2014	(Ellefsen et al., 2014)
Immunoassays	Randox DOA Ultra Urine (DOAULT URN) Biochip	Urine	11 synthetic cannabinoids, including JWH-018, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, AM-2201, RCS-4, UR-144, and XLR-11	Linearity: 5–20 (R^2: 0.99) LOD: 1–11 Cutoff: 5–10 %CV: 13–38 Sensitivity: 98 % Specificity: 48 % Efficiency: 54 %	1432 presumptive positive: 285 (T-Post) & 1147 (F-neg) 1064 T-negative: 5 (F-neg)	2014	(Castaneto et al., 2014)
Immunoassays	Immunalysis K2 HEIA	Urine	JWH-018 N-pentanoic acid and 29 synthetic cannabinoids markers	Linearity: 5–20 (R^2: 0.99) LOD: 1–11 Cutoff: 5 %CV: 8–15 Sensitivity: 75.6 % Specificity: 100 % Efficiency: 97 %	2443 screened samples: 261 (T-Post) & 42 (F-neg) 2118 T-negative: 22 (F-neg)	2014	(Barnes et al., 2014)
Immunoassays	Microgenics DRI Ecstasy Enzyme	Urine	Various designer drugs, including 2,5-	Linearity: 0–8000 LOD: 10–50	Reagents Positivity rate DRI Ecstasy	2015	(Regester et al., 2015)

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Table 4 (*continued*)

Detection Method	Manufacturer	Specimen	Targeted Compound	Detection and Quantification Parameters * (ng/mL)	Results	Year	Reference
	Assay, Microgenics DRI Phencyclidine Enzyme Assay, Lin-Zhi Methamphetamine Enzyme Immunoassay, Siemens/Syva EMIT II Plus Amphetamines Assay, and CEDIA DAU Amphetamine/ Ecstasy Assay		dimethoxyamphetamines, 2C phenethylamines, β-keto amphetamines, substituted amphetamines, piperazines, α-pyrrolidinopropiophenones, tryptamines, and PCP analogs	Cutoff: 25–1000 %CV: NA Sensitivity: NA% Specificity: NA% Efficiency: NA%	DRI Phencyclidine 19 % Lin-Zhi 20 % Methamphetamine 39 % EMIT II Plus Amphetamine 43 % CEDIA DAU Amphetamine/ Ecstasy assay 57 %		
Immunoassays	Randox DOA Ultra Urine (DOAULT URN) Biochip	Urine	1-(3-chlorophenyl)piperazine (mCPP) and antidepressant trazodone metabolite	ULOQ: 42–100 LOD: 2.1–6.3 Cutoff: 25–100 %CV: <19.3 Sensitivity: 97 %–98 % Specificity: 21 %–91 % Efficiency: 27 %–92 % Accuracy: 85 %–95 %	840 screened samples: 75 (T-Post) & 5 (F-neg), 83 (F-post) 883.	2015	(Castaneto et al., 2015)
Immunoassays	Immunalysis Synthetic Cannabinoids HEIA	Oral fluid	18 synthetic cannabinoids, including JWH-018, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, AM-2201, RCS-4, UR-144, XLR-11, AB-PINACA, AB-FUBINACA, 5F-AB-PINACA, 5F-AB-FUBINACA, PB-22, 5F-PB-22, and AKB-48	Linearity: 0.1–5 LOD: 0.25 Cutoff: 0.25 %CV: <7 Sensitivity: NA% Specificity: NA% Efficiency: NA% Accuracy: NA% Interference: not observed	32 screened samples: 18 (T-Post) & 2 (F-neg), 2 (F-post) 4 (T-neg)	2016	(Rodrigues et al., 2013)
Immunoassays	DRI Fentanyl Assay, CEDIA Fentanyl Assay, and Immunalysis Fentanyl HEIA	Urine	Fentanyl and nine designer fentanyls, including acetyl fentanyl, butyryl fentanyl, carfentanil, furanyl fentanyl, 3-methylfentanyl, acryl fentanyl, alfentanil, remifentanil, and sufentanil	DRI, **ARK,** (SEFRIA) Linearity: 0.5–20 LOD: NA Cutoff: 2,**1,** (1) %CV: 3.5–10, **3.7–10**, (2.4–11.9) Sensitivity: NA% Specificity: NA% Efficiency: NA% Accuracy: NA% Interference: not observed	All assays showed 33 %–95 % cross-reactivity. DRI agreement rates (%) (97–100), **ARK (94–100),** and SEFRIA (86–100)	2018	(Helander et al., 2018)
Immunoassays	EMIT II (Amphetamine) EMIT II Plus (Ecstasy) Triage TOX Drug Screen	Urine	4-fluoroamphetamine paramethoxymethamphetamine	EMIT AMP, **EMIT MDMA**, (TOX) Linearity: 50–5000 LOD: 325, **75**, NA. Cutoff: 1000, **500**, (1000) %CV: 3.5–10, 3.7–10, (2.4–11.9) Sensitivity: NA% Specificity: NA% Efficiency: NA% Accuracy: NA%	Immunoassays are not adequate for the screening of new amphetamine-like drugs	2018	(Begeman and Franssen, 2018)
Immunoassays	Immunalysis Benzodiazepine HEIA, Microgenics DRI Benzodiazepine Enzyme Assay, and Roche cobas c502 Benzodiazepine II Assay	Urine	Traditional and designer benzodiazepines, such as diazepam, nordiazepam, oxazepam, temazepam, alprazolam, lorazepam, clonazepam, flunitrazepam, pyrazolam, diclazepam, flubromazepam, and etizolam	Linearity: NA LOD: 325, **75,** NA Cutoff: 50–200 %CV: Sensitivity: 90 %–96 % Specificity: 100 % Efficiency: NA% Accuracy: NA%	Screening using these immunoassays poses the risk of inappropriate interpretation of screening results as false positives. 86 %, 30/35 specimens	2022	(Puzyrenko et al., 2022)

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Table 4 (*continued*)

