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REVIEW

Forensic Analysis of Illicit Drugs and Novel Psychoactive Substances in Wastewater
A review of toxicological, chemical and microbiological aspects

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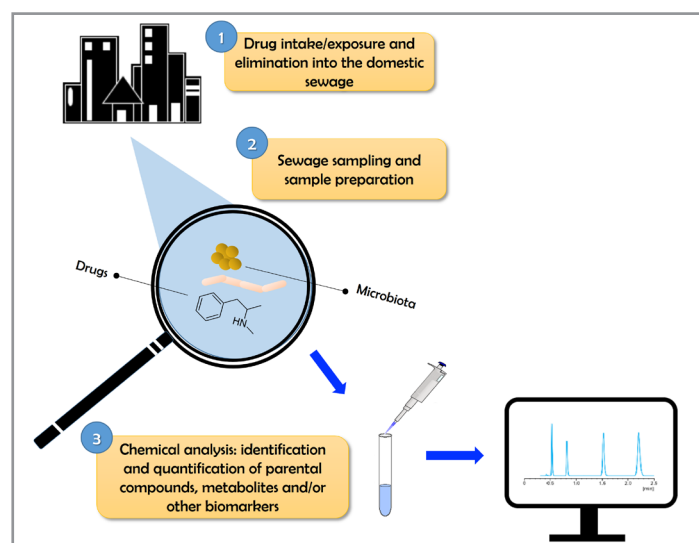
Wastewater-based epidemiology has emerged as a new analytical strategy for monitoring licit and illicit drug use in a population by measuring the levels of biomarkers in wastewater. The main concept of this approach is that chemical substances ingested by the population will be excreted in urine and feces, which will be discarded into the sewage network and may accumulate at the wastewater treatment plant. Several licit and illicit substances such as ethanol, nicotine, cocaine, amphetamine, methamphetamine and morphine have been investigated and reported in wastewater in worldwide. In recent years, this approach has also been explored for environmental monitoring of novel psychoactive substances (NPS) as well, since analyses of wastewater represent a fast and cost-effective way to evaluate collectively drug intake in a given population served by a sewage network. In this paper, a comprehensive and interdisciplinary review of the forensic, toxicological, chemical and microbiological aspects of the analysis of "traditional" drugs of abuse and NPS in wastewater and examples of applications reported in recently published papers is provided. Wastewater analysis is a very promising strategy in monitoring drug use in the context of Forensic Chemistry and Toxicology, and has been implemented by many researchers in the analysis of drugs of abuse, as supported by many recent literature reports.

Keywords: wastewater-based epidemiology, illicit drugs, novel psychoactive substances, forensic toxicology, forensic chemistry

INTRODUCTION

According to the United Nations Office on Drugs and Crime (UNODC) (2020), drug use has increased, from estimated 210 million users in 2009 to 269 million users in 2018 [1]. The illicit drug market is also becoming more complex [1], with the emergence of novel psychoactive substances (NPS). UNODC defines NPS as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat" [2]. NPS are new drugs of abuse that are usually produced to avoid legislation and scheduling controls [3], which can lead to the emergence of new, "legal" synthetic drugs, if they are not prohibited or scheduled yet. Many classes of NPS have been reported, such as novel stimulants, designer opioids, designer benzodiazepines, synthetic cannabinoids and novel hallucinogens [3]. Until December 2019, more than 950 NPS were reported to UNODC [2]. Since many of these drugs are unknown, information regarding their mechanisms of action, effects, metabolism and poorer. This is a significant challenge for forensic chemists and toxicologists, physicians, coroners, law enforcement agents and several other professionals involved in this field, as these new synthetic drugs represent a great threat in public health and safety. Therefore, several strategies are required to endure the NPS problem.

The analysis of drug residues in wastewater is an innovative analytical and epidemiological approach used in the estimation of drug use proposed in 2001 [4], explored for the first time in 2005 [5], which has been utilized in several other studies since then [6]. This approach called wastewater-based epidemiology (WBE) can provide useful public health information by measuring human biomarkers of drug use excreted in urine [7]. Any chemical substance consumed by humans may be excreted into urine and feces in the unmodified form and/or as metabolite(s) and eliminated into a particular sewage network or directly into surface waters [4,8]. Drugs and/or metabolites accumulated at the wastewater treatment plant (WWTP) during certain period should represent the compounds excreted by a given population into the sewage network that reached the WWTP in the same period, if these substances are stable in wastewater and efficiently transported through sewage networks [9,10]. Considering drug's pharmacokinetics and environmental fate, these amounts of drugs and/or their major metabolites can be used for estimating drug intake by a given population [5]. Therefore, the analysis of drugs in wastewater is a valuable analytical strategy that can aid in the assessment of drugs consumed by a community covered by a particular sewage network [8–10], providing anonymous population-normalized data [4,11], in a non-invasive [4,5] and timely approach [12].

DRUG TESTING IN WASTEWATER AND FORENSIC APPLICATIONS

The analysis of illicit drugs in wastewater can be an alternative/complementary approach to monitoring drug use in a given population in Forensic Chemistry and Toxicology [13]. For example, combining the analysis of wastewater with chemical profiling of seized materials can be a valuable strategy to expand the knowledge regarding the illicit drug use and market [14]. These studies can be used for the direct analysis of wastewater to monitor variations of drug use due to special events [8], which has been evidenced by some studies during music festivals [15], holidays [16], sports competitions [14] and, more recently, the pandemic of COVID-19 [17]. For example, WBE has been explored in the study of spatial and temporal trends of alcohol (ethanol), tobacco and illicit drugs use [7]. The investigation of clandestine laboratories may also be supported by the analysis of chemical waste in the sewage, such as the specific chemical profile of wastewater due to the disposal of waste from illicit production of stimulants [18]. Analysis of chemical markers in wastewater provide association to specific synthetic routes of amphetamine [18]. The advantages of wastewater analysis over other epidemiological approaches include more objective estimations, reduced costs [8], guarantee of the anonymity and privacy of people [4], and almost real-time assessment of drug use [14], with no need to collect biological specimens from individuals. However, wastewater analysis is associated to some uncertainties that should always be considered. The analysis in wastewater itself cannot inform data on drug use pattern and prevalence and purity of drugs [19]. Especially in Forensic Chemistry and Toxicology applications, it is noteworthy that drugs present in wastewater may

reach the sewer and WWTP from different sources besides human excretion, including direct disposal of the drugs and other synthesis products [4]. For example, drugs in the form of powders, tablets or vegetable materials, usually forms they are available for illicit use, can be discarded through the sewer, dissolve in wastewater and reach the WWTP. In this context, it is important to consider this possibility and a recommended approach is to include products of the human metabolism of these drugs in the scope of the method, to avoid biases [9]. On the other hand, metabolism studies may be required for some drugs, especially new synthetic drugs. In addition, enantiomeric profiling needs to be used in the analysis of chiral compounds, to obtain information related to the source of a particular drug (e.g., illicit use, metabolism or direct disposal), such as in the analysis of amphetamine-like drugs [9,20]. Several countries and agencies have been already implementing monitoring tools through wastewater analyses, for example, the National Wastewater Drug Monitoring Program (NWDMP) [21], the Sewage Analysis CORe group Europe [22] and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [19].

