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Review

Wastewater-Based Epidemiology: Assessing Illicit Drug Usage and Impact through an Innovative Approach

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Abstract: The abuse of illicit drugs, which is usually associated with violent crimes and public health issues, has evolved into a significant problem that the whole of society must address collectively. With the benefits of high productivity, convenience, objectivity, and semi-real time, wastewater-based epidemiology (WBE) has been demonstrated to be a powerful tool and has been utilized on a global scale for monitoring illicit drug use. In this review, we briefly introduce the development and application of WBE. Then, the stability of biomarkers is summarized, and methods to improve stability are introduced. We highlight recent advances in analytical techniques, from three aspects of chromatography–mass spectrometry, optical methods, and electrochemical strategies. The research progress of illicit drug abuse assessment based on WBE is summarized. Finally, we summarize the research hotspots and challenges on illicit drug abuse assessment based on WBE.

Keywords: addictive substances; wastewater fingerprinting; analysis methods; evaluation of drug consumption

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1. Introduction

Illicit drugs are a global pandemic, and their abuse is increasing worldwide [1]. In 2020, approximately 284 million people aged 15–64 worldwide had used illicit drugs in the past 12 months, an increase of 26% from 2010. The massive abuse of illicit drugs not only endangers personal health and family harmony, but it is also closely linked to crime, and it affects social stability, which has become a common challenge that the international community must resolve [2]. The key to tackling this situation lies in developing efficient methods to evaluate and supervise illicit drug abuse.

Wastewater-based epidemiology (WBE) is emerging as an innovative approach to estimate drug abuse by detecting chemical components in sewage. Daughton devised the initial concept of WBE for investigating pharmaceutical consumption based on municipal wastewater analysis in 2001 [3]. It is based on the idea that pharmaceuticals or any given substance that is consumed (irrespective of whether it is swallowed, inhaled/smoked) will be excreted (either in the chemical form it is consumed and/or in a chemically modified form that is referred to as a metabolite). The expelled substance or metabolite will eventually enter the sewer system, shown in Figure 1. Relating these drugs to population-scale use, consumption, or rate of exposure might provide valuable qualitative or quantitative information on the activity of inhabitants within a certain wastewater treatment plant (WWTP). In 2005, Zuccato successfully put this idea into practice, and for the first time, assessed the abuse of cocaine in some Italian cities by detecting the concentration of

cocaine and its metabolites in sewage and surface water. Since then, the development and application of WBE have experienced rapid growth. By systematically sampling and analyzing chemical residues in wastewater, drinking water, or river water, WBE can evaluate the consumption of exposure to chemicals on a population level.

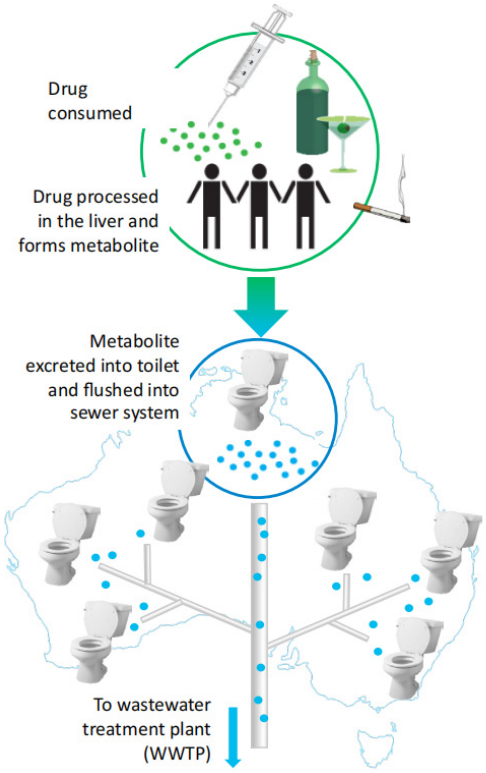

Figure 1. Schematic illustrating the process of drug transportation, starting from the ingestion of chemicals to the production of metabolized waste products that are eventually discharged into the sewer system. This schematic is adapted from ref. [4].

With the advantages of high productivity, convenience, objectivity, and semi-real time, WBE has been widely used to assess the usage of licit drugs such as cigarettes and alcohol [5–7], personal care and household products [8,9], and pharmaceuticals [10,11]. The majority of WBE research has compared chemical loads over time, across communities, or some combination of the two. These data can be used to track changes in population chemical use or exposure over time, such as changes in drug usage in response to public health habits [12,13] and government policies such as legalized retail sales on cannabis [14]. Chemicals in wastewater can also reflect the respective populations' social, demographic, and economic characteristics [15]. Recently, WBE has been employed for COVID-19 surveillance and prediction [16,17].

