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Investigation of Common Analysis Methods for Detecting Illicit Drugs in Wastewater

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Abstract

In this study, research was carried out using analytical devices used to detect illicit drugs that threaten public health. Articles on detecting illicit drugs worldwide were reviewed in the last five years. In 2022, one in 18 people, or approximately 292 million people, had used illicit drugs in the past year, and this number has increased by more than 20% considering the past decade. Approximately 30 million people used amphetamines, and 60 million people used opioids in the past year. Drug use, especially opioid use, remained the largest global burden of disease. Wastewater-based epidemiology is an innovative and promising discipline that has recently been used for individual biomonitoring and estimating the amount and type of illicit drug use in the population. Many disciplines, including analytical chemistry, physiology, biochemistry, sewage engineering, spatial epidemiology, statistics, and pharmaceutical/public health epidemiology, are used to estimate the prevalence of illicit drugs and their metabolic products in wastewater. This study aimed to review the articles on the detection of illicit drugs in wastewater, determine the most used analytical devices between 2017-2023, present a summary of the devices used for illicit drug detection, and provide a quick overview of the literature.

Keywords: *Illicit drugs, Wastewater based epidemiology, Analytical methods, Analytical devices*

Atık Sularda Yasa Dışı Uyuşturucu Maddelerin Tespitinde Kullanılan Yaygın Analiz Metotlarının İncelenmesi

Özet

Bu çalışmada halk sağlığını tehdit eden yasa dışı uyuşturucu maddelerin tespitinde kullanılan analitik cihazların araştırılması yapılmıştır. Son beş yılda dünya çapında yasa dışı uyuşturucuların tespitine yönelik makaleler incelenmiştir. 2022 yılında geçen bir yıl boyunca 18 kişiden biri veya yaklaşık 292 milyon kişi yasa dışı uyuşturucu madde kullanmıştır ve bu sayı son on yıla göre % 20'den fazla artmıştır. Geçen bir yılda yaklaşık 30 milyon kişi amfetamin, 60 milyon kişi opioid kullanmıştır. Uyuşturucu madde kullanımı, özellikle de opioid kullanımı, en büyük küresel hastalık yükü olmaya devam etmektedir. Atık su epidemiyolojisi, son zamanlarda bireysel biyoizleme ve popülasyondaki yasa dışı madde kullanımının miktarını ve türünü tahmin etmek için kullanılan yenilikçi ve umut verici bir disiplindir.Analitik kimya, fizyoloji, biyokimya, atık su mühendisliği, mekansal epidemiyoloji, istatistik ve farmasötik/halk sağlığı epidemiyolojisi dahil olmak üzere farklı disiplinler atık sularda yasa dışı madde ve metabolik ürünlerinin prevelansını tahmin etmekte kullanılmaktadır. Bu çalışmada, 2017-2023 yılları arasında atıksularda yasa dışı uyuşturucu maddelerin tespiti ile ilgili makalelerin incelenmesi, en çok kullanılan analitik cihazların belirlenmesi, yasa dışı uyuşturucu madde tespiti için kullanılan cihazların özetinin sunulması ve literatüre hızlı bir genel bakış sağlanması amaçlanmıştır.

Anahtar kelimeler: *Yasa dışı uyuşturucu maddeler, Atık su bazlı epidemiyoloji, Analitik metotlar, Analitik cihazlar*

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1. Introduction

Modern societies consume a wide variety of chemical products that can potentially cause toxic effects on receiving water, including pharmaceuticals, illegal drugs, and food additives, among others. Some of these illicit drugs are emerging contaminants of concern (CECs). The development and application of analytical methodologies, such as multi-residue methods, enable the detection of the presence and concentration of various chemicals in the wastewater and affected water systems. Such studies are used in early warning systems and/or to evaluate different chemical classes in spatiotemporal variations [1-3]. The consumption of different types of illicit drugs and new psychoactive substances (NPS) has risen in recent years and has become a global problem due to its effects on public health, safety, and the economy [4].

Epidemiology, and especially wastewater-based epidemiology, have great importance in identifying the rates of illicit drug use in wastewater, as well as providing more reliable, objective, and realistic results. Wastewater-based epidemiology makes an effective detection using illicit drug biomarkers in wastewater samples. Analytical devices used to detect illicit drugs in wastewater are becoming increasingly important for the measurements to be precise and reliable [5, 6].

1.1. Illicit drug use in the world

Illicit drug use is on the rise, with different types every year around the world. In 2021, 1 in 17 people in the age group of 15-64 used illicit drugs in the past 12 months. The number of people using illicit drugs in 2021 is estimated to be 296 million. Cannabis was the most used illicit drug, with 219 million users in 2021, and the number of cannabis users has increased by 21% in the last decade. Illicit drug use is increasing worldwide, and although men use cannabis more than women globally (approximately 70%), gendered patterns of illicit drug use vary in some subregions. In North America, 42% of cannabis users are women. According to the 2023 World Drug Report, it is estimated that 22 million people used cocaine, 36 million people used amphetamine, and 20 million people used "ecstasy" type drugs in the past year in 2021. Opioids remain the group of illicit drugs with the highest rates of serious drug-related harm, including fatal overdoses. Approximately 60 million people used non-medical opioids in 2021, of whom 31.5 million used opiates [7-8]. The data from the 2024 World Drug Report shows 292 million people in the world used drugs in 2022, and the number of people using drugs has increased by approximately 20% for more than 10 years. In 2022, drug use disorder was detected in 64 million people in the world, and only 1 in 11 people received treatment. In 2022, there were 13.9 million people who injected illegal drugs. Cannabis (228 million users) is the most used illicit drug worldwide. 60 million people used opioids, amphetamines used by 30 million people, and 23 million people used cocaine, and ecstasy is used by 20 million people in 2022 [9].