WBE has potential as a promising to support forensic intelligence strategies, including drug enforcement and control [23]. In general, studies on drug testing in wastewater have shown agreement and correlation with other epidemiological data, being able to complement data from reports on drug seizures, population surveys and more [8]. In the field of drugs of abuse, epidemiological analysis in wastewater can be applied in different aspects, such as monitoring and rapidly assessing drug trends, efficacy of drug abuse control programs and comparison with population surveys [9]. Data provided by wastewater analysis can lead to a comprehension of the size and the changes of drug market, making possible an indirect assessment of the impact of specific criminal groups after dismantled by law enforcement [23]. From a forensic intelligence perspective, the combination of all these data, including results from wastewater analysis, can support a better understanding of the drug abuse problem, supporting strategic control and treatment initiatives [13,23]. Data obtained by law enforcement operations and investigations can strengthen the results obtained by wastewater analysis, which are subjected to uncertainties [23]. Considering the emergence of NPS, the approach of combining data provided by wastewater analysis, law enforcement investigations/operation, drug seizures and toxicological analysis of intoxication cases could be a very useful and interesting strategy [23]. The analysis of wastewater can be an additional early warning system, to communicate potential new abuse substances available in the drug market. Nonetheless, for the analysis of NPS in wastewater, the toxicokinetics and the drug fate in wastewater need to be known and reference materials are required, in particular for targeted methods; alternatively, high resolution mass spectrometry (HRMS) can be used for NPS identification in non-targeted methods, including retrospective analysis [12]. These aspects will be discussed in details in the following sections.

GENERAL CONSIDERATIONS

Quantification of parent drugs or metabolites in wastewater can provide data related to the amount of compounds reaching the WWTP and to the mass load, which can provide useful information on the estimated amount of drugs being consumed by a population, considering toxicokinetics and fate of each drug and characteristics of the sewage network [9]. Combining target analytes concentration in influent wastewater and toxicokinetics, environmental and WWTP data, back-calculation can be done to estimate drug consumption, in mass/day or doses/day, and further normalized to 1,000 inhabitants, based on the population served by the WWTP [4,9,19], enabling the comparison of data from different locations [9].

The back-calculation of drug intake based on wastewater levels is based on the concentration, which is defined as the amount of the parent compound or one of its metabolites found in wastewater (in ng/L), the flow rate corresponding to that of the sewage network (in L/day), the correction factor related to the metabolism/excretion of each analyte and the population is the total population served by the WWTP [6,12]. The correction factor is based on drug's toxicokinetics/pharmacokinetics, accounting for the drug's excretion rate and the molecular mass ratio between parent drug and its metabolite [12], in the case of using a metabolite as biomarker.

The toxicokinetics of each target drug (absorption, distribution, metabolism and excretion) is one of the key factors determining the amount of each drug/metabolite that will be eliminated into the sewage network [4]. Toxicokinetics, in turn, is influenced by some factors such as the type of drug, dose, route of administration and individual characteristics (e.g., age and health conditions). Moreover, the gut microbiota may play a role in the biotransformation of xenobiotics in the body, influencing the formation of metabolites that might be excreted into urine/feces and eventually reach the sewage [4]. It is important to consider that potential biases in the estimation of drug intake by a population may influence interpretation of results and there is a need for minimizing the uncertainty of this estimate [24]. Information on chemical identity of relevant metabolites, excretion rates in parental or metabolite forms and proportion parent/metabolites especially in urine need to be considered [25], for the selection of the target analytes in the sample and for further calculations. In back-calculation, specific correction factors account for the metabolism and excretion (mainly urinary) of a drug [12]. Some authors recommend is to refine the correction factors by extensive review and study of pharmacokinetics data available, in order to select the proper correction factor data for estimations [12]. The selection of metabolites (instead of the parent drug) as biomarkers in wastewater analysis is also very important since it may distinguish human active consumption of a drug from direct disposal or synthesis [4], such as in case of cocaine (parent)/benzoylecgonine (BE) (major metabolite). The estimation of drug intake based on doses may also have some associated uncertainties since the "standard dose" is highly variable according to the drug, administration route and use patterns (chronic, occasional and heavy users) [9]. Therefore, pharmacokinetics data are required. A challenge in the analysis of drugs of abuse in wastewater is that human toxicokinetics/pharmacokinetics data on traditional drugs of abuse are limited and for NPS, data are even scarcer [25]. Pharmacokinetics studies involving drugs of abuse are very complex due to safety and ethical constraints and are conducted only in authorized research centers [12].

Processes that may cause structural modifications of the drug/metabolite from the point of disposal of excreta to the point of sampling is another aspect to consider [26]. Processes of mass transfer (including sorption, partitioning and transportation), besides chemical and biological reactions, can occur in wastewater and define the fate of each target drug, affecting their final concentrations in wastewater [24]. The adsorption of drugs into suspended particulate material may also affect the overall concentration of these drugs in wastewater [24]. Furthermore, it should be taken into account that wastewater samples are usually adjusted to acidic pH at the time of collection and that acidification potentially modifies the partitioning of drugs between liquid phase and particulate matter [27]. Therefore, when the particulate fraction of wastewater is not analyzed, the intake can be underestimated for some drugs, such as reported for methadone and cannabis [28].