The method of WBE has several successive steps that enable researchers to obtain accurate results [18]. First, the major excretion product (metabolite or unmodified parent drug) is chosen as biomarker for the determination and assessment of wastewater. Then, the appropriate procedures are used for the collection, transportation, and storage of sewage samples. In the third step, the sample needs to be pre-treated before analysis, since wastewater contains various impurities and chemical substances that will interfere with the determination. After obtaining the concentration of the biomarker, combined with some basic data such as the daily flow rate of wastewater, the degradation rate of the biomarker, and the metabolic correction coefficient, the levels of drug consumption are determined. Finally, to compare the daily intake of different cities, the overall consumption value will be

averaged across the entire population, which is often reported in daily doses per thousand people using an equation such as Equation (1).

$$Daily\ chemical\ consumption_i \left(\frac{mass}{\frac{day}{1000\ people}} \right) = \frac{C_i \cdot F \cdot \frac{R_i}{E_i}}{P} \quad (1)$$

Here, C_i is the concentration of biomarker measured in raw wastewater samples, F is the total wastewater volume during the sampling period (typically 24 h), P is the number of people in the catchment, R_i is the ratio of the molar mass of parent drug to its metabolite, and E_i is the average excretion rate of a drug residue i [19].

Considering the non-invasive, objective nature of WBE and its ability to operate on a population scale, it is highly suitable and extensively employed for monitoring illicit drug consumption in numerous countries and regions [20,21], including the USA [22], UK [23], Colombia [24], Canada [25], Arica [26,27], and New Zealand [28]. Compared with the traditional methods of investigating illicit drugs, such as self-report user surveys [29], seizure and arrest data [30], and medical statistics [31], WBE provides a practical approach to the regulation and suppression of illicit drugs abuse. In 2018, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) proposed that WBE can be used for drug monitoring to improve the assessment of drug abuse trends. The Australian government has funded a program (The National Wastewater Drug Monitoring Program) that releases wastewater analysis reports every four months to facilitate resource allocation and policy adjustments based on drug consumption [32]. China is promoting drug-analyzing technology in sewage and applying it as a routine method to the daily management of drug policing [33]. The World Drug Report published by the United Nations Office on Drugs and Crime (UNODC) in recent years has referred to the WBE drug monitoring results of some relevant countries. As research has continued, drug testing techniques based on influent epidemiology are playing an increasingly important role in preventing and intervening in drug consumption, combating drug crime, and ensuring social stability.

In this review, we first demonstrate the common biomarkers that can be used to evaluate the abuse of illicit drugs and their stability in wastewater. Then, we focus on recent advances in analytical techniques. Based on the differences in its technical principles, we classify the analytical techniques used in WBE into chromatography mass spectrometry, optical methods, and electrochemical strategies. Third, we described the progress of illicit drug assessment. Finally, we discuss the challenges and opportunities in the evaluation of illicit drug consumption based on WBE.

2. Biomarkers and Their Stability

The essence of wastewater epidemiology is a big data mining effort based on chemistry, environmental science, and social science. Therefore, it is important to determine the source of the data, i.e., the type and degree of metabolism of the drugs that are the targets (biomarkers) of the assessment. This requires a study of human metabolism based on drug metabolism kinetics, the identification of substances excreted in urine and feces, and the selection of the most highly excreted substances as detection targets. In addition to metabolism in the body, drugs or their metabolites may undergo a series of physical processes such as transportation, mixing, agitation, adsorption, deposition, biological absorption, and chemical transformation processes such as biodegradation and hydrolysis after being discharged from the body into the sewage network. Factors affecting the detection of biomarkers, such as the sewage pipe residence time, biodegradation, and hydrolysis processes, will have a greater impact on its detection concentration. The stability of biomarkers in wastewater is usually classified as high (0–20%), medium (20–60%), low (60–100%), and variable (different studies have different data), and the stabilities of select biomarkers are shown in Table 1. When performing drug detection in wastewater, substances with a high percentage of discharge and good stability are usually selected as detection targets.

Various illicit drugs have been monitored in recent years through their biomarkers based on WBE. Here, we briefly summarize some common biomarkers in wastewater samples for illicit drugs in Table 1. Parent compounds are the most commonly used biomarkers, particularly for drugs whose metabolism is unclear, such as new psychoactive substances (NPs). However, this may result in an overestimation of the use of the substance directly disposed of in sinks or toilets. Using specific urinary metabolites may overcome this limitation. Some parent compounds metabolize rapidly in the body or are unstable in the environment. Many researchers are eager to investigate suitable metabolites as biomarkers [34].

Table 1. Common biomarkers in the wastewater samples for illicit drugs. (Stability data were obtained from ref. [35]).

Drug Consumed	Biomarkers Measured	Stability	Reference
Methylamphetamine	Methylamphetamine	High	[20]
	Amphetamine	Variable	
Amphetamine	Amphetamine	Variable	[36]
MDMA	MDMA	High	[34]
	MDA	High	
Heroin	Morphine	High	[37]
	Codeine	High	
Cocaine	Cocaine	Low	[38]
	Benzoylecgonine	Medium–high	
THC	THC-COOH	Variable	[39]
	THC-OH		
Ketamine	Ketamine	High	[40]
	Norketamine	High	