Türkiye continues to be an important transit country for drug trafficking due to its geographical location. Illicit drug seizures are increasing in Türkiye. In addition, drugs are an important problem not only in the world but also in Türkiye. According to Turkish Drug Report, when individuals receiving treatment in 2023 were examined in terms of the types of substances they used for treatment; 28% were found to have received treatment for heroin, 37.1% for methamphetamine, 11.2% for marijuana, 4.9% for synthetic cannabinoids, 4.2% for other opiates, 4% for cocaine, 0.7% for ecstasy, 1.1% for volatile substances, and 8.9% for other substances. The average age of individuals applying for treatment was determined as 29.78. When the distribution of those receiving treatment by age group was examined, it was determined that those applying for treatment were concentrated between the ages of 25-34. When the distribution of those receiving inpatient treatment by gender was examined in 2023, it was reported that 90.3% were male and 9.7% were female [10].

1.2. Wastewater-based epidemiology

Illicit drugs and their metabolic products in municipal wastewater are used to estimate the prevalence of use in the community, employing various research disciplines, including analytical chemistry, physiology, biochemistry, sewage engineering, spatial epidemiology, statistics, and drug/public health epidemiology. Wastewater-based epidemiology is a promising discipline that has recently been used for individual biomonitoring and estimating the quantity and type of illicit drug use in society [11, 12].

Wastewater-based epidemiology also plays a key role in detecting new-generation psychoactive substances used in educational institutions and determining the prevalence of illegal drug use during weekends, national holidays, festivals, and epidemics such as COVID-19 [5].

According to the European Monitoring Centre for Drugs and Drug Addiction/ European Union Drug Agency (EMCCDA/EUDA) wastewater analysis explanations (Figure 1), the illicit drug to be detected is carried out by taking samples from wastewater treatment points that represent the population at certain times. Subsequently, daily drug use amounts are calculated using common calculation methods [11].

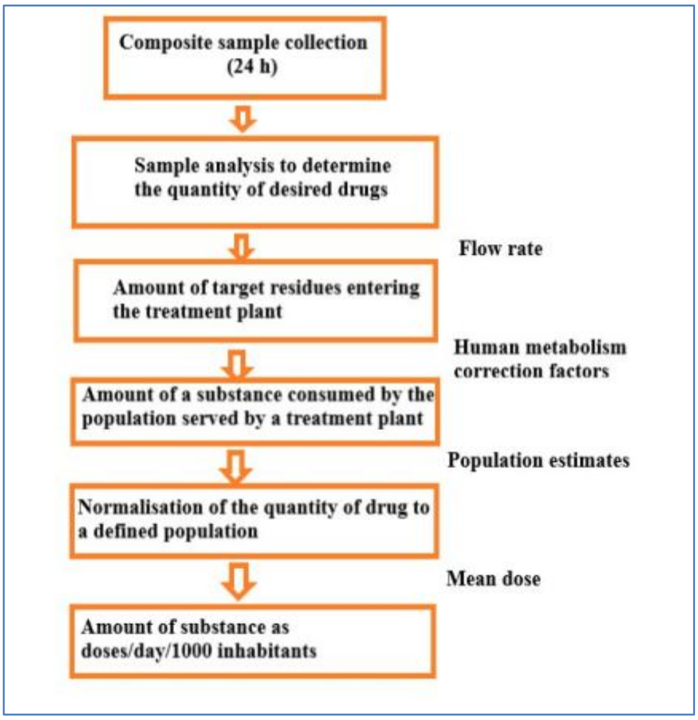

Figure 1. Steps to determine illicit drugs in wastewater [11]

According to the calculation method described above, composite samples are collected for 24 hours. The samples are analysed with the help of the specified analytical device to determine the concentrations of the drug residues to be determined. Following this, illicit drug use is estimated by back calculation by multiplying the concentration of each target drug residue (nanogram/liter) by the corresponding sewage flow (liter/day). A correction factor is taken into account in the calculation for each illicit drug. In the last step, the obtained data is divided by the population served by the wastewater treatment plant, which indicates the amount of substance consumed per day per 1000 people. Population estimates can be calculated using different biological parameters, census data, the number of houses in the residential area, etc. It should be noted that the general variability of different estimates can often be very high. Although this method represents a certain population use, it can be applied in festival periods, Ramadan periods, prisons, small settlements, workplaces, and schools. When used in small communities, this method can pose an ethical risk because it can detect a certain possible group in the small community, and for this reason, the SCORE group has published ethical guidelines for wastewater-based epidemiology and related fields [11].