Abiotic and biotic processes occurring in the environment can be responsible for the conversion of emerging pollutants into transformation products (TP) [29], which may also apply to illicit drugs and metabolites present in the aquatic environment, particularly in the sewage. Known processes primarily inducing the formation of TP are reactions of oxidation, hydroxylation, hydrolysis, conjugation, cleavage, dealkylation, methylation and demethylation [29]. Biotransformation occurring within human, animal and microbial metabolisms, in natural or engineered systems, are considered biotic processes whereas abiotic processes include hydrolysis and photolysis occurring in the natural environments and WWTP [29]. The microbiome of wastewater can induce the biotransformation of drug metabolites excreted by humans, directly affecting the interpretation of analytical findings [30]. For example, in-sewage biotransformation has a role in the final concentration of cocaine and its biomarkers and thus in the back-calculation of cocaine use [26]. In influent wastewater, there is a high diversity of bacteria [31] and the high diversity of wastewater microbiome leads to many potential microorganisms being responsible for the transformation of drugs and their metabolites, and having a role in the overall microbial metabolome [30]. Microorganisms can also affect pharmacological active substances exhibiting chiral properties [32]. Illicit drugs can undergo microbial biotransformations in wastewater under aerobic or anaerobic conditions, which may be mediated by bacterial enzymes [24]. Enzymatic reactions occurring in microbial metabolism include hydroxylations,

N-oxidations, *S*-oxidations, dealkylations, dehalogenations, nitro reductions and hydrolysis of amides and carboxylesters, among others [29]. For example, a recent study studied showed that the biotransformation of pyrrolidinophenone-type psychoactive substances in incubation with a *Pseudomonas putida* strain isolated from wastewater, reporting that a similar TP was formed also when the drug was incubated with wastewater as inoculum [30]. It is noteworthy though that human metabolites and microbial TP may present common metabolic pathways, converging into the formation of similar compounds, which makes challenging to discern the origin of metabolites [29]. Another consideration is that drugs and metabolites may be subjected to wildlife biotransformation as well [29].

There are other factors that are considered in wastewater analysis. The features and conditions of the sewage network/WWTP need to be assessed and considered in WBE studies as well [9]. The daily flow rate, dissolved oxygen concentration, pH, presence of sediments and temperature can model the conditions and composition of wastewater [24], which ultimately may affect the stability of drugs. For calculations, the total population covered by the WWTP and the flow rate are required [9]. A limitation is assessing the total population served by a given WWTP [8], which can be challenging since the population may exhibit fluctuations in specific seasons [9,19]. Two combined approaches, the estimation based on chemical markers in wastewater or census and sewage capacity data, can be used for determining the population served by a sewage network and WTTP [12]. Census and sewage capacity data may not account for seasonal fluctuations in the total population served by a given WWTP [33]. The parameters of water quality (e.g., chemical oxygen demand, biological oxygen demand, total nitrogen and total phosphorus) may be used but non-anthropogenic sources can affect the estimations of population based on these markers [33]. Another marker recently proposed is ammonium ion, which could be less sensitive to non-human sources [33]. However, more research is required to establish biomarkers to calculate the population served by the WWTP and to monitor eventual fluctuations [12].

Another factor is that processes occurring within the WWTP might play a role in the fate of drugs and metabolites in wastewater, particularly when the analysis is performed in effluent (treated) wastewater. A conventional WWTP is designed to provide the removal of any pathogens and coliforms present in wastewater and to reduce loads of carbon, nitrogen and phosphorus [34]. Chemical treatment in WWTP and hydrolysis/photolysis naturally occurring in the environment thus can also lead to TP [29]. For example, a study explored the effects of photolysis by simulated sunlight or UV irradiation to cocaine and metabolites, unveiling the formation of several TP [35]. Usually, wastewater is not exposed to sunlight or UV radiation but two products from cocaine and benzoylecgonine identified in photolysis experiments were also detected in influent and effluent wastewater samples in that study [35]. The authors concluded some of these products might be result from *in vivo* elimination, but other products might be derived of other processes occurring in sewage, such as bacterial biotransformation [35]. In another study, the effects of hydrolysis, chlorination and photolysis to 11-Nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) were investigated [36]. The identified products of transformation of THC-COOH were not found in influent wastewater samples but hydrolysis and photolysis products were detected in effluent wastewater and surface water samples [36]. Some of the WWTP use processes of UV irradiation, chlorination and ozonation [36], which might lead to physicochemical transformations of drugs and metabolites.

ANALYTICAL CONSIDERATIONS

Chemical analysis of illicit drugs and metabolites in wastewater is paramount for WBE studies, since the quantification of these compounds in the wastewater is needed for back-calculations and drug intake estimations [37]. Thus, Analytical Chemistry is one of the bases of WBE [12]. Wastewater is a high-complexity matrix, which contains solids, dissolved and particulate matter, microorganisms, nutrients, metals and micro pollutants [24,38]. Drugs and metabolites are usually present at very low concentrations in wastewater, in the range of ng L^{-1}, much lower than in human biological fluids, which adds another level of complexity to the chemical analyses of this matrix [9,37]. Therefore, the analysis of chemical substances in this type of specimen may be challenging and requires analytical techniques

of high sensitivity combined with sample preparation prior the analysis. Another important consideration is the quality control in wastewater analysis. Method validation is of greater importance, using reference materials and quality controls, assessing figures of merit (e.g., limit of detection and limit of quantification) and confirming positive findings, to assure the quality of the results [12]. In addition, it is recommended to include chemical markers of wastewater in the scope of the method, as a quality control and normalization factor [4]. These markers can be either indicators of either human use of substances (as caffeine or nicotine) or human activity (as coprostanol present in feces or creatinine present in urine) [4]. The use of high sensitivity, accuracy and precision, validated analytical methods is needed. Inter-laboratory exercises are also recommended in order to standardize analytical procedures and calculations [12,24].

Wastewater sampling is a critical step in WBE studies and the selection of a proper type and frequency of collection can avoid misinterpretation of the findings [12]. The active collection of wastewater samples can be performed mainly by composite or grab sampling [9,39]. Composite wastewater samples consist in a pool of influent, raw wastewater collected during 24 h, to be representative of an entire day of elimination into the sewage [9,39]. Composite samples are representative of the average daily conditions of the wastewater during the period when sampling was occurring [40]. Grab samples are collected as a single sample or a set of samples collected over a period no longer than 15 minutes, and it should reflect the conditions of wastewater at the moment of sampling [40]. The major limitation of using grab samples is that these samples may be biased by fluctuations in concentrations, especially due to special events or environmental conditions [4].

Another possibility consists in a passive sampling, in which a polymeric-based sorbent material is deployed at the WWTP for longer periods (days or weeks) and provides a long-term accumulation of chemical substances present in sewage [11]. An example of this approach is the use of polar organic integrative samplers (POCIS) [9]. This approach is particularly interesting for monitoring NPS considering some of these drugs might be used in a low rate by a population [11] or the prevalence fluctuations related to cycles of emergence-disappearance common to some NPS. However, passive sampling methods required calibration and quantification, for a better understanding on the mechanism by which compounds are collected and potential variability [11].