After the sample has been collected, it is usually not immediately available for testing and needs to be stored. Therefore, there is a need to avoid the impact on analytical results caused by the decomposition of the assay target during storage. As the first substance to be tested by WBE, cocaine (COC) is considered to be unstable under many conditions [41]. According to the experimental results of Devault et al. [42], When the pH was 7.6 or 6.5, the COC concentration decreased faster, regardless of the temperature, and the degradation rate was as high as 80% for 24 h. However, the stability of COC can be improved when the sample preservation conditions are changed. Since the rate of COC hydrolysis is influenced by pH, COC in the samples is more stable at lower pH values. When the pH was 5, the degradation rate of COC was less than 15% after 24 h, regardless of the temperature. Therefore, acidification of the samples can better preserve the COC in the influent. In addition, the antioxidant sodium metabisulfite ($Na_2S_2O_5$) or the preservative sodium azide (NaN_3) can be added to increase the stability of the samples. There is also a way to maintain the stability of COC in the sample, i.e., to extract and enrich the detection target in the influent sample on solid-phase extraction (SPE) cartridges for long-term storage. As the main metabolite of COC, benzoylecgonine (BZE) is able to maintain good stability in sewage samples under various conditions, including room temperature and sewage. Therefore, BZE is a better biomarker for the assessment of COC use. For example, Silva et al. evaluated COC abuse in Brasilia using BZE as the biomarker [38].

The main active ingredient of cannabis is tetrahydrocannabinol (THC), but there is almost no THC excreted in human urine. Its metabolites are generally chosen as targets for detection as tetrahydrocannabinolic acid (THC-COOH) or tetrahydrocannabinol hydroxide (THC-OH). THC-COOH in the influent (pH 7–8) has a high stability over 72 h and is not significantly affected by temperature changes. However, after longer storage (7 days) under refrigeration (4 °C), the degradation rate will be significantly higher [43]. Freezing of the samples can effectively slow down the degradation of THC-COOH. Heuett et al. showed that THC-COOH in frozen samples can be stored stably for more than 4 months [44]. However, the acidification conditions can affect the stability of THC-COOH and THC-OH

in water samples. Senta et al. showed that THC-COOH was more readily adsorbed on suspended particulate matter under acidic conditions, and the degradation rate of THC-COOH in wastewater under acidic conditions (pH 2) increased from 10% to 54% at 24 h compared to neutral conditions (pH 7.4) [45].

Heroin is hydrolyzed in humans by blood esterizes to monoacetylmorphine (6-monoacetylmorphine, 6-MAM) and is further hydrolyzed in the liver to morphine (MOR) [46]. Heroin and 6-MAM exhibit less stability, and MOR, which has a high stability in wastewater, is usually used as biomarker for heroin detection. However, MOR is a widely used pain medication and a metabolite of other opioids such as ethyl morphine, 6-MAM, and codeine (COD). COD is also a highly stable substance in wastewater. Therefore, the investigators determined both MOR and COD concentrations and estimated the amount of MOR originating from heroin abuse release by deducting the effects of COD concentration on MOR as a way to assess heroin utilization [37,46].

Amphetamine-type drugs, including methamphetamine, amphetamine, 3,4-methylenedioxy-amphetamine (MDA), and 3, 4-methylenedioxy-methamphetamine (MDMA) have a high stability in sewage, whether stored at room temperature for short periods, in refrigerated storage, or frozen for long periods. Ketamine and its main metabolite norketamine also have high stability under different conditions. Lin et al. evaluated the stability of different biomarkers under six different conditions—untreated, the addition of sodium metabisulfite, the addition of hydrochloric acid, filtration, and their combination at different temperatures, and some of the results are shown in Figure 2 [47].