Potential risks in prisons in the context of detecting illicit drugs in wastewater may include developing strategies to prevent drug consumption by prison staff, such as eliminating contact visits for prisoners' families. In this case, if emotional problems may occur as the precautions to be taken are increased, and if they harm those who do not consent to the study, an ethical problem may occur. Another problem is the stigmatization of prisoners, ex-prisoners, and their families. If the WBE research findings are not managed effectively in media reports about illicit drug consumption in prisons, this may cause negative feelings in society, prisoners may be embarrassed, and problems may occur in the process of reintegrating ex-prisoners into society [11].

Studies on schools and workplaces may result in the stigmatization and labelling of people in these places because of the findings being included in the relevant reports. Other risks include negative impacts on the reputation of schools or workplaces and economic damage to workplaces. It may be necessary to plan how the research results will be communicated to the media clearly and concisely. Media organizations should be encouraged to use language within the research plan that will persuade participants to emphasize the benefits of the research rather than stigmatizing or labelling [11]. In some cases, mixing samples from multiple sites can be used to overcome the problem of reporting only aggregate data. Or it may be possible to simply omit the name and location where the study was conducted. Additionally, taking samples without permission from a specific building (e.g., prison, school, workplace, hospital) may be an offense or a regulatory problem. This may negatively impact the reputation of the WBE field and the willingness of authorities to support or collaborate with WBE researchers [11].

The method used cannot provide information on the prevalence and frequency of use, main classes of users, and purity of drugs. Additional difficulties arise from uncertainties associated with the behavior of selected biomarkers in sewage, different back-calculation methods, and different approaches to estimating the size of the tested population [13, 16]. For example, caveats in the selection of analytical targets for heroin make monitoring this drug in wastewater more complex than for other substances. Illicit drugs from composite samples are usually analysed for their major urinary metabolites (i.e., substances produced when the body breaks down drugs). However, the specific metabolite of heroin, 6-monoacetylmorphine, is not stable in wastewater. Therefore, the only alternative is to use morphine, which is not a specific biomarker and can be detected after therapeutic use. This highlights the importance of obtaining the most accurate information on morphine use from prescription and/or sales reports [11, 17]. Furthermore, the purity of street drugs varies unpredictably over time and in different locations. Calculating the total amounts of drugs consumed into the corresponding average dose is complex because drugs can be taken by different routes (oral, intravenous) and in widely varying amounts, and their purity levels vary [18].

In particular, differences in flow rates that may occur in rainy and stormy weather and failure to obtain samples of the desired quality may negatively affect the analysis and calculations, or, since the purity of drugs produced illegally and counterfeit cannot be determined, such situations may change the accuracy and precision of the calculations. In the summer months, short-term visits by people who do not live in certain tourist areas and failure to determine the instantaneous or weekly net population may cause differences in the calculations. This can make it difficult to follow the general trend [18].

1.3. Analytical methods

The wide variety of components in wastewater, chemical stability of the substances in wastewater, and various properties such as pKa and hydrophilicity can be a complex process in analysis studies. Therefore, the selection of appropriate analytical methods is essential for accurate determination, so analytical methods are gaining importance in detecting illicit drug accurately [20-22].

Ultra-performance Liquid Chromatography (UPLC), Ultra-High-Performance Liquid Chromatography (UHPLC), and High-performance Liquid Chromatography (HPLC) are included in LC systems. In UPLC and UHPLC systems based on HPLC techniques, a stationary phase with a smaller chromatographic particle diameter and greater pressure in the column is used. High sensitivity and fast analysis results are obtained from these systems [5, 11-21, 22]. HPLC is of great importance for quantitative and qualitative analysis, playing an important role in the evaluation of pharmaceutical samples, as it is one of the fastest, most flexible, and safest chromatographic analytical techniques in the quality control of pharmaceutical ingredients and chemicals [22]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is one of the most selected methods to identify drug residues and drug quantity in wastewater. This analytical approach integrates the techniques of LC with the analysis properties of mass spectrometry. In terms of the low concentrations of drug residues and complexity in wastewater, LC-MS/MS is one of the effective devices due to its effectiveness, selectivity, robustness, and sensitivity. Electrospray ionization (ESI) interfaces generally provide the desired quality of sensitivity [23]. Due to its remarkable reduction in analysis time, rapid separation, and high throughput capacity, UHPLC is attractive for environmental, food, chemical, and pharmaceutical analyses. Ultrafast liquid chromatography (UFLC), while shortening the analysis time, is used with the MS detector for trace amounts of compounds. Its high-pressure resistance, fast scanning speed, enhanced throughput, and less solvent consumption make this device attractive. UFLC operates at a much faster rate than HPLC, providing faster analysis and shorter run times. UFLC provides more accurate and reliable data for complex sample analysis. UFLC often exhibits improved sensitivity and is useful in applications where identifying low-abundance analytes is critical. UFLC typically uses less solvent. As a result, costs are reduced, and a greener strategy is implemented [22].

UPLC has several advantages over HPLC. These include increasing sensitivity while reducing analysis time, reducing solvent usage and operating costs, reducing cycle times in processes, and enabling more throughput using existing resources. Another advantage is that it maintains the resolution performance while providing the selectivity, sensitivity, and variability of LC analysis and using cutting-edge separation materials with small particle sizes for faster analysis [22].