Sample preparation is a very important step in wastewater analysis. It is used to concentrate the target analytes and to reach low limits of detection (LOD) and of quantification (LOQ), besides acceptable recoveries [9], since the levels in wastewater are usually in reduced magnitude in comparison to the levels at the moment of excretion (caused by dilution, microbial degradation/biotransformation and sorption to particulate material) [4]. Preparation of wastewater samples is also required to remove matrix interferences that can affect the analysis, especially considering the ionization in liquid chromatography coupled to mass spectrometry (LC-MS) based methods [9].

After the collection, wastewater samples are kept and stored at low temperatures (4 ºC or -20 ºC, according to the estimated time) [9]. Usually, the pH of wastewater samples is adjusted by acidification, right after sampling [9] or prior to sample preparation. This procedure is recommended to improve sample stability, by decreasing bacterial activity [27]. For example, it has been reported the acidification of filtered or non-filtered wastewater samples increases the stability of many classes of NPS [11]. In addition, acidification of wastewater samples is also required if a solid phase extraction (SPE)-based method using mixed-mode cation exchange phase is performed to extract basic drugs [27]. However, acidification of wastewater samples can promote the biotransformation of THC-COOH [37]. The addition of sodium metabisulfite has also been explored for preserving wastewater specimens [37], such as to improve the stability of cocaine [24] and synthetic cannabinoids [11]. Therefore, stability studies are required to assess the optimal conditions for storing wastewater samples, to avoid degradation of target compounds and misinterpretation of results.

For sample preparation, several studies described in the recent literature have combined filtration, centrifugation and solid phase extraction (SPE). Filtration with membrane of glass fiber filters or centrifugation is required in order to remove all solid components present in wastewater samples [9,37,39]. SPE is well

known as a high selectivity extraction technique, which provides both good clean-up and pre-concentration of target compounds. In addition, SPE is a traditional technique adopted by many forensic laboratories. Offline and online SPE have been used in many studies for extracting target drugs and metabolites from wastewater samples, but offline SPE is the most commonly used approach in wastewater sample preparation reported in the literature [12,37]. Other solid phase-based sample preparation techniques have also been used, such as solid phase microextraction (SPME) and molecular imprinted polymers (MIPs)-SPE [41]. MIPs-based SPE resulted in high selectivity, accuracy and precision for the analysis of amphetamines and methylenedioxy derivatives in wastewater [41]. In another study, SPME was used for extracting Δ9-tetrahydrocannabinol (THC) and THC-COOH from wastewater samples, obtaining satisfactory precision and accuracy [42]. Liquid-liquid extraction (LLE) has also been used in some studies, and, for example, in comparison with SPE, minimal differences in the recovery of cannabinoids from wastewater have been reported for both methods [43]. Wastewater samples collected using POCIS are usually processed using a different approach. POCIS present sorbents similar to those of SPE cartridges in the membrane, enabling the collection of hydrophilic drugs [9]. In a recent study published in the literature, the extraction was performed from POCIS using methanol, two times, and combined both extracts, which were further analyzed by LC-MS/MS [44]. According to the authors, findings obtained from samples collected with POCIS showed an underestimation in comparison to 24-h composite collected samples, which could be explained by the potential blockage of the POCIS surface with solid materials during filtration and consequent reduced trapping of drugs [44].

Drug testing in wastewater has become plausible due to great advancements in analytical technologies, making it possible to detect trace levels of drugs and metabolites in this type of sample [8]. Coupling chromatography and mass spectrometry is the best analytical strategy for chemical analysis of wastewater, in order to reach the needed sensitivity and selectivity [37]. Several studies have used LC-MS techniques for detection and quantification of drugs in wastewater samples [12], with recent studies reporting both high-performance and ultra-performance liquid chromatography (HPLC and UPLC, respectively). Ionization in LC-MS methods are usually performed by electrospray (ESI) in both positive and negative modes, depending on the type of analyzed compounds. For example, in recent studies, ESI in a negative mode was used in the detection of ethyl sulfate (EtS), metabolite of ethanol, whereas other illicit drugs were detected using ESI in positive mode [17,45]. Several detection systems in mass spectrometry (MS) have been used, including hybrid and high-resolution systems. Low-resolution MS including ion trap and triple quadruple analyzers are the most used techniques in quantification of illicit drugs and metabolites in wastewater [37]. However, the use of HRMS has been increasingly explored in recent years [37]. Some examples include triple quadrupole mass spectrometry (e.g. [45–47]), quadrupole-ion trap mass spectrometry (QTrap) (e.g. [16,17,48]), quadrupole-orbitrap mass spectrometry (e.g. [49,50]) and quadrupole-time-of-flight mass spectrometry (QTOF) (e.g. [49]). These techniques can provide high sensitivity and selectivity, reaching good results, especially considering the complexity of wastewater samples. Particularly LC-HRMS-based methods can provide a comprehensive screening of illicit drugs and NPS, their metabolites and TP [37]. Direct analysis by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) systems (without extraction) has also been reported. In a study published in the literature, wastewater samples were directly injected into LC-MS/MS after filtration without any extraction, reaching limits of detection (LOD) between 0.05 and 30 ng L^{-1} and median limit of quantification (LOQ) of 31 ng L^{-1} [51]. The authors found that using SPE for clean-up did not increase the sensitivity of the method in comparison to direct injection and exhibited decreased sample throughput, adding more time to the process [51].

Gas chromatography-mass spectrometry (GC-MS) is another technique of high selectivity and sensitivity but it requires a derivatization step for many compounds of forensic interest, which can extend the analytical workflow [37]. In the literature, GC-MS has been used by some studies. For example, GC-MS with an ion trap detector was recently used in enantiomeric profiling of several compounds including amphetamine, methamphetamine, 3,4-methylenedioxy-methamphetamine (MDMA) and norketamine [52]. Wastewater samples were filtered, acidified and further extracted using SPE followed by chiral derivatization

with (R)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((R)-MTPA-Cl) [52]. Authors obtained a good separation for several diastereomers of the target analytes, without using a chiral column [52]. In another study, GC-Ion trap-MS/MS was used in combination with SPE and MSTFA derivatization for the determination of illicit drugs in grab samples collected from 5 WWTPs [53]. Cocaine and its metabolite, benzoylecgonine, THC and its metabolite THC-COOH, codeine and morphine were unequivocally detected in wastewater by GC-MS/MS [53]. However, the authors stated that the method used was not able to detect the methadone metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and requires more time for analysis than LC-MS-based methods, considering the derivatization step of 90 min [53]. In addition to chromatographic-spectrometric techniques, other techniques have been explored in wastewater analysis. A recent example is in which the application of surface-enhanced Raman spectroscopy (SERS) sensor was used in the detection of methamphetamine in wastewater [54]. According to the authors, the SERS-based method exhibited results comparable to those obtained by a LC-MS-based method, high sensitivity, good selectivity to methamphetamine and good reproducibility [54].