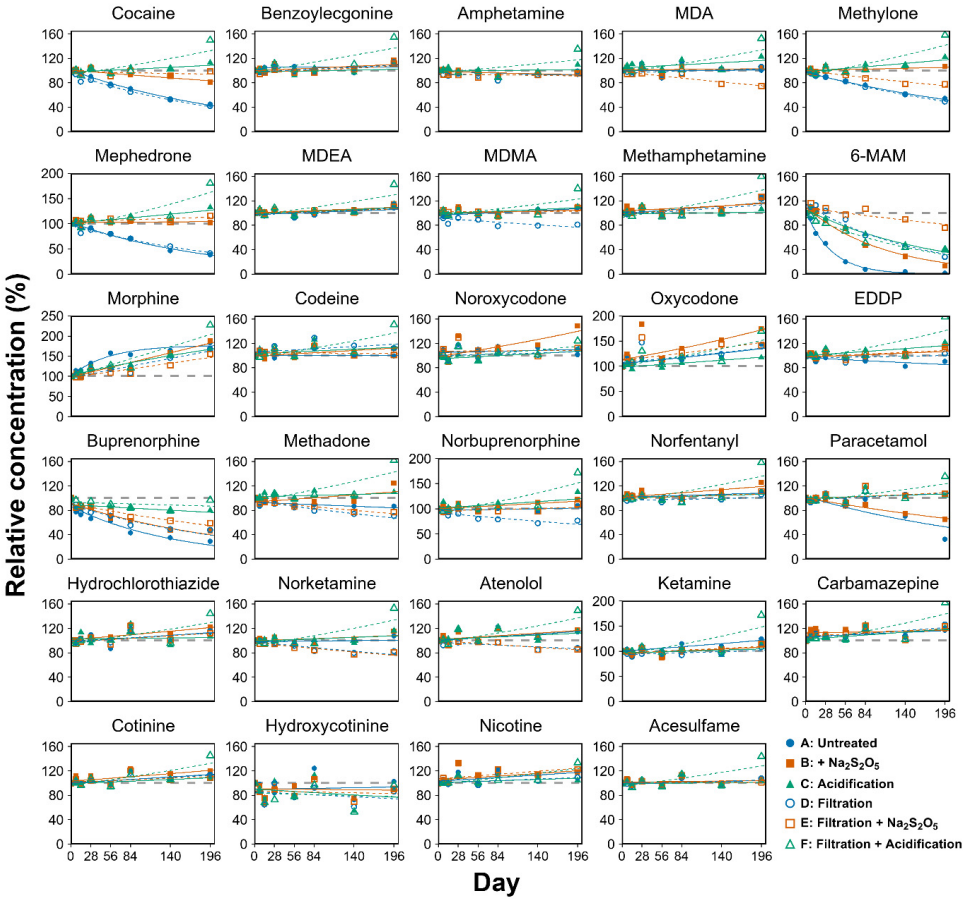

Figure 2. The relative concentrations of select biomarkers at −20 °C storage, adapted from ref. [47].

Overall, the main factors affecting the stability of biomarkers in wastewater samples include temperature, pH, microbial counts, and the amount of suspended particulate matter. Microbial activity can be inhibited by adding biocides or antioxidants to prevent microbial degradation. To prevent adsorption, etc., the influent sample can be filtered

through a membrane before storage, or the target to be measured can be enriched with an SPE column first for storage. When filtering or SPE enrichment is performed, there is a possibility that the concentration of the biomarker may be affected, so isotopically labeled internal standards are generally added to improve the accuracy of the assay. Acid treatment of the sample ensures the stability of most of the assay targets but affects the stability of THC-COOH. Cryopreservation helps to maintain the stability of the assay targets.

3. Analytical Techniques

The concentration of biomarkers in wastewater are often low to ng/L and the interfering components are extremely complex; therefore, highly sensitive analytical techniques are needed to achieve accurate qualitative and quantitative analysis and to provide an accurate database for subsequent drug abuse assessment. The most widely used method for the detection of biomarkers is chromatography–mass spectrometry.

In recent years, various technologies have been employed for the analysis of illicit drugs in sewage, including nanomaterial-based aptamer sensors [48,49], gas chromatography–mass spectrometry [50,51] and surface-enhanced Raman spectroscopy [52], have been reported to be used for the analysis of illicit drugs in sewage. These methods have the advantages of easy operation, rapidity, and low cost, providing automated, miniaturized, and easily scalable technical means for future sewage epidemiological detection of drug abuse. However, due to factors such as detection sensitivity and reproducibility, these techniques have been applied less in drug abuse assessment practice.

3.1. Chromatography–Mass Spectrometry

Chromatography–mass spectrometry is the most widely used method for the analysis of drug components in wastewater, and it is well suited to the requirements of WBE in terms of sensitivity, selectivity, and identification.

Gas chromatography–mass spectrometry (GC-MS) has a high selectivity and sensitivity and can be used for the determination of detection targets in wastewater [50]. Wang et al. used GC-MS to detect methamphetamine in wastewater with a limit of quantification (LOQ) of 2.86 ng/L [53]. Nascimento et al. developed a novel semi-automated liquid–liquid microextraction device which allows nine illicit drugs to be detected usin GC-MS without derivatization, with an LOQ of 34.9–73.3 ng/L. However, most of the biomarkers in wastewater are highly polar and cannot be analyzed directly using gas chromatography. Therefore, derivatization is required to remove the active hydrogen. The process, however, is time consuming and may produce a series of by-products. Therefore, GC-MS is used less in the quantitative analysis of wastewater samples, despite its advantages in terms of instrument maintenance, popularity, and cost of use.

In contrast, liquid chromatography–mass spectrometry (LC-MS) enables the determination of polar, low volatile, and/or thermally unstable compounds through relatively simple pre-treatment steps and shorter chromatographic analysis times, enabling highly sensitive and accurate analysis of biomarkers in wastewater [54]. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) has become the technique of choice for the quantitative determination of biomarkers in sewage because of its powerful detection capabilities, good stability, and wide range of applications. LC-MS/MS uses liquid chromatography as the separation system and two-tandem mass spectrometry as the detection system to achieve highly sensitive detection. The first stage of the technique, MS1, is used to analyze filtered precursor ions, and the second stage, MS2, is used to analyze fragment ions generated after the fragmentation of the precursor ions.