Direct injection (DI) of samples represents a new trend that takes importance in the increased sensitivity of mass spectrometry (MS) devices [24]. Micro-liquid chromatography-tandem mass spectrometry (μLC-MS/MS), which can be utilized for cocaine, ATS, and NPS analysis, has higher ionization efficiency and sensitivity compared to conventional UHPLC due to the low flow rate [25].

Liquid chromatography coupled with high-resolution tandem mass spectrometry (LC-HRMS) is used to determine markers of illicit stimulants in wastewater. LC-HR/MS can be used to detect levamisole, a veterinary anthelmintic found in street cocaine, herbal medicines containing only natural ingredients, designer drugs, and doping agents. LC-HR/MS has successfully detected mislabeled or misrepresented street drugs. Detection of new designer amines, stimulants found in “bath salts” and synthetic cannabinoids is well suited for LC-HR/MS. Liquid chromatography coupled with High-resolution mass spectrometry (LC-HRMS) can be utilized for highly sensitive and reproducible detection of hundreds or thousands of metabolites in a single sample [26, 27]. LC-HRMS is robust, flexible, sensitive, practical, and useful for modern high-throughput laboratories [26, 30].

Flow injection analysis (FIA) combined with high-resolution mass spectrometry (HRMS) is used for the rapid analysis of psychoactive substances found in recreational (illicit drugs, human and veterinary medicines, legal highs) and licit/illicit drugs. FIA and HRMS operating conditions can be adjusted to detect a wide range of psychoactive compounds [28]. QTOF-HRMS technique is a practical method for fast screening of pharmaceutical drugs as it leads to the combination of high-resolution full-scan analysis [29]. Although LC-MS/MS has high sensitivity and selectivity, some limitations exist for multi-compound and multi-class analyses. The most current QqQ instruments available in MS/MS methods allow for low dwell times and a significant increase in the number of passes obtained at a time. Even when working with thousands of contaminants, the acquisition time of each pass is still a limitation in wastewater. The duration of each transition may limit the number of analytes to be detected. The use of LC-MS/MS methods is insufficient to make a general comment regarding the drugs in question. In the LC-MS/MS method, substances present at high levels other than the selected analytes are often ignored [30].

In summary, the most important issue in the selection of devices used in the detection of illicit drugs is the rapid and precise measurement of the illicit drugs to be detected. Another important issue for analysts is that the analysis time is not prolonged. While GS-MS provides high selectivity and sensitivity, derivatization of most drugs and compatibility of biomarkers with GC may not be practical and fast enough. LC-MS can be used to detect more compounds, in addition to being faster and requiring less sampling. LC-MS/MS is frequently used in the detection of drugs in wastewater, and HRMS continues to be used to obtain accurate mass full-spectrum data with its advantageous screening and identification features.

2. Experimental Section

2.1. High-frequency keywords

The most searched words include illicit drugs, wastewater analysis, illicit drug(s), wastewater-based epidemiology, analytical methods, LC-MS/MS, UHPLC_MS/MS, LC-Orbitrap/MS, Direct Injection (DI), UPLC-MS/MS, Solid Phase Extraction (SPE), and LC/HRMS. The most searched words include illicit drugs, wastewater epidemiology, target drug metabolite, and wastewater-based analysis. The international electronic databases, including Google Scholar, Scopus, PubMed Science, and local databases from 2017 up to 2023, were searched. High-frequency keywords in the field of WBE are shown in Fig. 2.

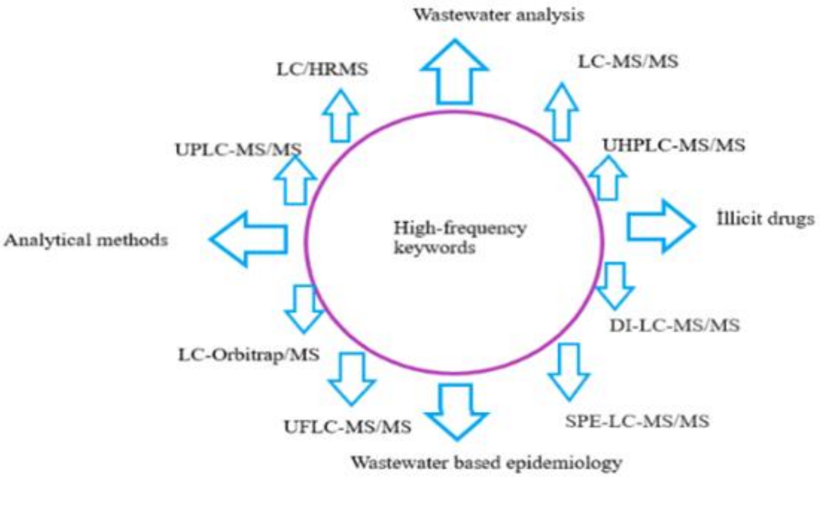

Figure 2. High-frequency keywords in the literature

2.2. Analytical laboratory devices in literature

According to the European Union Drug Agency (EUDA), Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the most frequently used analytical method to measure drug residues in wastewater. Additionally, considering the expected complexity and low concentrations in wastewater, LC-MS/MS is one of the most powerful techniques for this analysis due to its sensitivity and selectivity [11].