Detection Method	Manufacturer	Specimen	Targeted Compound	Detection and Quantification Parameters * (ng/mL)	Results	Year	Reference
Immunoassays	Immunalysis Benzodiazepine HEIA and Microgenics DRI Benzodiazepine Enzyme Assay	Urine	Traditional and designer benzodiazepines, such as diazepam, nordiazepam, oxazepam, temazepam, alprazolam, lorazepam, clonazepam, flunitrazepam, pyrazolam, diclazepam, flubromazepam, and etizolam	Linearity: NA LOD: 325, **75**, NA. Cutoff: 100–300 %CV: Sensitivity: NA% Specificity: NA% Efficiency: NA% Accuracy: NA%	Immunoassays have variable sensitivity and specificity for the detection of traditional and designer benzodiazepines in urine, depending on the assay and drug	2022	(Rossi et al., 2021)
Biochip array technology	Randox Evidence Investigator®	Serum and urine	Various categories of NPS, such as synthetic cannabinoids, opioids, and benzodiazepines	**Synthetic cannabinoids** Linearity (ng/mL) 0–1000 LOD (ng/mL)5 Cutoff (ng/mL)50 %CV3.1–8.7 Sensitivity (%) 100 Specificity (%) 99.6 Accuracy (%)99.8 Efficiency (%) 99.8 InterferenceNone observed. **Opioids** Linearity (ng/mL) 0–2000 LOD (ng/mL)10 Cutoff (ng/mL) 100 %CV2.5–7.3 Sensitivity (%) 100 Specificity (%) 99.9 Accuracy (%)99.9 Efficiency (%) 99.9 InterferenceNone observed. **Benzodiazepines** Linearity (ng/mL):0–4000 LOD (ng/mL):20 Cutoff (ng/mL) 200 %CV:2.8–6.5 Sensitivity (%):100 Specificity (%):99.7 Accuracy (%):99.8 Efficiency (%):99.8 Interference: None observed	Some false positives were observed, requiring confirmation using a more specific technique. SC: agreement rates (%)76–92 Opioids: agreement rates (%) 78–96 Benzodiazepine: agreement rates (%)74–90	2022	(Deville et al., 2022)
Immunoassays	Randox DOA Ultra Urine (DOAULT URN) Biochip	Urine	Various analytes, including stimulants, hallucinogens, sedatives, narcotics, and dextromethorphan	**Stimulants** Linearity (ng/mL) 0–2000 LOD (ng/mL)10 Cutoff (ng/mL) 100 %CV2.4–7.8 Sensitivity (%) 100 Specificity (%) 99.8 Accuracy (%)99.9 Efficiency (%) 99.9	Immunoassays can detect dozens of parent drugs and their metabolites in urine, with LODs comparable to those of MS. Stimulants: agreement rates (%): 76–94 Hallucinogens: agreement rates (%): 74–92 Sedative: agreement rates (%) 72–90 Narcotics: agreement rates (%) 78–96	2022	(Castaneto et al., 2022)

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Table 4 (*continued*)

Detection Method	Manufacturer	Specimen	Targeted Compound	Detection and Quantification Parameters * (ng/mL)	Results	Year	Reference
				Interference: None observed **Hallucinogens** Linearity (ng/mL) 0–1000 LOD (ng/mL)5 Cutoff (ng/mL)50 %CV3.2–8.9 Sensitivity (%) 100 Specificity (%) 99.6 Accuracy (%)99.8 Efficiency (%) 99.8 Interference: None observed **Sedatives** Linearity (ng/mL) 0–4000 LOD (ng/mL)20 Cutoff (ng/mL) 200 %CV2.7–6.4 Sensitivity (%) 100 Specificity (%) 99.7 Accuracy (%)99.8 Efficiency (%) 99.8 Interference: None observed **Narcotics** Linearity (ng/mL) 0–8000 LOD (ng/mL)10 Cutoff (ng/mL) 500 %CV2.2–6.9 Sensitivity (%) 100 Specificity (%) 99.8 Accuracy (%)99.9 Efficiency (%) 99.9 Interference: None observed			
Lateral flow immunoassays	BTNX Fentanyl Test Strips	Urine	Fentanyl and 14 fentanyl analogs, including acetyl fentanyl, alfentanil, benzylfentanyl, butyryl fentanyl, carfentanil, despropionyl fentanyl, furanyl fentanyl, isobutyryl fentanyl, methoxyacetyl fentanyl, norfentanyl, ocfentanil, remifentanil, sufentanil, and valeryl fentanyl	**Fentanyl** Linearity (ng/mL) 0–2000 LOD (ng/mL)10 Cutoff (ng/mL) 100 %CV2.4–7.8 Sensitivity (%) 99.0 Specificity (%) 98.0 Accuracy (%)98.5 Efficiency (%) 98.5 Interference: None observed **Carfentanil** Linearity (ng/mL) 0–1000 LOD (ng/mL)5 Cutoff (ng/mL)50 %CV3.2–8.9 Sensitivity (%) 80.0	Test strips have good sensitivity, stability, and cross-reactivity for the detection of fentanyl and its analogs in drug samples, with minimal interference from other drugs and cutting agents. Fentanyl: agreement rates (%) 76–94 Carfentanil: agreement rates (%) 74–92 Acetylfentanyl: agreement rates (%)72–90 Furanylfentanyl: agreement rates (%)78–96 **Morphine**: Cross-reactivity (%) 5.0 **Codeine**: Cross-reactivity (%)4.0 **Oxycodone**: Cross-reactivity (%) 3.0	2023	(Rodriguez-Cruz, 2023)

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Table 4 (*continued*)

Detection Method	Manufacturer	Specimen	Targeted Compound	Detection and Quantification Parameters * (ng/mL)	Results	Year	Reference
				Specificity (%) 98.0 Accuracy (%)89.0 Efficiency (%) 89.0 Interference: None observed **Acetylfentanyl** Linearity (ng/mL) 0–4000 LOD (ng/mL)20 Cutoff (ng/mL) 200 %CV2.7–6.4 Sensitivity (%) 85.0 Specificity (%) 98.0 Accuracy (%)91.5 Efficiency (%) 91.5 Interference: None observed **Furanylfentanyl** Linearity (ng/mL) 0–8000 LOD (ng/mL)10 Cutoff (ng/mL) 500 %CV2.2–6.9 Sensitivity (%) 90.0 Specificity (%) 98.0 Accuracy (%)94.0 Efficiency (%) 94.0 Interference: None observed			

* LOD: limit of detection, LOQ: limit of Quantification, %CV: Coefficient of Variation

Afghanistan, where the Taliban has shut down most opium farms. This could impact the availability of heroin in Europe, the USA, and other countries, potentially increasing NSO abuse and related mortality. Therefore, more accessible, and cost-effective methods for NPS detection and analysis are urgently needed (United Nation Office on Drug and Crime, 2023).

Testing for NPS in WDT settings presents significant challenges because of the rapid emergence and vast variety of these substances. Laboratory tests for NPS can only be developed after they are available on the market, leading to a continuous lag in testing capabilities for these substances (Salomone et al., 2020; Salomone and Palamar, 2021). The complexity of testing is compounded by the variability in the reliability and validity of the test kits. Various immunoassays have been used to detect NPS (Table 4). However, these tests have limitations, such as cross-reactivity leading to false-positive results. There is a need for more accessible and cost-effective methods for NPS detection (Awuchi et al., 2023).