The use of HRMS has introduced many possibilities in forensic analyzes. The many features of HRMS instrumentations including mass accuracy and sensitivity allows the investigation of a high number of analytes, including additional compounds present in the sample that were not previously targeted [37]. Time-of-flight (TOF) and Orbitrap are the most frequently used analyzers for HRMS [37]. Based on HRMS techniques, another approach in the analysis of wastewater is the suspect screening/analysis, in which any compound that is in the instrument's library is qualitatively analyzed in a sample [11,55]. In this approach, there is no need for selecting analytes and using reference materials for method development [11]. However, each entry in the instrument's library requires data of each compound (e.g., exact mass, retention time and fragments), which depends on the availability of reference materials; if there is no reference material available, the data for including the compound in the library is more limited but the tentative identification might still be possible by inspecting the high resolution mass spectra [37]. In addition, *in silico* models can be used for predicting chemical structure and properties of unknown compounds, which can include or exclude potential chemical structures and increase the level of confidence in the identification, in case no reference materials or data are available [11].

The application of the techniques mentioned above can be used for targeted and non-targeted analysis. Targeted analyses are performed for a limited number of compounds present in the scope of the method [55], which leads to high sensitivity and selectivity but do not detect any other compounds present in the sample that were not included in the scope of the method [37]. In general, targeted analyzes are performed using GC-MS or LC-MS techniques [37]. In wastewater samples, LC-MS/MS, with triple quadrupole or ion trap analyzers (low-resolution mass spectrometry), have been successfully used for analysis of drugs and NPS in wastewater samples [11,37]. On the other hand, non-targeted analyses consist in the investigation of any compound present in the sample detectable by the analytical technique in use [47], without selecting any analytes [11] and providing any information of the analyte of interest prior to the analysis [37]. In this approach, both chromatographic profile and accurate mass spectrum are thoroughly investigated for tentative identification [11]. This approach is difficult when applied to wastewater due to the complex chemical composition of the samples, the potentially high number of compounds present, and at low concentration [37].

In addition to parent drugs, human metabolites and TP may also be investigated in wastewater as biomarkers of drug use, in the absence of the parent or as a complement to the parent compound. Combining toxicokinetics and stability in wastewater data on drugs of abuse is a critical step to perform WBE studies since these data will be used for calculation and estimations [25]. The selection of the appropriate biomarker for a specific drug is paramount for chemical analysis of wastewater as well as for further back-calculations and estimations. In general, parent drugs or their metabolites present in urine are selected as biomarkers for illicit drugs use in the analysis of wastewater [37]. Data on human metabolism and identity of major metabolites is available for many known drugs of abuse whereas for NPS such information is much more limited [37]. In this context, studies on biotransformation occurring in-sewage are needed [12].

If the identity of a metabolite or a TP is known then it can be added to the scope of the method and in the library of an analytical instrument, to be considered for analysis in wastewater, routinely or retrospectively [29,37]. However, if the identities of those compounds are not described, alternative approaches need to be adopted. The structural elucidation of metabolites or TP might be done by the study of high-resolution mass spectra, based on fragmentation patterns of the parent drug and unknown compounds, but also on the chromatographic profile, especially if there are additional peaks at retention times corresponding to the fragments under investigation [37]. This can also be performed retrospectively, reanalyzing other compounds that were not initially targeted in previous data acquisition [37].

In vitro studies may be explored for characterization of potential human metabolites, which can further be used as biomarkers of drug intake in wastewater. *In vitro* models can be used to assess toxicokinetics data for classic and novel drugs of abuse, predicting the human metabolism of drugs [25]. There are several *in vitro* models available including cells, cell fractions and organs, such as human hepatocytes and human liver microsomes. In these models, the target parent drug is incubated within systems containing liver enzymes and samples are further analyzed to characterize potential metabolites [25]. Using *in vitro* models, there are no concerns regarding ethical and safety issues, in contrast to *in vivo* studies, and the costs are usually lower. On the other hand, for *in vitro* assays, reference materials of the drug of interest and high resolution analytical techniques are required (e.g. HRMS). In regards to NPS, especially recently emerged ones, reference materials may not be available yet [25]. *In silico* studies may also be performed to predict human metabolites or TP [37]. For example, the software SMARTCyp of the University of Copenhagen is an *in silico* platform that predicts the molecular sites where a potential Cytochrom P450 metabolic reaction can occur [25,56]. Another example is the EAWAG-BBD Pathway Prediction System, which predicts the microbial biotransformation of chemical substances based on its database and it has been successfully used for predicting the fate of environmental contaminants [25,57]. Once potential metabolites and TP are predicted, these compounds can be investigated in data obtained by HRMS, extracting the exact mass from chromatographic data based on several positive identification criteria and tentatively characterizing the structure based on fragmentation data [37]. Although both models (*in vitro* and *in silico*) are very useful to predict potential human metabolites, it is still possible they are not formed *in vivo* or excreted in urine, in a real user scenario. Nonetheless, these models are considered powerful tools to predict a list of potential metabolites that can be eventually be tested as target compounds using high-resolution mass spectrometric methods [25].

Research on drug stability in wastewater has been conducted over the recent years and there is information available in the literature for some drugs and their metabolites. In-sample stability has been assessed for some drugs and metabolites but in-sewage stability subjected to different conditions is not well-understood [24]. Therefore, studies assessing biotransformation/biodegradation kinetics, microbial kinetics and stability can be very powerful tools to characterize the fate of drugs, metabolites and TP in wastewater. In addition, characterization of the microbiome present in wastewater through metagenomics can support the investigation of the functional potential of the microbiome for the biotransformation/biodegradation of drugs. The combination of *in vitro* or *in vivo* metabolic profiling and biotransformation/degradation studies of drugs is a suitable analytical strategy to obtain data on known and unknown metabolites and TP, which might be further investigated in wastewater samples and assessed as potential biomarkers for WBE studies [37].