When using the LC-MS/MS method to detect biomarkers in wastewater, two main factors are considered: separation conditions and mass spectrometry conditions. The separation conditions are mainly influenced by the composition of the mobile phase, the flow rate, and the choice of the column. The main considerations of the mass spectrometry conditions are the selection of the ion source, the mass analyzer, and the selection of the qualitative ion pair of the target to be measured. Commonly used ion sources

are electrospray ionization sources (ESI) and atmospheric pressure chemical ionization sources (APCI). ESI sources are suitable for molecules of compounds with moderate to strong polarity, especially for compounds capable of pre-forming ions in solution and for macromolecules that can acquire multiple protons. APCI sources are commonly used for the analysis of small molecules or small compounds of moderate polarity. Despite the disadvantages of matrix effects and signal suppression of ESI sources, they are more widely used in the detection of illicit drug components in wastewater due to their higher sensitivity and reproducibility [55]. Both ion sources are available in positive and negative ion modes. The positive ionization mode is more suitable for alkaline compounds, and the sample is generally acidified by acetic acid or formic acid with an acid content of no more than 1% to improve the ionization efficiency of the target. The negative ionization mode is suitable for acidic compounds, using ammonia or triethylamine to alkalize the sample and improve the ionization efficiency of the target. The commonly used mass analyzers for LC-MS/MS are the triple quadrupole mass spectrometer (QqQ) method, hybrid quadrupole time-of-flight mass spectrometry (Q-TOF), etc. Table 2 lists some chromatographic and mass spectrometric conditions for the detection of drug components in wastewater using LC-MS/MS in recent years.

Table 2. Selected methods for the analysis of illicit drugs using LC-MS/MS.

Biomarkers	Chromatographic Column	Mobile Phase	Ion Source and Mass Detector	Limit of Detection (LOD, ng/L)	Reference
Multiclass illicit drugs (cocaines, opiates, amphetamines, and cannabinoids)	Acquity UPLC, BEH C_{18} (100 × 2.1 mm, 1.7 µm) column (Waters, UK)	Mobile phase A (0.1% *v*/*v* aqueous formic acid solution) and mobile phase B (0.1% *v*/*v* methanolic formic acid solution)	ESI+, QqQ MS/MS detector (Waters, UK)	0.8–9.4	[56]
10 kinds of illicit drugs including cocaine, MDMA, THC, etc.	Shimadzu CBM-20A UPLC, Allure Pentafluorophenylpropyl (PFPP) column (50 mm × 2.1 mm, 5 µm)	10 mM ammonium formate in water (solution A) and methanol (solution B)	ESI+, QqQ Mass Spectrometry (Shimadzu, Japan)	0.04–38	[57]
6 kinds of illicit drugs including fentanyl, codeine, MDMA, etc.	Ultimate 3000 UHPLC, Hypersil GOLD PFP column (2.1 mm × 100 mm, 3 µm, Thermo Scientific, USA)	Mobile phase A was 0.1% of a formic acid solution containing 20 mmol/L ammonium acetate	ESI+, QqQ MS/MS detector (Thermo Scientific, USA)	0.3–8.7	[58]
89 drugs including lysergic acid diethylamide (LSD), morphine, MDMA, etc.	HPLC, Zorbax SB-Aq column (150 mm × 2.1 mm, 3.5 µm; Santa Clara, CA, USA) serially connected to a Javelin guard column (Betasil C_{18}, 2.1 mm × 20 mm, 5 µm; Thermo Electron Corp.)	Methanol (A) and water containing 0.1% (*v*/*v*) formic acid (B).	ESI+, QqQ MS/MS detector (Applied Biosystems, USA)	0.11–202	[59]
More than 1000 licit and illicit drugs	Acquity UPLC, BEH C_{18} (100 × 2.1 mm, 1.7 µm) column (Waters, UK)	Mobile phase A was water with 0.01% formic acid and mobile phase B was methanol with 0.01% formic acid	ESI in both positive and negative, Q-TOF mass spectrometer (Waters, UK)	-	[60]

In recent years, the application of high-resolution mass spectrometry (HRMS) has provided a powerful technique for WBE-based illicit drugs analysis, enabling the screening of large numbers of compounds, the analysis of unknown compounds, the identification of new metabolites and degradation/transformation products, and the quantitative analysis of low-concentration detection targets. HRMS can acquire accurate mass full-spectrum data in the influent. These data allow the analyst to screen compounds in a post-target manner, without the need to pre-select one or more compounds as analytes. Even if the presence of an analyte was not initially considered, the screening can be further expanded by simply re-processing the raw data without the need for re-analysis. This accurate-mass full-spectrum property of HRMS also offers the possibility to study non-detectable targets in wastewater samples, but the complexity of sewage samples and the low concentrations

of analytes requires a complex process to match the chemical structures of unknown compounds. Detection targets are important for drug detection in the influent, but for some new psychoactive substances (NPs), the metabolites or detection targets are often unknown. Using the powerful analytical capability of HRMS provides an effective strategy to solve this problem by analyzing the fragments that are common between parent compounds and metabolites in combination with in vivo and in vitro metabolism experiments.

The analysis of illicit drugs in wastewater using chromatography–mass spectrometry usually requires a complex operational procedure including sample collection and pretreatment. There are two main types of wastewater collection: continuous sampling and discontinuous sampling. Continuous sampling includes time proportional sampling and flow proportional continuous sampling. Discontinuous sampling includes random single sampling, flow proportional discontinuous sampling, time proportional discontinuous sampling, and volume proportional discontinuous sampling. Usually, the flow proportional continuous sampling method is used, which can eliminate some errors caused by randomness in the sampling process; however, there are high requirements for the continuous working ability of the equipment [61].