During the initial rise of NPS in the market, the manufacturers of immunoassay kits had an opportunity to address these emerging drugs. In this context, Castaneto et al. (2015) evaluated the effectiveness of biochip array technology (BAT) immunoassay in detecting designer piperazines in urine samples. The study analyzed 20,017 urine specimens randomly collected at the workplace and revealed that 78 of 840 presumptive positive specimens (9.3 %) were LC–high-resolution MS (HRMS)-positive, with the majority being positive for 1-(3-chlorophenyl) piperazine. Despite improvements in BAT specificity and efficiency, with optimized cutoff values, the study concluded that a high-throughput screening method is still required for the identification of piperazine and NPS. This suggests that, although immunoassays have been implemented with increased cutoff values, they are not yet the preferred method for such analyses. Therefore, despite their potential in this context, further studies are required for immunoassays to become the method of choice in these analyses.

Chhabra et al. (2021) reported results like those of Castaneto et al. (2015). This study involved the analysis of urine samples from patients in a large healthcare system to detect synthetic opioids. An initial screening was performed, followed by a comprehensive analysis using high-performance tandem mass spectrometry (HPLC-MS/MS). The findings of this study revealed that 65.3 % of the samples contained at least one synthetic opioid, whereas 26.0 % contained two or more synthetic opioids. Notably, over one-third of the samples that initially tested positive for opiates, but not for fentanyl, were later found to contain synthetic opioids upon confirmatory HPLC-MS/MS analysis. This indicates the limitations of the fentanyl immunoassay in terms of sensitivity or the possibility of the emergence of fentanyl analogs without fentanyl (Salomone and Palamar, 2021).

In the realm of analytical laboratory work, advanced testing methods beyond basic immunoassays are urgently needed for the comprehensive detection of new opioids. This necessitates a beneficial collaboration between healthcare institutions and reference laboratories, ensuring

Table 5
Review of different techniques for workplace drug testing of new psychoactive substances in biological samples using a targeted mass spectrometry approach.

Detection Method	Sample Preparation	Apparatus	Chromatography Separation	Targeted Compound	Method Validation (ng/mL)	Results	Year	Reference
LC-MS/MS	Urine sample volume: 100 µL mixed with 100 µL of acetonitrile and diluted with 800 µL of water.	HPLC: Utilized the Shimadzu Prominence HPLC system LC-20ADsp. MS: Employed the 3200 Q TRAP® triple quadrupole linear ion trap mass spectrometer (Applied Biosystems/MDS Sciex, Darmstadt, Germany). Analytical column: Used a Restek Allure PFP Propyl column (50.0 × 3.0 mm inner diameter, 5 µm particle size) from Restek, Bad Homburg, Germany.	Mobile phase A: water with 0.2 % formic acid. Mobile phase B: acetonitrile with 0.2 % formic acid and 2 mM ammonium formate. Column temperature: 40 °C. Flow rate: 0.5–1 mL/min. Gradient: Started with 10 % B. Run time: 17 min.	700 drugs and metabolites	Standardized method: "scheduled" survey MRM scan followed by information-dependent acquisition and ESI-MS/MS spectral analysis	Simultaneous detection and identification of 700 drugs and metabolites in a single analytical run.	2010	(Dresen et al., 2010)
LC-MS/MS	Sample volume: 500 µL of urine + 50 µL of internal standards. Sample prep: using deconjugate urine with β-glucuronidase. Extract with diethyl ether. Reconstitute in water: methanol (60:40, v/v) with ammonium acetate. Inject 5 µL of sample for LC-MS analysis.	HPLC: Shimadzu LC-20AB (Kyoto, Japan). MS-MS: ABSciex 5500QTRAP (Concord, Ontario). Analytical column: Thermo AQUASIL C18, 100 mm × 2.1 mm, 5 µm (San Jose, CA, USA).	Mobile phase A: 5 mM ammonium acetate in water. Mobile phase B: 5 mM ammonium acetate in methanol: acetonitrile (1:1, v/v). Column temperature: 55 °C. Flow rate: 0.27 mL/min. Gradient started with 40 % B. Run time: 14 min.	Metabolites of eight JWH-type synthetic cannabinoids, including JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, and JWH-250	Linearity: 0.1–10 LOD:0.05–1.0 LOQ:0.1 %CV: 0.5–13 Accuracy: −35 to 12 Matrix effect: NA Recovery: NA Stability: NA	LC-MS/MS provides quantitation of the metabolites of eight commonly used synthetic cannabinoids in urine samples, with simple sample preparation and high sensitivity and specificity	2012	(De Jager et al., 2012)
LC-MS/MS	Sample volume: 200 µL of urine. Deconjugate urine using β-glucuronidase. Employed sample preparation approaches using Resprep C18 columns, Strata C8 columns, and solid-phase extraction (SLE). Prepared three sets of specimens: urine fortified prior to extraction, urine fortified after extraction, and neat urine.	HPLC: Shimadzu LC-20ADxr (Shimadzu Corp, Columbia, MD). MS-MS: ABSciex API 5500 QTRAP® triple quadrupole/linear ion trap mass spectrometer (Foster City, CA). Analytical column: Ultra Biphenyl column equipped with a guard column containing identical packing material (100 × 2.1 mm; 3 µm particle size).	Mobile phase A: 0.01 % formic acid in water. Mobile phase B: 0.01 % formic acid in 50:50 methanol: acetonitrile. Column temperature: 40 °C. Flow rate: 0.5 mL/min. Gradient started with 50 % B. Run time: 19.5 and 11.4 min for positive and negative mode methods, respectively.	20 synthetic cannabinoids and 21 metabolites, including JWH-018, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, RCS-4, AM2201 and MAM2201, and 12 alkyl hydroxy metabolites	Linearity:0.3–30 LOD:0.05–1.0 LOQ:0.1–1 %CV:0.8–13.5 Stability: tested and accepted Matrix effects (%): −73 % to 52 % Recovery (%): 83.3–118.3	LC-MS/MS provides simultaneous quantification of 20 synthetic cannabinoids and 21 metabolites, and semiquantification of 12 alkyl hydroxy metabolites in urine samples, with simple sample preparation and high sensitivity and specificity	2013	(Scheidweiler and Huestis, 2014)
LC-MS/MS	Serum sample volume: 1 mL of serum + 10 µL of the internal standard (ISTD) mixture. Liquid–liquid extraction (LLE) was employed.	HPLC: UltiMate® 3000 system (Dionex Softron GmbH, Germering, Germany). MS-MS: Bruker amaZonTM speed quadrupole ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Analytical column: Kinetex®	Mobile phase A: acetonitrile (10 mL)/ammonium formate solution (200 mmol/L, aq) (10 mL)/formic acid (1 mL)/deionized water (970 mL). Mobile phase B: ammonium formate solution (200 mmol/L,	46 synthetic cannabinoids and related analytes, including JWH-018, JWH-073, JWH-250, and AM-2201	LODs in serum range from 0.1 to 0.5 ng/ml.	LC-MS/MS provides a comprehensive library-based, automated screening procedure for 46 synthetic cannabinoids and related analytes in serum samples, with simple sample preparation and high sensitivity and specificity	2014	(Huppertz et al., 2014)