The analysis of drugs, metabolites and TP in real sewage would be ideal for understanding the biological, physical and chemical behavior of these substances, under sewage networks in normal operation conditions; however, this is a complex and limited approach that would require several studies to obtain accurate data [24]. In-laboratory studies on drugs' stability in wastewater should consider: (1) inclusion of biofilms present in sewage during stability assessments; (2) physical-chemical characterization of wastewater to assure the reproducibility; (3) effective spiking concentrations considering the purpose of the study; (4) quality controls (positive control, negative control and abiotic controls); (5) suitable experimental design and sampling [14]. A detailed discussion on each of these recommendations can be found elsewhere [24].

DRUGS REPORTED IN WASTEWATER

The applications of WBE in forensic research has been performed since the middle-2000s, with one the first studies on the analysis of cocaine and BE in wastewater published in 2005 [5]. Since then, many researchers have evaluated licit and illicit drugs in wastewater around the globe. Among licit substances, nicotine is a substance highly available in the world, which can be measured itself in wastewater. Additionally, nicotine can be used as a marker of human consumption, for quality control and data normalization purposes [4]. In wastewater samples, there are several studies available in the recent literature detecting nicotine metabolites cotinine or trans-3′-hydroxycotinine to assess nicotine use (e.g. [17,45,46,50,59–65]). Ethanol is one of the most commonly consumed substances in the world [59] and ethanol intake can also be measured in wastewater. In the body, ethanol is mainly metabolized to acetaldehyde and acetic acid, with minor fractions metabolized to ethyl sulfate (EtS) and ethyl glucoronide (EtG) [66]. EtS and EtG are common metabolites of ethanol found in urine after alcohol intake [59,66]. It has been suggested that EtS is a more recommended metabolite for ethanol consumption estimation based on wastewater levels, since EtG instability in effluent wastewater has been previously reported [67]. Additionally, it is important to consider that the source of ethanol present in wastewater can be the direct disposal of alcoholic beverages and other products (e.g. hand sanitizers) [67]. This might be a factor for wastewater research conducted during the pandemic of COVID-19, since there are many ethanol-based hand sanitizers being used. However, in the presence of ethanol in sewage, the probability of formation of EtS in wastewater is minimal, thus not affecting the selection of EtS as a biomarker [67]. In wastewater samples, EtS has been proposed for estimating ethanol consumption (e.g. [17,45,61,64]).

Cannabis is the most used drug reported by UNODC, with 192 million users around the world in 2018 [1]. Trends in cannabis use have been influenced by its legalization in some countries, and according to the UNODC, it will take time to assess the impacts of non-medical use legalization measures and the cannabis market should be under close monitoring [1]. In regards to cannabinoids, THC-COOH is the THC metabolite commonly used as biomarker of cannabis use in wastewater [43], usually at greater concentrations in influent wastewater in comparison to effluent wastewater [36]. THC-COOH has been detected in wastewater, in several studies (e.g. [15,17,43–45,47,49,50,61,64,65,68–73]). In the literature, the detection of THC itself was also reported (e.g. [63,71]). Another metabolite of THC, THC-OH has also been reported in wastewater (e.g. [68,71]). It is important to consider that due to their lipophilicity, metabolites of THC may be eliminated through the feces, adsorb and deposit to particulate content present in wastewater [43]. THC-COOH may interact with particulate material present in wastewater and failure in measuring its content in this fraction of wastewater may lead to underestimations [28]. CBD is another cannabinoid present in cannabis, with therapeutic but not psychoactive properties. CBD is excreted in urine mostly in the parent form [74]. In a recent study, CBD and the metabolites CBD-7-OH and CBD-7-COOH were searched in wastewater but only CBD was detected [43]. Considering the current landscape of legalization of cannabis for medical and/or recreational purposes, the concentrations of THC-COOH could increase in environmental waters [74], and this could eventually be observed with other cannabinoids present in cannabis.

In the literature, stimulants are a class of drugs frequently detected and reported in wastewater. In recent days, cocaine is still one of the most largely produced drugs, with estimated 19 million users in 2018 [1]. In wastewater samples, cocaine and/or benzoylecgonine have been detected (e.g. [13,17,44–46,49,51,60–65,68–71,73,75–78]. Other cocaine metabolites have been reported in some studies: a few examples include ecgonine methyl ester (EME) [63], norcocaine [68,71], anhydroecgonine methyl ester (AME) [60] and cocaethylene [60,68,69,71]. Another group of stimulants, amphetamines seized between 2009 and 2018 has significantly increased [1]. The estimated number of amphetamines and prescription stimulants users in 2018 was 27 million [1]. In wastewater samples, amphetamine and/or methamphetamine have been detected (e.g. [13,15–17,44–52,54,60–65,68,70,71,73,75–81]. However, these drugs are metabolites of others such as fenproporex, selegeline and famprofazone, and these findings in wastewater can overestimate the use of amphetamine or methamphetamine [9,82]. Therefore,

it is noteworthy to highlight that by enantiomeric analysis it is possible to differentiate between legal and illegal sources of amphetamine-like compounds [82]. The amphetamine-like drugs MDMA and MDA have also been determined in wastewater samples (e.g. [13,15–17,44–47,50–52,60–65,68–71,75–78,81]. According to the UNODC, in 2018 21 million people have used ecstasy [1]. MDMA metabolites, HMMA and HMA, have been reported in wastewater samples in some studies as well (e.g. [7,60,83]). Some authors even recommend including HMMA [83,84] and HMA [83] in the scope of analytical methods to estimate MDMA use. 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), another illicit stimulant similar to MDMA, has been also reported in some studies (e.g. [7,68,71]). An important consideration regards to the chirality of methylenedioxy derivatives, such as MDMA and MDA. For example, chiral analytical methods can help to understand the source in wastewater of MDMA (MDMA use vs. direct disposal) and MDA (MDA use vs. MDMA metabolism) [20].