The SCORE group has been organizing an annual monitoring campaign for systematic data assessments through wastewater analysis since 2011. This comprehensive analysis, which supports wastewater analysis, is published by the European Centre for Drugs and Drug Addiction (EMCDDA), European Union Drug Agency (EUDA) in Lisbon, to communicate the results to the scientists or institutions that support it [31,32].

Reliable estimation of accuracy is important, so some laboratories validate laboratory results with the SCORe group to ensure the accuracy of their work. In the study titled "Wastewater-based Monitoring of Illicit Drugs in Cyprus by UPLC-MS/MS: The Impact of the COVID-19 Pandemic", it was stated that the validation of the results was provided by participation in the annual SCORe group validation [35]. In the article titled “Three Years of Wastewater Surveillance for New Psychoactive Substances from 16 Countries”, the WBE protocol was applied for sample collection, storage, and analytical methodology [43].

The validated analytical method of the article “Comparison of Community Illicit Drug Use in 11 Cities of Turkey through Wastewater-based Epidemiology Study” passed the interlaboratory cross-comparison of the SCORE group [49]. In the article titled "Monitoring of Changes in Illicit Drugs, Alcohol, and Nicotine Consumption During Ramadan via Wastewater Analysis”, it was stated that the reliability and accuracy of the sample preparation and LC-MS/MS device complied with the quality criteria determined by the SCORE group [50]. It was stated that all analytical methods used in the study titled "Assessment of Illicit Drugs in Wastewater and Estimation of Drugs of Abuse in Adana Province, Turkey" were validated and cross-checked with the SCORE interlaboratory free comparison test [54].

The analytical method was validated in terms of Limit of Quantification (LOQ), Limit of Detection (LOD), linearity, recovery, and instrument and method repeatability. Instrument and method repeatability were controlled by analysing each sample three times. The linearity of the calibration curves with coefficient of determination $R^2 > 0.9900$, for all illicit drugs [35].

Instrumental detection limits (IDLs) and instrumental quantification limits (IQLs) were obtained by direct injection of decreasing quantities of each illicit drug. IDLs and IQLs were calculated as the concentration-giving peaks for which the signal-to-noise ratio was 3 and 10, respectively. Limits of detection and quantification, intra-day and inter-day precisions, accuracies, and recovery studies were applied to determine the performance of the method [41].

In the study titled “Occurrence of Z-drugs, Benzodiazepines, and Ketamine in Wastewater in the United States and Mexico During the Covid-19 Pandemic”, method detection limits were calculated according to the US Environmental Protection Agency (USEPA, 2016) revised protocol [37]. In the article "Dilute-and-Shoot Approach for The High-Throughput LC-MS/MS Determination of Illicit Drugs in the Field of Wastewater-based Epidemiology" accuracy and precision were evaluated for each influent wastewater (IWW) samples (n = 5) at four concentrations of 0.1, 0.5, 2.5 and 10 $\mu g\ L^{-1}$:

Mean recoveries ranging from 70% to 120% were considered suitable. Individual quality controls (QCs) recoveries ranging from 60% to 140% were considered efficient [39].

In the analytical method study conducted in 11 provinces, it was determined that the method was linear and the correlation coefficient was greater than 0.99. In the studies, the method recovery was determined to be between 85-114% for the illicit drugs studied [39, 41]. Limits of detections (LODs) and LOQs of illicit drugs obtained using US Environmental Protection Agency (USEPA) guidelines (USEPA 2016) [50].

It has been stated that sources such as the International Conference on Harmonization (ICH), the European Medicines Agency (EMA), the International Union of Pure and Applied Chemistry (IUPAC), the Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry, and the Scientific Working Group for Forensic Toxicology (SWGTOX) can be used for analytical validation [41].

The acceptable deviation between empirical and library data was selected using the guideline document "Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed SANTE 11312/2021" [51].

Table 1 includes studies carried out to detect illicit drugs in various countries of the world and at various times. Analytical laboratory devices are selected depending on the type of drug desired, its sensitivity, and factors such as the detection time of metabolites. In general, the LC-MS/MS device was chosen more intensively in the articles examined within the scope of this study.

In addition to Table 1, artificial intelligence and machine learning (ML) methods also play an important role in illicit drug detection from wastewater. In the study, different machine learning models (Support Vector Machines (SVM), eXtreme Gradient Boosting (XGB), Random Forest, Gradient Boosting, K-Nearest Neighbors (KNN)) were successfully used to classify illegally produced drugs according to their infrared spectrum due to their effective, easy and fast applicability [56]. In other studies, conducted on illicit drug detection, it was determined that different models, such as various artificial neural networks, deep learning, elastic net, decision trees, and logistic regression, were used. The determined models were used for different substance classes, including alcohol, cannabis, hallucinogens, tobacco, opioids, sedatives, and hypnotics. Various substance use data from social media platforms and risk factors such as socioeconomic and demographic data, behavior characteristics, and psychopathology data were used for model training, and then the model was tested. Using artificial intelligence models to develop data-driven smart applications can reduce negative consequences related to the process and accelerate the early implementation of interventions. This could help healthcare professionals and drug enforcement officials effectively screen, assess, and predict illicit substance use. Also, the usage of AI and ML supports developing timely preventive activities [57-59].