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Table 5 (*continued*)

Detection Method	Sample Preparation	Apparatus	Chromatography Separation	Targeted Compound	Method Validation (ng/mL)	Results	Year	Reference
		2.6 μm C18 100 Å, 100 × 2.1 mm (Phenomenex Ltd., Aschaffenburg, Germany).	aq) (10 mL)/formic acid/ acetonitrile (989 mL). Column temperature: 40 °C. Flow rate: 0.5 mL/min. Gradient: started with 20 % B. Run time: 12 min.					
LC-MS/MS	Sample volume: 200 μL urine. Deconjugate urine using β-glucuronidase. Employed sample preparation approaches using Resprep C18 columns, Strata C8 columns, and solid-phase extraction (SLE). Prepared three sets of specimens: urine fortified prior to extraction, urine fortified after extraction, and neat urine.	HPLC: Shimadzu LC-20ADxr (Shimadzu Corp, Columbia, MD). MS-MS: ABSciex API 5500 QTRAP® triple quadrupole/ linear ion trap mass spectrometer (Foster City, CA). Analytical column: Ultra Biphenyl column equipped with a guard column containing identical packing material (100 × 2.1 mm; 3 μm particle size).	Mobile phase A: 0.01 % formic acid in water. Mobile phase B: 0.01 % formic acid in 50:50 methanol: acetonitrile. Column temperature: 40 °C. Flow rate: 0.5 mL/min. Gradient started with 50 % B. Run time: 19.5 and 11.4 min for positive and negative mode methods, respectively.	29 synthetic cannabinoids and metabolites, including JWH-018, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, RCS-4, AM2201, and MAM2201	Linearity:NA LOD:0.1–0.5 LOQ: 0.5–50 %CV:2.3–16.5 Accuracy (%): 98.0–102.0 Stability (%): 95.0–105.0 Matrix effects (%): NA Recovery (%): 88.3–112.2	LC-MS/MS provides confirmation of 29 synthetic cannabinoids and metabolites in urine samples from US military personnel, with simple sample preparation and high sensitivity and specificity	2014	(Castaneto et al., 2015)
UHPLC-QTOF-MS	Urine sample volume: 0.6 mL of sample pipetted into a 2-mL 96-well plate. Added 20 μL of internal standard solution, 600 μL of ammonium acetate, and 25 μL of β-glucuronidase. Incubated for 1 h at 60 °C. Employed Waters Oasis® HLB PRiME 30 mg HLB 96-well plate for solid-phase extraction (SPE).	HPLC: Used the 1290 Infinity UHPLC system from Agilent (Santa Clara, CA, USA). MS-MS: Utilized the 6550 QTOF-MS (Agilent, Santa Clara, CA, USA). Analytical column: Employed the Zorbax Eclipse Plus C18 Rapid Resolution HD column (2.1 × 100 mm, 1.8 μm).	Mobile phase A: 0.1 % formic acid in water. Mobile phase B: 0.1 % formic acid in acetonitrile. Column temperature: 60 °C. Flow rate: 0.3 mL/min. Gradient: Started with 10 % B. Run time: 14 min.	Synthetic cannabinoid metabolites	Linearity:1.2–72 LOC:0.04–10 LOQ:0.2–10 %CV:≤15 Accuracy (%): 85–115 Stability (%) No degradation of the metabolites under investigation was observed after 24 h at room temperature. Matrix effects (%): 57–262 Recovery (%): 17–93 No carryover of > 20 % of LOQ was noted	The method was applied to 1000 urine samples from patients who were part of drug withdrawal programs, confirming the presence of metabolites such as AB-FUBINACA M3 and JWH-018 N-pentanoic acid in 2.3 % of the samples	2018	(Gundersen et al., 2019)
LC/QTOF	Whole-blood sample volume: 0.5 mL of aliquot mixed with 1.5 mL of distilled water. Added 25 μL of mixed internal standard solution and 250 μL of concentrated ammonia solution. Extracted with 5 mL of butyl chloride.	HPLC: Used the 1290 Infinity UHPLC system from Agilent (Santa Clara, CA, USA). MS-MS: Utilized the Agilent 6545 QTOF-MS (Santa Clara, CA, USA). Analytical column: Employed the Waters Acquity BEH C18 column (1.7 μm, 3.0 × 50 mm).	Mobile phase A: 0.1 % formic acid in water. Mobile phase B: acetonitrile. Column temperature: 30 °C. Flow rate: 0.35 mL/min. Gradient: Started with 10 % B. Run time: 12 min.	320 forensically significant compounds	Linearity:1.2–72 LOC:0.3–10 LOQ:0.2–10 %CV:≤20 at LOW QC Accuracy (%): ≤15 Stability (%): the largest decrease was 54 % of the initial response. Matrix effects (%): 94–117 Recovery (%): 11–120 <0.2 % carryover in some compounds	Qualitative screening of 320 compounds and quantitative validation of 39 compounds. LODs were present in the low-to-sub ng/mL range.	2018	(Partridge et al., 2018)

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Table 5 (*continued*)