Opioids are a class of substances largely used in the treatment of moderate to severe pain [85]. This class includes pain reliever prescribed drugs such as oxycodone, hydrocodone, codeine, morphine and others, as well as heroin (an illegal drug) and other synthetic opioids such as fentanyl [86]. In 2018, it was estimated that 57.8 million people used opioids, including opiates and pharmaceutical opioids [1]. Morphine (e.g. [17,44,45,48,60–64,68,71,73,75,76]), codeine (e.g. [44,48,60–63,65,71,75,81]), methadone and its metabolite EDDP (e.g. [13,17,44,50,60,62,63,65,68,69,71,75,76,81]) are some of the most commonly detected opioids in wastewater, which in turn is one of the main sources of opioids present in superficial waters [39]. In two recent studies, normorphine [60,63] was also targeted and reported in wastewater analysis. Heroin was also measured in wastewater in some studies (e.g. [15,71]) and, although 6-MAM is a unique heroin metabolite, this compound may not be commonly reported in wastewater samples due to its low levels in these samples [9]. For example, concentrations of 6-MAM in wastewater were recently as low as 15.4 ng/L [50]. 6-MAM was also reported in wastewater in other studies (e.g. [60,64,70,71,76]). In wastewater analysis, it is challenging to estimate heroin use based on morphine, considering that morphine can be present in wastewater resulting from therapeutic use of morphine and codeine [9] or illicit use of morphine. When estimating heroin use based on morphine levels, the amount of morphine used therapeutically and the amount of morphine from codeine metabolism need to be taken into account [9,60]. It is also important to consider that the ingestion of poppy seeds can result in the formation and excretion of morphine in urine [87]. Norcodeine, a codeine metabolite, has not been usually targeted in the wastewater codeine testing, which can be explained due to its low levels in wastewater specimens, requiring high sensitive analytical techniques [88]. However, examples of studies detecting norcodeine in wastewater are available (e.g. [60,63]). Another metabolite of methadone, 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP), was also targeted [6].

In addition to morphine, codeine and methadone, other opioids have been reported in wastewater studies. Hydromorphone is available in the pharmaceutical market, which poses an analytical challenge to assess whether the source of hydromorphone present in urine is hydromorphone intake or hydrocodone metabolism [89]. The detection of hydromorphone in urine though is not necessarily an indicator of hydromorphone use [90]. Therefore, this should be considered in the analysis and interpretation of hydrocodone and hydromorphone levels in wastewater and consumption estimates. Hydromorphone has been detected and reported in several recent studies in the literature (e.g. [44,60,68,71]). Dihydrocodeine, a metabolite of hydrocodone, and dihydromorphine, a hydromorphone metabolite, were also detected in wastewater samples [60]. Similarly, hydrocodone and norhydrocodone were detected in wastewater samples, according to some studies available in the literature (e.g. [44,63,68,71,75]). Oxycodone is another semisynthetic opioid, derived from codeine [91]. In wastewater specimens, oxycodone, noroxycodone (major metabolite of oxycodone) and oxymorphone (a minor metabolite) [92], have been detected in some studies (e.g. [44,50,51,60–62,65,68,71,75]). Tramadol is an orally active, synthetic opioid [93], analog of codeine [94], with pharmaceutical use. The illicit (non-medical) use of tramadol has also been reported, such as in some countries in West, Central and North Africa [1]. Tramadol, in its parent form, has also been found in wastewater samples (e.g. [17,44,50,60,62,65,76,79]). The metabolites *N*-desmethyltramadol and

O-desmethyltramadol have been detected in wastewater samples as well (e.g. [63,75]). A compound named *O*-*N*-bisdesmethyltramadol was also reported in wastewater [63] but no additional information on this compound was found, and it may be the metabolite *O*-*N*-didesmethyltramadol of tramadol reported elsewhere [95]. An analog of tramadol, tramadol-*N*-oxide was also reported [63]. Buprenorphine is a semisynthetic opioid, derived from thebaine, medically used in pain management and in the treatment of opioid dependence [96]. Buprenorphine and/or its metabolite norbuprenorphine have been determined in wastewater (e.g. [44,46,61,68,71,75]). Additionally, the conjugated metabolite norbuprenorphine-glucoronide was detected in wastewater samples [50,65]. Finally, another opioid highly relevant in Forensic Toxicology and Chemistry is fentanyl, characterized by its high potency (80 times higher than morphine) and reduced duration of action [97]. However, the illicit use of fentanyl and the emergence of illicit analogs have been causing public health problems in many regions, including the US and Europe [98]. In 2018, fentanyl was associated with two thirds of 67,367 deaths by overdose in the USA [1]. In recent studies available in the literature, fentanyl and/or its metabolite norfentanyl have been detected in wastewater (e.g. [61,63,68,71]). Fentanyl detection in wastewater can be analytically challenging. Its elimination occurs in urine and feces, mainly in the form of the inactive metabolites (primarily norfentanyl) [99,100], with small fractions corresponding to the unchanged and free forms of fentanyl [99–101]; thus this drug might not be detected in wastewater samples due to its low levels. However, in case fentanyl is detected in wastewater, it might be present as a result of direct disposal, similarly to other drugs. In addition, fentanyl can also be present in other drugs (e.g., heroin, cocaine, methamphetamine and MDMA) as adulterant [86], which is particularly important when estimating drug intake and comparing it with other epidemiological or seized data.

Benzodiazepines comprise a number of drugs, which includes diazepam, oxazepam, temazepam, alprazolam and more [102]. The frequent prescription of benzodiazepines is made due to their pharmacological properties, useful in the treatment of anxiety, insomnia, convulsions, as sedative, amnesic and relaxant agent [102,103]. However, misuse of benzodiazepines has also been reported [104]. One of these drugs is diazepam, which exhibits a complex metabolism, with other active metabolites, including nordiazepam and temazepam (minor), which can be both further metabolized to oxazepam, another active compound that is conjugated in Phase II metabolism (oxazepam glucuronide) [102,103]. The metabolism of oxazepam occurs mainly by glucuronidation [105]. Oxazepam and temazepam are also pharmaceutical drugs. In recent literature, the detection of diazepam and nordiazepam have been reported in wastewater (e.g. [51,63,68,71]). Oxazepam and/or temazepam have been detected in wastewater as well in some studies (e.g. [17,44,46,50,51,60,62,63,65,68,71,75]). Alprazolam is another benzodiazepine drug, with short-duration action [106], used mainly in the treatment of anxiety and panic disorders [102]. Alprazolam has been detected in wastewater samples in several studies (e.g. [63,68,71]). The metabolite α-OH-alprazolam was also quantified in wastewater samples [107]. Clonazepam is a benzodiazepine prescribed in the treatment of anxiety and seizures [108]. A few studies have reported the detection of clonazepam in wastewater, [34,65]. Clonazepam's metabolite, 7-aminoclonazepam, was also detected in WBE studies (e.g. [75]). In blood, clonazepam and other nitrobenzodiazepines exhibit instability, which is especially remarkable in postmortem blood contaminated with bacteria [108]. In a similar context, the microbiome of sewage might play a role in the stability of clonazepam in wastewater, similarly to biological fluids such as blood. Although it is not a benzodiazepine, zolpidem exhibits a mechanism of action similar to benzodiazepines [105] and it is used therapeutically as hypnotic [109], being part of the group called "Z-drugs" [110]. These drugs, including zolpidem, have been associated with several cases of misuse, dependence and even fatal intoxications [110]. In the literature, zolpidem and its metabolite zolpidem 4-phenyl carboxylic acid were detected in wastewater in some studies [79,111].