The complex composition of wastewater can interfere with the analysis and the concentration of biomarkers for which low pre-concentrations are often required, making pre-treatment particularly important. Typically, after removing large suspended particulate matter from wastewater through filtration or centrifugation, samples are cleaned and concentrated using an SPE cartridge, liquid–liquid extraction (LLE), or solid-phase microextraction (SPME). SPE was developed by combining liquid–solid extraction and column-liquid chromatography techniques with the advantage of high recovery rates. SPE is most widely used in the pretreatment of wastewater, as it can effectively separate analytes from interfering components and is relatively simple to operate. Pretreatment of wastewater samples using the SPE technique generally requires four steps: activation equilibrium, sample loading, washing, and elution. In this process, the appropriate adsorbent type, adsorbent activation process, drenching solution type, eluent type, and blow-dry reagent type should be selected according to the type and nature of the test target to achieve the best pretreatment effects.

3.2. Optical Methods

Despite the excellent stability, high sensitivity, and selectivity of chromatography–mass spectrometry, it requires a long analysis time, a tedious operation process, expensive equipment, and professional operators. Researchers have attempted to develop a faster, simpler, and inexpensive analytical method for detecting illicit drugs in sewage, preferably in the field, to facilitate immediate assessment and traceability of drug abuse.

SERS is an ultra-sensitive molecular spectroscopy technique that is widely used for the detection of low-concentration substances [62]. Zhang et al. prepared gold nanoparticle (AgNP) substrates based on diatomite which can achieve SERS detection of fentanyl in sewage at the sub-parts-per-billion level and verified the feasibility of using a portable Raman spectrometer to detect drugs in sewage [63]. However, this method requires immersion of the SERS substrate in the water sample for 24 h, which is difficult to meet the requirements of rapid and efficient sewage drug testing.

The accuracy and ease of drug detection in wastewater continue to improve with the maturation of biosensor technology in conjunction with optical methods. Mao et al. developed a biosensor coupled with the SERS method to detect drugs in sewage (Figure 3a) [64]. In this method, a sequence of oligonucleotides with a high binding affinity, acting as a DNA aptamer, was used to generate SERS signals using 4-mercaptobenzoic acid-labeled Au@Ag core-shell nanoparticles to generate SERS signals. These signals were produced by 4-mercaptobenzoic acid-labeled Au@Ag core-shell nanoparticles. This approach showed good linearity in the range of 0.5–40 ppb, and the detection limit was 0.16 ppb, demonstrating the advantages of biosensors for accurate sample identification.

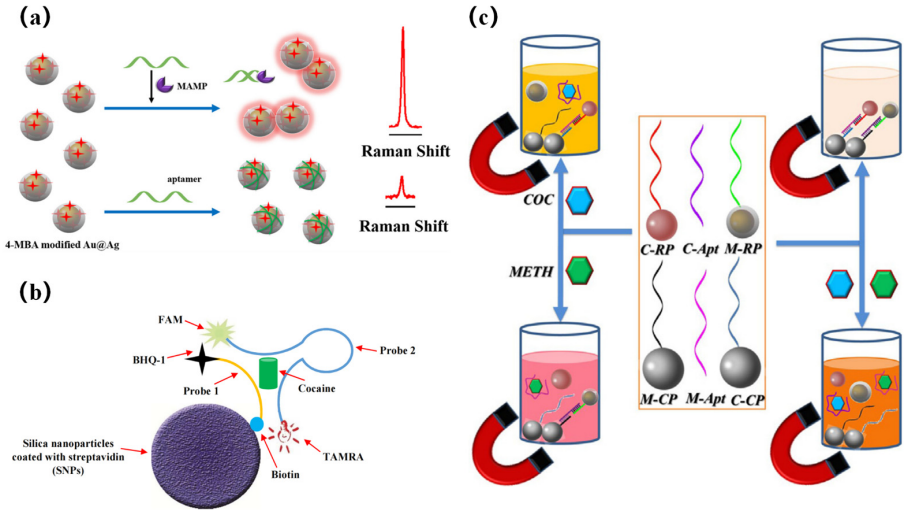

Figure 3. Optical methods for the detection of illicit drugs. (**a**) SERS aptasensors. (**b**) Fluorescent aptasensors. (**c**) Colorimetric aptasensors.