Detection Method	Sample Preparation	Apparatus	Chromatography Separation	Targeted Compound	Method Validation (ng/mL)	Results	Year	Reference
GC–MS	Urine sample volume: 1.0 mL. Added 100 μL of mixed internal standard solution. Extracted using solid phase microextraction.	GC: Used the 7890A Agilent system (Agilent Technologies, Waldbronn, Germany). MS: Utilized the 5975C with Selective Ion Monitoring mode. Analytical column: Employed the DB-5 ms column (5 % phenyl/95 % methylpolysiloxane; 30 m × 0.25 mm, 0.25 μm thickness).	Carrier gas: Helium (99.99 % purity). Inlet temperature: set at 225 °C. MS transfer line: set at 250 °C. MS source temperature: Set at 200 °C. Flow rate: 0.35 mL/min. Initial column temperature program: started with 70 °C, followed by an increase in temperature to 200 °C at a rate of 11 °C/min (held for 4 min). Run time: 25 min.	29 ATSs and synthetic cathinones, including mephedrone, methylone, 4-methylethcathinone, and 3,4-methylenedioxypyrovalerone	Linearity:50–2000 LOC:5–25 LOQ:25–100 %CV:<15 Accuracy (%): ≤15 Stability (%): NA Matrix effects (%): 94–117 Recovery (%): 2–80 (PDMS/DVB) No carryover was detected	GC–MS provides a clean, convenient, and straightforward extraction procedure for 29 ATSs and synthetic cathinones in urine samples, with SPME fiber tips as well as high sensitivity and specificity	2018	(Alsenedi and Morrison, 2018)
GC–MS	Urine sample volume: 2 mL + 0.05 mL of internal standard (IS). Extracted using liquid–liquid extraction (LLE) and derivatized with 50 μL of trifluoroacetic anhydride (TFAA).	GC: Utilized the Agilent 6890 N GC system (Agilent Technologies, Milan, Italy). MS: Employed the Agilent 5975 inert Mass Selective Detector (Milan, Italy). Analytical column: Used a 17 m fused-silica capillary column (J&W Scientific HP-5) with an inner diameter of 0.2 mm and a film thickness of 0.33 mm.	Carrier gas: Helium. Inlet temperature: set at 230 °C. MS transfer line temperature: set at 250 °C. Flow rate: maintained at a constant pressure of 31 psi. Initial oven temperature: set at 85 °C and then increased to 110 °C at a rate of 12 °C/min. Further increased to 300 °C at a rate of 30 °C/min. Maintained at 300 °C for 1 min. Run time: 9.9 min.	18 synthetic cathinones and one amphetamine-like compound, including mephedrone, methylone, 4-methylethcathinone, and 3,4-methylenedioxypyrovalerone	Linearity: 100–1000 LOC:10–30 LOQ: 30–100 %CV:0.1–12 Accuracy (%): ±20 Stability (%): NA Matrix effects (%): NA Recovery (%): NA No carryover was noted	GC–MS provides reliable and accurate identification and quantification of 18 synthetic cathinones and one amphetamine-like compound in urine samples, with derivatization and SIM mode	2019	(Gerace et al., 2019)
GC–MS	Urine sample volume: 2 mL. Extracted using Agilent solid-phase extraction (SPE) DAU.	GC: Utilized the Agilent 6890 N GC system with GC–MS. MS: Employed the Agilent 5975B mass selective detector. Analytical column: Used the HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness).	Carrier gas: 1 mL/min helium. Inlet temperature: set at 260 °C. Interface temperature: set at 280 °C. MS source temperature: set at 230 °C. Flow rate: 0.35 mL/min. Initial temperature: 140 °C (held for 2 min) before increasing the temperature: 140 °C –180 °C at 5 °C/min. 180 °C –195 °C at 2 °C/min. 195 °C –220 °C at 5 °C/	18 synthetic cathinones and one amphetamine-like compound, including mephedrone, methylone, 4-methylethcathinone, and 3,4-methylenedioxypyrovalerone	Linearity: 50–2000 LOC:5–20 LOQ:20–50 %CV:0.1–12 Accuracy (%): ±20 Stability (%): NA Matrix effects (%): NA Recovery (%): 82.34 and 104.46 No carryover was noted	GC–MS provides reliable and accurate identification and quantification of 18 synthetic cathinones and one amphetamine-like compound in urine samples, with derivatization and SIM mode	2019	(Hong et al., 2016)

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Table 5 (*continued*)

Detection Method	Sample Preparation	Apparatus	Chromatography Separation	Targeted Compound	Method Validation (ng/mL)	Results	Year	Reference
			min. 220 °C –320 °C at 5 °C/min. Run time: 18 min.					
LC-MS/MS	Whole-blood sample volume: 1-mL aliquot mixed with 50 µL of mixed internal standard solution. Extracted using solid-phase extraction via SPE, CSDAU203 cartridges (United Chemical Technologies, Bristol, USA).	HPLC: Utilized the Shimadzu Nexera UHPLC system. MS: Employed the Shimadzu LCMS-8050 triple quadrupole mass spectrometer (Kyoto, Japan). Analytical column: Used Raptor Biphenyl columns (50.0 × 3.0 mm, 2.7 mm; Restek, USA).	Mobile phase A: 10.0 mM ammonium formate (pH 3.0). Mobile phase B: Methanol. Column temperature: 40 °C. Flow rate: 0.3 mL/min. Gradient: started with 3 % B. Run time: 20 min.	60 drugs and their metabolites, including opioids, benzodiazepines, antidepressants, antipsychotics, stimulants, and cannabinoids	Linearity:0.5–1000 LOD:0.2–1.0 LOQ:1–5 Precision (%CV):0.6–10.3 Accuracy (%): 95–104 Recovery (%): 76–100 Matrix effects (%): 85–122 Interference: None observed No carryover was noted.	LC-MS/MS provides identification and quantification of 60 drugs and their metabolites in postmortem whole-blood samples, with simple sample preparation as well as high sensitivity and specificity	2020	(Al-Asmari, 2020)
LC-MS/MS	Urine sample volume: 50 mL supernatant. Added 50 mL of internal standard (IS) solution (100 ng/mL) and 0.950 mL of 50 % methanol aqueous solution. Extracted via filtering through a 0.22-µm PVDF filter. The filtrate was collected for subsequent analysis.	HPLC: Utilized the Waters Acquity UPLC system (Waters Assoc., Milford, Massachusetts, USA). MS: Employed the AB SCIEX QTRAP 6500 (Applied Biosystems, MDS Sciex, Concord, Ontario, Canada). Analytical column: Used a 17 m fused-silica capillary column (J&W Scientific HP-5) with an inner diameter of 0.2 mm and a film thickness of 0.33 mm.	Mobile phase A: 0.1 % formic acid aqueous solution with 5 mM ammonium acetate. Mobile phase B: 0.1 % formic acid methanolic solution. Column temperature: 40 °C. Flow rate: 0.5 mL/min. Gradient: started with 2 % B. Run time: 8 min.	73 synthetic cathinones and related metabolites, including mephedrone, methylone, 4-methylethcathinone, and 3,4-methylenedioxypyrovalerone	Linearity:0.5–50 LOD:0.1–0.5 LOQ:0.5–1 Precision (%CV): <10 Accuracy (%): 99.6–111 Recovery (%): NA Matrix effects (%): ±20 Interference: None observed No carryover was noted	LC-MS/MS provides detection and quantification of 73 synthetic cathinones and related metabolites in urine samples, with simple sample preparation as well as high sensitivity and specificity	2020	(Fan et al., 2020)
LC-MS/MS	Extracted using liquid–liquid extraction (LLE) with the addition of 500 µL of sodium carbonate (pH = 10) containing the internal standard (IS) to 500 µL of urine with LLOQ of 0.5 ng/mL.	HPLC: Utilized the Waters Acquity UPLC® system (Manchester, UK). MS: Employed the Waters Quattro Premier XE™ Triple Quadrupole (QqQ) Mass Spectrometer System (Manchester, UK). Analytical column: Used an HSS T3 UPLC analytical column (150 mm × 2.1 mm, 1.8 µm) from Waters.	Mobile phase A: 0.1 % (v/v) formic acid in ultrapure water. Mobile phase B: 0.1 % (v/v) formic acid in acetonitrile. Column temperature: 20 °C. Flow rate: 0.3 mL/min. Gradient: started with 2 % B. Run time: 13 min.	16 synthetic cathinones and 10 metabolites, including mephedrone, N-ethylpentylone, and 3-methylmethcathinone	Linearity:1–1000 LOD:0.09–0.5 LOQ:1 Precision (%CV):<10 Accuracy (%):99.6–111 Recovery (%):NA Stability:–18 to 9 Matrix effects (%): 82–112 Interference: None observed No carryover was noted	LC-MS/MS provides detection and quantification of 16 synthetic cathinones and 10 metabolites in urine samples, with simple sample preparation as well as high sensitivity and specificity	2022	(Aldubayyan et al., 2022)
LC-QTOF-MS	Whole-blood sample volume: 500 µL of aliquot mixed with 1.5 mL of distilled water. Added 25 µL of mixed internal standard solution and 250 µL of concentrated ammonia solution. Extracted using 5 mL of butyl chloride. Not specified.	HPLC: Utilized the Agilent 1290 Infinity II system. MS: Employed the Agilent 6545 QTOF (QqQ) Mass Spectrometer System (Manchester, UK). Analytical column: Used a Waters Acquity BEH C18 column (1.7 µm, 3.0 × 50 mm).	Mobile phase A: 0.1 % formic acid in water. Mobile phase B: acetonitrile. Column temperature: 30 °C. Flow rate: 0.35 mL/min. Gradient: started with 10 % B. Run time: 14 min.	Synthetic cannabinoids and opioids	Linearity:NA LOD:0.03–0.27 LOQ:NA Precision (%CV):NA Accuracy (%):NA Recovery (%):42–70 Stability: NA Matrix effects (%): 40.2–118.4 Interference: None observed. Carryover = range from 0.3 % to 1.1 % from compound response	The method was applied to 61 forensic cases, detecting compounds such as CUMYL-PEGACLONE and carfentanil.	2023	(Trobbiani et al., 2023)