Other substances eventually involved in forensic casework have also been reported in wastewater. Lysergic acid diethylamide (LSD) is a semi-synthetic hallucinogen, derived from lysergic acid present in fungus ergot *Claviceps purpurea* [112]. In wastewater, both substances, the parent and its metabolite 2-oxo-3-OH-LSD, have been detected in studies available in the recent literature (e.g. [50,65]). Ketamine

is a derivative of phencyclidine (PCP) and shows anesthetic, analgesic, hypnotic and amnesic properties [113]. It is well described that ketamine is responsible for inducing dissociative anesthesia [114]. However, similarly to other drugs, the illicit use of ketamine for recreational purposes is well known. Ketamine and norketamine have been determined in studies recently published on wastewater (e.g. [44,48–51,60,65,70,75,76,81]). It is important to highlight that clinical and veterinary prescriptions, as well as illicit use of ketamine can all contribute for its release into the environment [115], which includes into the sewage. gamma-hydroxybutyric acid (GHB) is a chemical substance endogenously produced, resulting from the gamma-aminobutyric acid (GABA) metabolism [116]. However, GHB has been used illicitly, as a drug of abuse, since the 1990s [117] and as a dietary supplement and sleep inducer [118]. GHB has also been used as a chemical agent in drug-facilitated crimes (DFC) [119]. In wastewater, as expected, GHB can be excreted into sewage as a product of endogenous metabolism, as a component of dietary supplements or as an illicit drug [120]. In a recent study, GHB has been detected in wastewater and authors concluded that the GHB present is probably from endogenous metabolism, based on its levels [120].

In the context of Forensic Chemistry and Toxicology, NPS represent a challenge in clinical, toxicological, public health and public safety aspects. As a result of its emergence in the drugs of abuse market, this group of "new" drugs have been frequently reported in biological samples collected from intoxication cases and in seized materials. Therefore, it is not surprising that some of these substances also started to be reported in wastewater. Examples of recent studies reporting NPS are summarized in Table I. However, the detection of NPS in wastewater can be especially challenging for many reasons. Some NPS may be present in wastewater either after being directly disposed through the sewage network or also as a contaminant within "traditional" drugs. For example, fentanyl analogs can be used as adulterants of heroin, cocaine and other drugs and also as fake pharmaceutical opioids [1]. Another example is 4-ANPP, which can be either a metabolite or a precursor of fentanyl analogs in synthetic processes [121]. Since the metabolism of some new drugs is still unknown, it is difficult to target potential human metabolites in wastewater, as it has been done with other classical drugs. Some NPS or their metabolites may not have been reported until now in wastewater samples due to their unknown identity. Thus they are not known and not currently being targeted, or because of unavailability of reference materials for identification and method development purposes.

Table I. Examples of studies in the recent literature covering the detection of NPS in wastewater

List of NPS reported in wastewater	Reference
Ethylone, mephedrone and N-ethyl-pentylone	[16]
Methcathinone, 4-methyl-pentedrone, 1-(3-chlorophenyl) piperazine (mCPP), 4-methyl-amphetamine and 4-ANPP	[68]
4-methylethcathinone (4-MEC), methedrone and mephedrone	[51]
Methylone	[61]
Mephedrone	[60]
Carfentanil, methoxyacetylfentanyl, furanylfentanyl, MAB-CHMINACA, methcathinone, 4-methylpentedrone, 2-methyl-4'-(methylthio)-2-morpholinopropiophenone (MMMP), mCPP and 5-(2-Aminopropyl) Indole (5IT)	[122]
25-iP-NBoMe, 3,4-dimethylmethcathinone (3,4-DMMC), 4,4'-Dimethylaminorex (4,4-DMAR), α-methyltryptamine, buphedrone, methcathinone, mephedrone and ephenidine (NEDPA) *Detected only:* 2-phenethylamine, 25E-NBOMe, 4-chloro-α-PPP and 2,5-dimethoxy-4-isopropylamphetamine (DOiP)	[49]

Table I. Examples of studies in the recent literature covering the detection of NPS in wastewater (Continuation)

List of NPS reported in wastewater	Reference
Mephedrone	[70]
Cathinone, mephedrone and 1,3-benzodioxolyl-N-methylbutanamine (MBDB)	[50]
2C-D, dimethoxyamphetamine (3,4-DMA), 4-methyl-pyrrolidino-propiophenone (MPPP), cathine/ norpseudoephedrine and para-fluorofentanyl	[63]
Methylone, ethylone, butylone and mephedrone	[64]
Cathinone, mephedrone and MBDB	[65]
5.6-methylenedioxy-2-aminoindane (MDAI), AB-CHMINACA, methoxetamine, 4′-Methyl-α-pyrrolidinopropiophenone (MePPP), methedrone, 5-OH-DMT, dimethyltryptamine (DMT), 2-phenetylamine, *N*-ethylamphetamine, methoxyphenamine, methylbenzylpiperazine (MBZP) and ethylphenidate	[120]
5-fluoro-APINACA, JWH-073 (4-hydroxypentyl) and MDMB-CHMICA	[43]
Dipentylone	[78]
3-MMC, 4-FA, 4-MEC, Alpha-PVP, butylone, ethylone, mephedrone, methiopropamine, methoxetamine, methylone, *N*-ethylpentylone, pentedrone, pentylone, PMA and eutylone	[123]
3-MMC, 4-FA, 4-MEC, ethylone, methylenedioxypyrovalerone (MDPV), mephedrone, methcathinone, methylone, N-ethylpentylone and pentylone. 4-chloromethcathinone, 4-fluoromethamphetamine, acetyl fentanyl, mitragynine and eutylone	[124]

CONCLUDING REMARKS

Wastewater analysis is very promising in Forensic Chemistry and Toxicology. Much information can be extracted from the analysis of these specimens and data obtained from WBE studies can support multiple strategies in public health and security. Although the goal of this study is not a systematical and exhaustive review of the literature, rather it is a comprehensive review on this topic, many reports in the literature support that classic and novel drugs of abuse could be monitored in wastewater. However, studies involving the analysis of wastewater need to address many considerations and there are current limitations and uncertainties, which require further research. It is important to consider the stability and fate of drugs in wastewater, features of the sewage network and WWTP and environmental conditions. Although data required for calculations and estimations is available for some drugs such as excretion rates and parent/metabolite ratios, data availability is still very limited for new synthetic drugs, indicating that gathering more data will aid in estimations based on wastewater levels.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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