As small analytical devices, biosensors can also be combined with optical methods such as colorimetric and fluorometric methods to achieve trace detection of illicit drugs in different complex matrices. Abnous et al. constructed an amplified double-quenching sensor using aptamers for the detection of cocaine [65]. When cocaine is present in the sample, it can trigger the formation of a double-fragment cocaine aptamer, which makes the quenching molecule TAMRA fluorescent group close to the streptavidin surface and the FAM fluorophore close to the quenching molecules, resulting in an enhanced fluorescent signal, as shown in Figure 3b. The fluorescent aptamer sensor has rapid detection (30 min) and high sensitivity to cocaine, with a LOD as low as 84 pM. Mao et al. reported a simple and rapid colorimetric biosensor for the repeat detection of methamphetamine and cocaine, the principle of which is shown in Figure 3c [66]. DNA reporter probes (RPs) for methamphetamine and cocaine were used to functionalize AuNPs and Au@Ag NPs. Magnetic beads (MBs) were bound to capture probes (CPs) of methamphetamine and cocaine, respectively. The respective RPs and CPs were designed to hybridize with each illicit drug-binding DNA aptamer through DNA–DNA hybridization, forming a sandwich structure. Due to the presence of magnetic beads, this sandwich structure can be removed in the presence of an applied magnetic field. When performing the assay, due to the high affinity of DNA aptamers for drugs, the intercalation structure is disrupted when drugs are present in the solution and AuNPs corresponding to methamphetamine or Au@Ag NPs corresponding to cocaine are released, resulting in a change in the color of the supernatant. By processing the data, it is possible to quantitatively analyze both drugs simultaneously. The LOD of methamphetamine is 0.3 nM, and the LOD of cocaine is 3.3 nM. This method has a high sensitivity and selectivity and requires a short period of time (60 min), demonstrating the potential in the determination of illicit drugs in sewage. Although this report can only detect two drugs simultaneously, the aptamer used in this protocol is synthetic single-stranded DNA that is capable of specifically binding to a variety of respective targets with good selectivity and sensitivity. By simply substituting the corresponding aptamer, it is able to detect a variety of different detection targets.

3.3. Electrochemical Strategies

Electrochemical detection is used to measure the content of a substance based on an electrochemical reaction between the electrode and the substance, which can be used to detect illicit drugs in sewage. In drug detection based on WBE, electrochemical detection can be used to detect the main components of some drugs, such as cocaine and heroin. The main components of these drugs are usually charged ions, which can undergo an electrochemical reaction on the electrode surface to create a current or potential change. By

measuring the magnitude of the change in current or potential, the content of the substance to be measured can be determined. In addition to detecting the main components of drugs, electrochemical detection can also be used to detect the levels of some drug metabolites, such as methamphetamine (meth) metabolite uracil content. In this case, the principle of electrochemical detection is to react metabolites with reactants on the electrode surface to generate current or potential changes, which are used to measure the content of the substance. In general, electrochemical detection has a high sensitivity and accuracy in sewage drug detection; therefore, it is widely used in drug detection and crime detection.

Electrochemical detection technology has the advantages of simplicity, rapid detection, high sensitivity, low cost, and good environmental compatibility; therefore, it is also widely used in the determination of illicit drugs, and it has good prospects for application in sewage drug detection [67]. Hashemi et al. reported a method for the electrochemical detection of cocaine based on three-dimensional reduced graphene oxide/polyaniline/gold nanoparticle composites modified with SPE electrodes [68]. This method can be reused many times and requires less electrolyte and probe solution, with an LOD of 0.029 nM.

4. Illicit Drug Abuse Assessment

With continuous improvements in theories and analytical techniques, the application range of illicit drug assessment based on WBE has been developed rapidly, including estimating the trends and assessing the scale of drug abuse.

After the analysis of the influent samples using the appropriate method, the concentration (c) of each biomarker is obtained, and the drug consumption in mg/(1000 person·days) can be found using Equation (1). In sewage drug testing, the R_i/E_i in Equation (1) is usually replaced by a correction factor f. The correction factor f is not fixed or unique for a particular illicit drug and needs to be adjusted for data based on the biomarker, abusers characteristics, or drug metabolism. Zuccato et al. used BZE as the biomarker, calculated the COC consumption from its concentration, and first proposed a value of 2.33 for its correction factor f [69]. These data use pharmacokinetic data, as 45% of COC is metabolized as BZE and excreted in the urine [70]. Thus, by dividing the molecular weight ratio COC/BE (303/289) by 0.45, the value of the correction factor obtained is 2.33. Subsequent studies have shown that BZE production and correction factor values are related to different routes of administration and to the user preferences of COC abusers in different regions. The use of different correction factors has a significant impact on the assessment of drug consumption. For example, Damien et al. integrated the regional COC consumption habits of the Fort de France urban area (Martinique, Caribbean) and used a BZE discharge rate of 18.5% to derive a correction factor of 5.67 for the assessment of COC use [71]. Subsequent studies have shown that BZE production and correction factor values are related to different routes of administration and to the user preferences of COC abusers in different regions (Table 3).

Table 3. Different correction factors for biomarkers.

DTRs	Excretion Factor (%)	Correction Factor f	Reference
COC/BZE	45	2.33	[72]
COC/BZE	25	4.19	[73]
AMP/METH	36	2.77	[74]
METH	27	3.7	[53]
AMP	30	3.3	[75]
MDMA	26	3.8	[76]
Methadone	27.5	3.6	[77]

After calculating the regional drug consumption, it is possible to compare different regions and types of illicit drug abuse. By compiling global sewage drug data from 2012–2019, Zarei et al. concluded that the most abused drug is cannabis or tetrahydrocannabinol, with a consumption of up to 7417.9 mg/day/1000 people. The next in line were

COC (655.7 mg/day/1000 people), morphine (384.9 mg/day/1000 people), and methamphetamine (296.2 mg/day/1000 people). The comparison of drug consumption can better reflect the abuse situation of different kinds of illicit drugs, which can be used to compare the effectiveness of anti-drug work and to improve the targets of drug control [33]. The results of drug abuse calculations are based on the average amount of abuse in the total population and do not facilitate comparisons of drug abuse across countries and regions. One of the more common indicators is drug prevalence, which is the percentage of the total population that consumes a certain drug at a particular time. Calculating drug prevalence requires calculating the number of people who consume drugs based on the results of sewage drug tests (drug concentration, flow, population), combined with data on drug abuse patterns, frequency of abuse, typical average dose, etc. [78].