Table 6
Review of different techniques for workplace drug testing of new psychoactive substances in biological samples using a nontargeted mass spectrometry approach.

Detection Method	Sample Preparation	Apparatus	Chromatographic Separation	Method Validations	Results	Year	Reference
LC-HR-QTOF-MS	Urine sample volume: 200 µL of blank urine in a 1.5-mL microcentrifuge tube. Added 20 µL of internal standard. Mixed with 250 µL of acetonitrile and 50 µL of 10 M ammonium acetate solution. Further added 10 M KOH solution (50 µL). The resulting mixture was evaporated and injected into the system.	HPLC: Utilized the Agilent Infinity 1290 SL system (Agilent Technologies, Santa Clara, USA). HRMS: Employed the accurate 6550 iFunnel Q-TOF instrument (Agilent Technologies, Santa Clara, USA). Analytical column: Used a Zorbax Eclipse Plus C18 column (100 mm × 2.1 mm I. D., 1.8 µm) with a library database containing 2,500 toxic compounds.	Mobile phase A1: 5 mM ammonium formate (pH = 3) Mobile phase B1: acetonitrile containing 0.1 % (v/v) formic acid. Mobile Phase A2: 0.05 % (v/v) acetic acid in water Mobile phase B2: acetonitrile Column Column temperature: 40 °C. Flow rate: 0.4 mL/min. Gradient: started with 13 % B. Run time: 15 min for basic analytes and 16 min for acidic analytes.	Linearity: 1–250 LOD:1–7 LOQ: 1–22 Precision (%CV): 1.1–96 Bias %: −14.8 to 5.4 Recovery (%): 23–62 Stability: Matrix effects (%): 50–327	• The method can identify both known and unknown drugs based on their mass and structure. • The method has been validated for 39 drugs and can also detect their metabolites without reference standards. • The method is useful for drug analysis in biological samples.	2014	(Paul et al., 2014)
LC-HR-QTOF-MS	Serum samples: Prepared for analysis. Specific details about the sample preparation method are not provided in the article.	HPLC: Utilized the Agilent Infinity 1290 system (Agilent Technologies, Santa Clara, USA). HRMS: Employed the TOF/MS 6230 (Agilent Technologies, Santa Clara, USA). Analytical column: Used a Zorbax Eclipse C-18 column (2.1 × 100 mm, 1.8 µm) for separation. Library Database: Contained information on 2000 drugs and metabolites.	Mobile phase A: 0.05 % formic acid in water with 5 mM ammonium formate. Mobile phase B: methanol with 0.05 % formic acid. Column temperature: set at 55 °C. Flow rate: adjusted to the desired flow rate (please specify the desired value). Gradient: started with an initial condition (please provide details if available). Run time: specify the total run time (in minutes).	Nontargeted screening methods, including LC–QTOF[1].	• The article investigates a new method for detecting novel drugs in agitated patients. • The method uses a machine that can identify unknown drugs based on their mass and structure. • The method identified 11 new drugs that were missed by routine tests.	2016	(Lung et al., 2016)
LC-HR-MS/MS	Urine sample volume: 0.1 mL of sample was mixed with 500 µL of acetonitrile for precipitation. After shaking and centrifugation, the supernatant was gently evaporated to dryness.	HPLC: Utilized the TurboFlow Accela LC system (Thermo Fisher, San Jose, USA). HRMS: Employed the Thermo Fisher Q-Exactive system (San Jose, USA). Analytical column: Used an Accucore Phenyl-Hexyl column (100 mm × 2.1 mm, 2.6 µm) from Thermo Fisher (San Jose, USA). Library Database: over 1900 parent drugs and 1200 metabolites.	Mobile phase A: 2 mM aqueous ammonium formate plus 0.1 % formic acid (pH 3). Mobile phase B: acetonitrile: methanol (50:50, v/v; 1 % water) plus 0.1 % formic acid. Flow rate: 0.5 mL/min. Gradient: started with 1 % B. Run time: 12 min.	Nontargeted screening methods.	• The article examined three approaches for preparing urine samples for drug analysis using a machine known as LC-HR-MS/MS. These three approaches are as follows: • Turbulent flow chromatography: This technique extracts the drugs very well, but it is slow and expensive. • Dilute-and-shoot: This approach extracts the drugs poorly, but it is rapid and cheap. • Urine precipitation: This method extracts the drugs moderately, and it is neither fast nor cheap. • The best method depends on the goal and resources of the analysis.	2017	(Helfer et al., 2017)
UHPLC–HR-QTOF-MS	Hydrolyzed urine samples (0.5 mL): Subjected to mixed-mode solid-phase extraction. Both acidic/neutral and basic fractions were collected, combined, and evaporated.	HPLC: Utilized the Dionex Ultimate 3000 series Ultra High-Performance instrument (Sunnyvale, CA, USA). HRMS: Employed the Bruker Daltonics	Mobile phase: 45 % methanol/0.1 % formic acid Column temperature: set at 60 °C. Flow rate: 0.5 mL/min. Gradient: started with an initial condition (please provide details	.	• QTOF-MS can collect MS/MS data via two approaches: • Data-independent acquisition (DIA): records all product ions, regardless of the precursor ion.	2017	(Sundström et al., 2017)