Table 1. Analytical methods used in the detection of illicit drugs globally

Analysis device/ method	**Location**	**Year/ Sampling period**	**Types of illicit drugs & NPSs**	**First author& References**
Ultra-High-Performance Liquid Chromatography/ High-resolution mass spectrometry (UHPLC–HRMS)	Taiwan	2023/ 7 March 2023	**Illicit drugs** • Amphetamine • Methamphetamine • Norketamine • Ketamine • Morphine • Codeine • Tapentadol • Meperidine • Levorphanol • Tramadol • Hydromorphone • Dihydromorphine • 4-Methy N, Ndimethylcathinone • Methylenedioxypyrovalerone (MDPV) • 5-(2-Aminoethyl)-2,3-dihydrobenzofuran (5-AEDB) • Bufotenin • N-methyl-2-aminoindane • 1,4-Androstadiene-3,17-dione • Methenolone • Ephedrine • N-Methylephedrine **NPSs** • Mephedrone 4’-chloro alphapyrrolidinopropiophenone (4-Cl-α-PPP)	Chen et al. [33]
Liquid Chromatography coupled to tandem mass spectrometry (LC-MS/MS)	Germany	2023/ from April 2020 to December 2021	• The metabolites of cocaine (Benzoylecgonine) • Methamphetamine • MDMA • Nicotine (cotinine) • Gabapentin • Metoprolol • Amphetamine • Carbamazepine	Oettel et al. [34]
UPLC-MS/MS	Cyprus	2023/ 20-26 April 2021, 19–25 July 2021, 11–17 October 2021, 25 December 2021–2 January 2022	• MDMA • Amphetamine • Cocaine • Methamphetamine	Psichoudaki et al. [35]

Table 1. Analytical methods used in the detection of illicit drugs globally (Continued)

Analysis method	**Location**	**Year/ Sampling period**	**Types of illicit drugs & NPSs**	**First author& References**
LC-HRMS	Cadiz Bay (Spain)	2023/Daily during 1 week in June 2021	• Cocaine and its metabolite benzoylecgonine • Tapentadol • Tramadol • Cannabidiol (CBD) • Delta 9-Tetrahydrocannabinol (THC) • 2C-E (2,5-Dimethoxy-4-ethyl phenethylamine) • Cocaethylene	Santana Viera et al. [36]
Liquid Chromatography	Mexico and USA (in different states from the US and 14 states from Mexico)	2023/July to October 2020	• Benzodiazepines • Ketamine	Adhikari et al. [37]
LC-HRMS LC-Orbitrap/MS	South Korean	2023	**Traditional Psychoactive Substances and Their Metabolites** • Ketamine • Zolpidem phenyl-4-COOH • Tramadol • Phenmetrazine • Phentermine • Methamphetamine • Codeine • Morphine • Phendimetrazine • Ritalinic acid • Ephedrine **NPSs** • 25E-NBOMe • N-methyl-2-AI • 25D-NBOMe	Lee et al. [38]
DI-LC-MS/MS LC-MS/MS SPE-LC-MS/MS	Spain	2024/ During 2022 and 2023	• The main urinary metabolite of cannabis, THCCOOH • Benzoylecgonine (BE) • Amphetamine • Ketamine (KET) • Methamphetamine (METH) • Cocaine metabolite • The unique metabolite of heroin, 6-acetyl morphine (6-MAM) • 3,4-methylenedioxymethamphetamine (MDMA)	Gracia-Marín et al. [39]

Table 1. Analytical methods used in the detection of illicit drugs globally (Continued)

Analysis method	**Location**	**Year/ Sampling period**	**Types of illicit drugs & NPSs**	**First author& References**
LC-MS/MS	Niterói Nova Friburgo Campos dos Goytacazes Rio de Janeiro Cabo Frio Resende Petrópolis	2023/December 27, 2022, and January 10, 2023	• Ecstasy • Amphetamine • Methamphetamine • Cocaine • Benzoylecgonine • Cannabis	Ferreira et al. [40]
LC-MS/MS DI-UPLC-MS/MS HPLC-MS/MS UPLC-MS/MS UFLC-MS/MS UHPLC-MS/MS DI-UHPLC-MS/MS	-	2023/ 2020-2022	• **Cocainics** Cocaine Benzoylecgonine Cocaethylene • **ATS** 3,4- methylenedioxy methamphetamine Amphetamine 3,4- methylenedioxy amphetamine Methamphetamine • **Opioids** Heroine 6-acetyl morphine Methadone Cannabinoids Morphine Codeine Other compounds	de Oliveira et al. [41]
Liquid Chromatography-Mass Spectrometry	Spain	2023/ New Year period (from 29-Dec-2021 to 4-Jan-2022) Summer Festival (from 29 June 2022 to 12 July 2022)	• Phenethylamines • Cathinones • Opioids • Benzodiazepines • Plant-based NPS • Dissociatives methamphetamine • MDA • MDMA • Ketamine • Heroin • Cocaine • Pseudoephedrine	Rousis et al. [42]

Table 1. Analytical methods used in the detection of illicit drugs globally (Continued)