WBE-based drug detection can also provide spatial distribution characteristics and temporal patterns of drug consumption. The preference of drug consumption in different cities and the scale of drug consumption in different periods can be visualized in wastewater. By monitoring the sewage in 13 Spanish cities (covering 6 million inhabitants and 12.8% of the total Spanish population), Bijlsma et al. found differences in drug consumption preferences between cities. For example, methamphetamine consumption was significantly higher in Barcelona than in other cities [79]. Foppe et al. monitored drug consumption during selected special events in the United States and found a significant increase in the consumption rates of amphetamines, methamphetamines, COC, morphine, and methadone during Independence Day and eclipse watching events. By analyzing the spatial distribution characteristics and temporal patterns of drug consumption, the targeted control and prevention of drug consumption can be enhanced [75].

In addition, WBE-based drug testing results can be used for drug source inference, drug synthesis route inference, for NP detection, and in other fields. Since the metabolism of illicit drugs is generally stable in the body, the ratio of metabolites in the influent can be tested to determine whether the drug originated from abuse or manufacturing. For example, it is common to test for both COC and BZE when assessing COC abuse, because the ratio of COC/BZE reflects the source of COC. When the ratio of COC/BZE is less than or equal to 0.1, it indicates that the COC in the influent is from consumption. When the ratio is relatively high (0.1–0.7), it indicates that the COC in the influent may come from other sources, such as direct dumping. In recent years, due to the strict control on drug raw materials, drug makers often change the synthesis route and seek alternative chemicals. The inference process of drug synthesis routes using influent epidemiology is mainly based on the analysis of drugs in influent and the raw materials required for drug synthesis using mass spectrometry, or the chirality of drugs and their metabolites in influent [80].

5. Conclusions and Perspectives

Wastewater-based epidemiology is an innovative method to assess the use and impact of illegal drugs by analyzing drug residues in wastewater. Studies have shown that wastewater-based epidemiology can provide objective, accurate, and real-time data for assessing drug use in communities or cities and as a tool for discovering new drug trends. WBE has several advantages over traditional methods of drug monitoring, including its objectivity, accuracy, and ability to provide real-time or near real-time data. The method of WBE involves several steps, such as identifying biomarkers, sampling, analyzing, and assessing. The stability of biomarkers in sewage can alter the concentrations of illicit drugs to varying degrees. Studying the stability of substances through various simulation experiments has become a hot topic, which provides criteria for choosing appropriate biomarkers for drug abuse assessment. At the same time, significant effort has been directed towards improving the stability of biomarkers, such as filtration, acidification, or low-temperature storage. Chromatography–mass spectrometry, especially LC-MS, is the most widely used analytical technology for detecting illicit drugs in wastewater, which can achieve a high sensitivity and accurate detection. The continuous maturation of HRMS technology has allowed for the development of non-targeted screening for NPs. Advances in pre-treatment

technology have also made chromatography–mass spectrometry technology more convenient and accurate. In recent years, the development of rapid inspection technology has provided a faster and more efficient option for the detection of drugs in sewage, the most typical of which is the optical method and electrochemical strategies. The application range of illicit drug assessment based on WBE has been developed rapidly, including estimating the trends and assessing the scale of drug abuse. Information such as consumption, prevalence, spatial distribution characteristics, and temporal patterns of illicit drugs can be obtained through analysis of WBE results.

However, there are still many challenges and opportunities in using WBE to detect drugs in sewage. (1) The stability of biomarkers in wastewater, especially in natural environments, remains to be further studied. (2) The analytical method needs to be improved continuously. The chromatography–mass spectrometry method is a complex, costly, and time-consuming operation, but it has broad prospects in the screening of NPs and unknown metabolites. Optical and electrochemical analysis is the development direction of future detection of illicit drugs in wastewater. However, further studies are needed to determine the aspects of stability, accuracy, and large-scale application. (3) It is necessary to establish relevant evaluation models for the study of error and uncertainty. How to extract more information from existing test results to support illicit drugs prevention and control is also a promising direction worthy of focus. Although mass spectrometry faces some technical, privacy, and ethical challenges, it still has great potential. In the future, we can look forward to the wider application of wastewater-based epidemiology in the field of drug abuse and public health and hope that this method can be further improved and developed to provide more accurate and reliable data support.

In conclusion, WBE has the potential to be an important tool for addressing the problem of illicit drug use on a global scale. While there are challenges associated with the use of WBE, ongoing research and development can help to overcome these challenges and improve the accuracy and reliability of the technique. By analyzing drug residues in wastewater, we can obtain real-time data on drug use in communities or cities, thereby improving prevention and intervention measures and monitoring emerging drug trends. With the continuous development of technologies and methods, wastewater-based epidemiology will play a more important role in future research and policy-making.

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