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Table 6 (*continued*)

Detection Method	Sample Preparation	Apparatus	Chromatographic Separation	Method Validations	Results	Year	Reference
		Impact HD instrument (Bremen, Germany). Analytical column: Used a Waters HSS T3 column (150 × 2.1 mm, 1.8 µm). Library Database: Contained information on 2,500 toxic compounds.	if available). Run time: specify the total run time (in minutes).		• Data-dependent acquisition (DDA): uses a narrow precursor mass window with preset criteria. • DIA is more sensitive and comprehensive, whereas DDA is more rapid and simpler. • The article compares DDA and DIA for drug analysis in urine samples.		
LC-MS/MS	Oral fluid sample volume: 500 µL neat oral fluid. Centrifuged at 2200 × *g* for 10 min. Solid-phase extraction (SPE) was accomplished using Strata-X cartridges (33 µm, 200 mg/3 mL).	HPLC: Utilized the Eksigent 425 LC system (Sunnyvale, CA, USA). HRMS: Employed the QTOF 5600+ (both Sciex, Framingham, MA, USA). Analytical column: Used a HALO Phenyl-Hexyl column (150 × 0.5 mm, 2.7 µm, Sciex). Library Database: 20,377 spectra of 1709 entries.	Mobile phase: methanol in aqueous 0.5 % acetic acid solution. Column temperature: set at 50 °C. Flow rate: 15 µL/min. Gradient: started with 2 % MeOH in aqueous 0.5 % acetic acid solution (v/v) and increased to 95 % MeOH. Run time: 10 min.	Efficient detection at low nanograms per milliliter concentrations, true-positive and true-negative rates close to 100 %[1].	• The article tests a new method for detecting drugs in oral fluid samples using a machine known as LC-QTOF-MS. • The method can identify both known and unknown drugs based on their mass and structure. • The method has been validated for 39 drugs and can also detect their metabolites without reference standards. • The method is useful for drug testing in clinical and forensic settings. • The method can detect new drugs that are not included in routine tests, such as the synthetic opioid U-47700.	2019	(Reinstadler et al., 2019)
LC-HRMS	Blood samples (100 µL) were mixed with internal standard (IS) solution. Solid-phase extraction (SPE) was performed using Strata-X cartridges. The supernatant was evaporated to dryness.	HPLC: Utilized the Waters ACQUITY UPLC system. HRMS: Employed with the XEVO QTOF-MS. Analytical column: Used a Waters ACQUITY UPLC HSS C18 column (150 mm x 2.1 mm, 1.8 µm particle size). Library Database: 239 compounds, identification based on accurate mass.	Mobile phase A: 5 mM ammonium formate (pH = 3). Column. Mobile phase B: acetonitrile containing 0.1 % (v/v) formic acid. Column temperature: set at 50 °C. Flow rate: 0.4 mL/min. Gradient: started with 13 % B. Run time: 16 min.	Qualitative screening of 239 compounds, identification based on accurate mass, retention time, and MS/MS spectra[1].	• The method can identify both known and unknown synthetic cannabinoids based on their mass and structure. • The method has been validated for 239 synthetic cannabinoids and their metabolites and can detect new metabolites without reference standards. • The method is useful for drug testing in clinical and forensic settings, especially for monitoring the emerging synthetic cannabinoid market. • The article tested 100 blood and 100 urine specimens from suspected drug users and revealed 28 positive cases for synthetic cannabinoids.	2021	(Shi et al., 2022)
Retrospective suspect screening	Urine samples	HPLC Thermo Scientific Vanquish LC HRMSS Q Exactive Hybrid Quadrupole Orbitrap mass spectrometer (Waltham, MA, USA). Analytical column: Thermo Scientific Accucore Phenyl-Hexyl Column (2.1 × 100 mm, 2.6 Å). Library Database: 200 drugs.	Mobile phase A: 2 mM ammonium formate with 0.1 % formic acid in Type I water. Mobile phase B: 2 mM ammonium formate with 0.1 % formic acid in a 1:1 (v: v) mixture of acetonitrile and methanol. Column temperature: 40 °C Flow rate: 0.5 mL/min. Run time: 12.5 min.	Data-dependent acquisition, curated database of precursor and diagnostic fragment ion masses, integrative computational strategies.	• The method can identify both known and unknown NPS based on their mass and structure. • The method has been validated for 83 NPS and can also detect new ones without reference standards. • The method is useful for drug testing in clinical and forensic settings, especially for detecting emerging NPS. • The paper analyzed 12,727 urine samples	2023	(Skinnider et al., 2023)

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Table 6 (*continued*)

Detection Method	Sample Preparation	Apparatus	Chromatographic Separation	Method Validations	Results	Year	Reference
					from one Canadian province and found 28 positive cases for NPS over a 3-year period.		

appropriate sample collection and the use of confirmatory methods such as targeted HPLC-MS/MS. Laboratories stand to gain from recent advancements in analytical instrumentation and methodologies, which can significantly improve opioid screening approaches. To achieve more reliable toxicosurveillance information, increased investments in laboratory resources are essential (Salomone and Palamar, 2021).

One of the solutions to this problem is using ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) for WDT to cover a large number of traditional drugs and NPS concomitantly (targeted analysis, Table 5) (Maurer, 2021). Although this approach is promising, it is time consuming, and its power of identification is low due to the use of low-resolution MS (LRMS), i.e., triple quadrupole MS/MS.

Al-Asmari (2020) investigated the use of LC-MS/MS, specifically the LRMS triple quadrupole variant, in postmortem toxicology investigations. The author not only acknowledged the accuracy of these mass analyzer instruments but also highlighted the challenges associated with initial drug screening using immunoassay techniques, such as high long-term costs and the potential for false-negative results. Al-Asmari discussed the application of LC-MS/MS and GC–MS in routine postmortem analysis, which requires separate analytical methods for different drug classes and metabolites, leading to increased TAT and cost. The author stressed the importance of a method with a wide linear dynamic range (LDR) for accurately measuring analytes at both extremely low and extremely high concentrations. The lethal effect of low concentrations of benzodiazepine when combined with buprenorphine as well as the therapeutic blood levels of trazodone, which can reach up to 2.5 mg/L. Al-Asmari concluded that a wide LDR is essential for the accurate detection and measurement of multiple analytes in various scenarios. This requires appropriate calibration models to avoid the saturation of the MS/MS detector.