Analysis method	**Location**	**Year/ Sampling period**	**Types of illicit drugs & NPSs**	**First author& References**
LC-MS/MS	Australia Belgium Brazil Cyprus New Zealand France Greece Italy the Netherlands' Iceland Spain Sweden Slovenia The Republic of Korea United States	2023/ New Year period in three consecutive years (2019–2020, 2020–2021, and 2021–22)	• Eutylone • Mephedrone • Mitragynine • N-ethylhexedrone • 2F-deschloroketamine • 3-MMC • Etizolam (Xanax) • Clonazolam • Para-methoxyamphetamine (PMA) • Pentylone • N-ethylpentylone • N-ethylhexedrone • Methylone • Methoxetamine • Methiopropamine • Mephedrone • Methylenedioxypyrovalerone (MDPV) • Eutylone • Ethylone • 4-Methylethcathinone (4-MEC) • 4-Fluoroamphetamine • 3-Methylmethcathinone • 2F-Deschloroketamine (2F-DCK)	Bade et al. [43]
LC-MS/MS	Southwest China (156 WWTPs in 21 different cities)	2023/ from October to November 2021	• Methamphetamine • Morphine • Ketamine • Codeine • Heroin	Wang et al. [44]
Solid Phase Extraction and Ultra-High Performance Liquid Chromatography coupled to tandem mass spectrometry	Eleven-time points in Reykjavik	2022/ from 2017 to 2020	• Amphetamine • Cocaine • Cannabis • Methamphetamine • 3,4-methylenedioxymethamphetamine (MDMA)	Löve et al. [45]

Table 1. Analytical methods used in the detection of illicit drugs globally (Continued)

Analysis method	Location	Year/ Sampling period	Types of illicit drugs & NPSs	First author& References
LC-MS/MS	Australia Entire population of a prison in Australia	2022/from March to December 2020	• Amphetamine • (Metabolite of methamphetamine and dexamfetamine, an attention-deficit hyperactivity disorder (ADHD) medicine; also used as an illegal drug), • 3,4-methylenedioxy-N-methylamphetamine (MDMA) • 3,4-methylenedioxyamphetamine (MDA) • Mephedrone • Ephedrine • Methamphetamine • Methylone • 3,4-methylenedioxy-N-ethylamphetamine (MDEA) • Tetrahydrocannabinol (THC) and its metabolite 11-nor-9-carboxy-Δ−9 tetrahydrocannabinol (THC COOH) • 6-Monoacetylmorphine (6-MAM), ketamine, and its metabolite nor-ketamine • Cocaine and its metabolite benzoylecgonine •	Wang et al. [7]
LC-HRMS	All states and territories in Australia, and both metropolitan and regional areas	202/ Bimonthly from October 2017–June 2018 and October 2019–February 2020	• 4-FA • Butylone • Ethylone • Mephedrone • Methoxetamine • 4-MEC • 3-MMC • Pentedrone • N-ethylpentylone • Methylone • PMA • Alpha PVP • Methiopropamine • Pentylone	Bade et al. [46]

Table 1. Analytical methods used in the detection of illicit drugs globally (Continued)

Analysis method	**Location**	**Year/Sampling period**	**Types of illicit drugs & NPSs**	**First author& References**
Solid-phase extraction, LC-MS/MS	Brasília	2022/ from March 1st to 15th 2019	• Benzoylecgonine (BE) • 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) • Cocaethylene (COE) • Cocaine (COC)	Sodré et al. [47]
Solid-phase extraction, LC-MS/MS	Turkey (11 cities) Adana, Ankara, Diyarbakır, Erzurum, Gaziantep, Kayseri, Konya, Mersin, Şanlıurfa, Trabzon, Van	2021/Between March 2019 and December 2019 (for a week)	• Marijuana • Heroin • Amphetamine • Cocaine • Ecstasy • Methamphetamine	Daglioglu et al. [48]
LC-MS/MS	Western United States- One micropolitan (Site A), and one rural (Site B)	2020/ April 2019 to June 2019	• Amphetamine • Morphine • 6-acetyl morphine • Codeine • Hydromorphone • MDA • Benzoylecgonine • Fluoxetine • Hydrocodone • Oxycodone • Ketamine • EDDP • Noroxycodone • Tramadol • Ritalinic acid • MDMA • Methamphetamine • Methadone	Bishop et al. [49]
LC-MS/MS	Adana	2022/ During Ramadan 21–27 May 2019	• Cocaine • 3,4-Methylenedioxy methamphetamine • Heroin • Marijuana (THC) • Alcohol • Amphetamine • Nicotine • Methamphetamine • Ecstasy	Guzel, E. [50]

Table 1. Analytical methods used in the detection of illicit drugs globally (Continued)