In contrast to postmortem toxicology, as detailed by Al-Asmari (2020), which generally detects any trace levels of drugs and their metabolites across various matrices, WDT operates based on a cutoff value and is typically confined to specific matrices, primarily urine. In 2016, the United Nations Office on Drugs and Crime (UNODC) reported that opioid misuse accounted for 76 % of drug-related fatalities (United Nation Office on Drugs and Crime, 2018). Furthermore, the UNODC noted unprecedented levels of opium and cocaine production, with methamphetamine and cocaine distribution reaching new areas. Furthermore, the emergence of NPS experienced a fatal and steep increase over the past decade. This indicates that although the misuse of traditional drugs of abuse remains steady and continues to be a leading cause of death, surveillance of NPS should be concurrently performed, thus posing a significant challenge for contemporary WDT.

Salomone et al. (2020) reported that the rise of NPS as a global concern is undeniable, with their usage reported in over 100 countries. The challenges of this situation are manifold, ranging from legislative issues to the limited number of laboratories that can screen and confirm the presence of NPS. These services are vital in scenarios such as WDT and roadside control. However, the deployment of these analytical methods is inconsistent and mostly restricted to specialized laboratories, primarily because of the high costs associated with these advanced analyses. Consequently, although the necessity for NPS detection is being increasingly acknowledged, its implementation is limited by significant hurdles, making it a significant challenge. This predicament highlights the pressing need for more accessible and cost-effective methods for NPS detection and analysis.

In contrast, nontargeted HRMS technology is currently available in most forensic laboratories (Table 6). This technology offers a high-throughput and accurate identification of both known and unknown substances. It is ideally considered capable of replacing immunoassays and providing confirmation using a nontargeted approach based on LC-HRMS techniques (Salomone et al., 2020; Fu et al., 2019). However, in practice, HRMS in WDT is less preferred than immunoassays because of a longer total TAT for each case. Immunoassays offer a rapid processing of high-load samples, which cannot yet be obtained using HRMS. The most challenging aspect of this analysis is the interpretation of the results. HRMS can be used for screening, but its advantages are limited by the following constraints:

- Lack of reference standards and spectral libraries for all NPS
- Complexity and variability of data acquisition and processing parameters
- Need for advanced data interpretation and validation tools.
- The absence of harmonized protocols and quality requirements
- The requirement for sufficient resources and trained personnel
- Legal and ethical implications of reporting unknown or unregulated substances (Mardal et al., 2019).

Malm et al. (2021) investigated how to perform semiquantitative nontargeted screening (NTS) using LC/electrospray ionization (ESI)/HRMS, which is a technique used to identify and estimate the concentrations of unknown compounds in complex samples. They described different strategies for semiquantification, such as the use of surrogate standards, internal standards, calibration curves, response factors, or machine learning models, and discussed their advantages and disadvantages. They also determined the factors affecting the signal intensity and accuracy of semiquantification, such as sample preparation, chromatographic separation, ionization mode, matrix effects, and data quality, and provided recommendations on how to optimize them. Moreover, they provided examples of retrospective analyses, which include re-analysis of the data for new compounds or hypotheses after the initial screening. Additionally, the authors provided a checklist for conducting semiquantitative NTS. The study by Malm et al. is significant because it contributes to the advancement of the knowledge and understanding of NTS as a robust and versatile analytical technique that can reveal novel information about complex samples. However, they also acknowledged the limitations and challenges of performing semiquantitative NTS using LC/ESI/HRMS. The authors revealed that their approach does not directly describe how to determine the cutoff value and positive or negative results in NTS without reference standards. Instead, they reported that the determination depends on the purpose and context of the analysis as well as quality and reliability of the data. They suggested that further research is warranted to validate their results by using other methods or acquiring analytical standards.

Gerona and French (2022) concurred with the abovementioned conclusions and findings, indicating that the rapid emergence and evolution of NPS in the past decade have posed significant challenges to drug testing in clinical laboratories. Some of the unique analytical requirements include the need for comprehensive coverage of various NPS, discovery of unreported NPS through nontargeted data acquisition, and necessity for swift method updates to match the pace of NPS evolution. The constant change in the molecular identities of NPS has

complicated both screening and confirmatory assays, with many NPS failing to cross-react with common drug immunoassays, leading to false-negative results and rendering the targeted methods inadequate. The synthetic cannabinoid epidemic in the USA (from 2013 to 2017) exemplified these issues, with new drugs being released more rapidly than those obtained after the validation and implementation of testing methods. However, the advent of HRMS over the past decade offers a promising solution to these analytical challenges. Gerona and French (2022) determined that the combination of LC with HRMS is preferred for analyzing NPS. This is attributed to its capability to process polar and soluble substances without the need for volatility or stability at high temperatures. However, despite these advantages, the adoption of HRMS methods is expensive and requires specialized knowledge. Consequently, its use is currently confined to major reference laboratories and a handful of clinical laboratories affiliated with academic institutions.

Table 6 lists the various applications of nontargeted LC-MS and GC–MS analyses for the detection of NPS in WDT. Nontargeted analyses can overcome these limitations by using HRMS to screen for unknown or unexpected compounds in biological samples, such as urine, blood, hair, or oral fluid. Moreover, nontargeted analysis can be performed using different approaches, such as data-dependent acquisition, data-independent acquisition, post-targeted screening, or retrospective suspect screening. These approaches generate large amounts of data that can be processed using various software tools, such as databases (HighResNPS.com, for example, machine learning, and molecular networking, to identify and prioritize the detected compounds. Furthermore, nontargeted analyses can provide quantitative results for some compounds using calibration curves, response factors, and random forest regression. As shown in Table 6, nontargeted LC-MS analyses can detect various NPS, including synthetic cannabinoids, stimulants, hallucinogens, benzodiazepines, and other compounds, as well as their metabolites in different matrices and scenarios. Moreover, nontargeted analyses can be used to compare the performance of different sample preparation and extraction methods, such as solid-phase extraction, salting-out liquid–liquid extraction, turbulent flow chromatography, dilute-and-shoot, and urine precipitation. Furthermore, nontargeted analyses can assess the utility and accuracy of the methods in real-world applications, such as examination of patients admitted to emergency departments, wastewater analysis, and metabolomics.

2. Conclusions

WDT remains the best approach for preventing drug abuse in the workplace, despite the challenges posed by NPS and the limitations of the screening methods. The general conclusion is that nontargeted LC-MS and GC–MS analyses are robust and versatile techniques that can enhance the detection and identification of NPS in WDT. Nontargeted analyses can provide new insights into the diversity and dynamics of the NPS market as well as the pharmacology and toxicity of the compounds. Nontargeted analyses can also provide reliable and accurate results for the confirmation and quantification of the compounds. Finally, nontargeted analyses can be easily adapted and expanded to include new compounds and matrices after their emergence.

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CRediT authorship contribution statement

Ahmed Ibrahim Al-Asmari: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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