Analysis method	Location	Year/ Sampling period	Types of illicit drugs & NPSs	First author& References
LC-IMS-HRMS	March-June 2019	2023/ Two Slovenian municipalities; the capital Ljubljana, and a smaller one (M1).	• 3-MMC • 1R-2S-(−)-Ephedrine (licit) • Ethcathinone (licit) • 5-IT • AMT N-methyltryptamine (licit) • Ephedrine • 6-IT (licit) • 3-MEC • Pentedrone • 4-chloro-α-PPP (licit) • Isopentedrone • 2,3-DMMC • N- Ethylbuphedrone • 2-NMC • N-acetylmethamphetamine (licit) • Levorphanol • Embutramide (licit) • Kavain (licit) • 2,4-DMMC • 4-MDMC • 2-MEC • 4-MPH	Verovšek et al. [51]
LC-MS/MS (Solid-phase extraction)	Centro, Mangue, Catumbi, Alegria, Faria-Timbó and São Cristóvão	2020/ Between the spring period (September/2018) and early summer (December/2018) a	• Cocaine (COC) • Methamphetamine (METH) • 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH, THC metabolite) • Benzoylecgonine (BE, cocaine metabolite) • Amphetamine (AMP)	Pacheco et al. [52]
LC-MS/MS (Sciex 5500 + QTRAP) with an electrospray ionization (ESI) interface coupled to the HPLC system	Southern China	2020/ from November 2017 to October 2018	• Amphetamine • Methamphetamine • MDMA • Methylone • Ketamine • Norketamine • EDDP • Codeine • Noroxycodone • Oxycodone • Norfentanyl • Morphine •	Zheng et al. [53]

Table 1. Analytical methods used in the detection of illicit drugs globally (Continued)

Analysis method	Location	Year/ Sampling period	Types of illicit drugs & NPSs	First author& References
Liquid Chromatography Mass Spectrometry (LC–MS/MS)	Seyhan and Yüregir Waste Water Treatment Plants	2021/ October 2016 and August 2017	• Heroin metabolite 6-acetyl morphine (6-MAM) • Amphetamine • 3,4-Methylenedioxymethamphetamine (MDMA) • Morphine • The main metabolite of Δ9-tetrahydrocannabinol (THC), 11-hydroxy (THC-OH) • Codeine • The metabolite of cannabis, 11-nor-carboxy-THC (THC-COOH), • 3,4-methylenedioxyamphetamine (MDA) • Methamphetamine • 3,4-Methylenedioxymethamphetamine (MDEA) •	Daglioglu et al. [54]
LC-MS/MS	Montana	April 15 to June 20, 2019	• Methamphetamine • Morphine • 6-acetyl morphine • 2-Ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine (EDDP) • Codeine • Ketamine • Benzoylecgonine • Amphetamine • Methadone • 3,4-methylenedioxymethamphetamine (MDMA) • Hydromorphone • Oxycodone • Noroxycodone • Fluoxetine • Tramadol • 3,4methylenedioxyamphetamine (MDA) • Ritalinic acid • Hydrocodone	Margetts et al. [55]

3. Results and Discussion

When the World Drug Reports and National Drug Reports are examined, it has been determined that illicit drug use is becoming more widespread in the world every day. The synthesis of new psychoactive substances and other illicit drugs is increasing every day and it is becoming difficult to track these substances instantly. From this perspective, the devices used to detect illicit drugs are very essential.

The Sewage Analysis CORe Group - Europe (SCORE), a European network, was established in 2010 to regulate the approaches used in wastewater analysis and standardize methods for participating countries. Subsequently, Turkey, like many other countries, contributed to these studies. Studies have been initiated to identify the illicit drugs used, not only by analyzing wastewater but also by analyzing the illicit drugs found in syringe residues. Pioneering studies on the detection of various narcotic substances in wastewater have been conducted in the world and Türkiye, and continue to be conducted. In addition, the studies investigated the effects of various parameters such as weekdays and weekends, national holidays, seasons, musical events, and pandemics.

In this literature research covering the last years, it has been seen that various countries analyze wastewater and choose the analysis method by taking into account the illicit drug structure, metabolites, analysis time, device sensitivity, and measurement accuracy. When all the articles examined are taken into consideration, it has been determined that many devices are used. Many devices and their various features are being developed in wastewater analysis and it is seen that LC-MS/MS is more widely used in wastewater analysis among these devices.

4. Conclusion

In these studies, which have been intensively examined worldwide, especially in the last five years, the analysis of various illicit drugs has been examined. Various methods and analysis devices were examined in these analyses. LC-MS/MS is one of the most preferred devices. Its sensitivity and desired illicit drug detection facilitate its use in studies. The type of illicit drugs, metabolite of the illicit drugs, half-life, and laboratory conditions are the determining factors in analysis methods. Considering the constantly produced new psychoactive drugs, method development, improving device properties, and following the literature are critical issues.

Sensitive results and illicit drug detection play an important role in the fight against addictions. For this reason, following the current literature and choosing effective devices are among the early prevention strategies in the fight against drugs. In addition, supporting these studies with artificial intelligence and machine learning helps develop preventive studies and early warning systems.

It is of great importance all over the world for scientists to detect newly synthesized or known illicit drugs in wastewater and report them to the relevant early warning systems. The early warning system will ensure that various law enforcement agencies are informed of the illicit drugs reported, which will also increase illicit drug screening throughout the country and thus seize the illicit drug before it is used. In addition, since drug use is a public health problem, the disease burden in the country will decrease as it will be prevented from being used. Increased data sharing between public health, hospital systems, first responders, and law enforcement is needed to ensure clarity in determining drug consumption.

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Declarations and Ethical Standards

The author(s) declare that they have no potential conflict of interest regarding the research, authorship, and/or publication of this article. The author(s) also state that the materials and methods used in this study do not require ethics committee approval and/or any legal-special permissions.

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