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Occurrence of Z-drugs, benzodiazepines, and ketamine in wastewater in the United States and Mexico during the Covid-19 pandemic

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HIGHLIGHTS

• First study to detect Z-drugs in wastewater from North America
• Ketamine consumption was high during the U.S. COVID-19 pandemic.
• First study to use Medicaid data and cross-validate drug levels found in sewage
• Discrepancy between Lorazepam RX and wastewater concentrations suggests abuse.

ABSTRACT

Z-drugs, benzodiazepines and ketamine are classes of psychotropic drugs prescribed for treating anxiety, sleep disorders and depression with known side effects including an elevated risk of addiction and substance misuse. These drugs have a strong potential for misuse, which has escalated over the years and was hypothesized here to have been exacerbated during the COVID-19 pandemic. Wastewater-based epidemiology (WBE) constitutes a fast, easy, and relatively inexpensive approach to epidemiological surveys for understanding the incidence and frequency of uses of these drugs. In this study, we analyzed wastewater (n = 376) from 50 cities across the United States and Mexico from July to October 2020 to estimate drug use rates during a pandemic event. Both time and flow
proportional composite and grab samples of untreated municipal wastewater were analyzed using solid-phase extraction followed by liquid chromatography-tandem mass spectrometry to determine loadings of alprazolam, clonazepam, diazepam, ketamine, lorazepam, nordiazepam, temazepam, zolpidem, and zaleplon in raw wastewater. Simultaneously, prescription data of the aforementioned drugs were extracted from the Medicaid database from 2019 to 2021. Results showed high detection frequencies of ketamine (90 %), lorazepam (87 %), clonazepam (76 %) and temazepam (73 %) across both Mexico and United States and comparatively lower detection frequencies for zaleplon (22 %), zolpidem (9 %), nordiazepam (<1 %), diazepam (<1 %), and alprazolam (<1 %) during the pandemic. Average mass consumption rates, estimated using WBE and reported in units of mg/day/1000 persons, ranged between 62 (temazepam) and 1100 (clonazepam) in the United States. Results obtained from the Medicaid database also showed a significant change (p < 0.05) in the prescription volume between the first quarter of 2019 (before the pandemic) and the first quarter of 2021 (pandemic event) for alprazolam, clonazepam and lorazepam. Study results include the first detections of zaleplon and zolpidem in wastewater from North America.

1. Introduction

The World Health Organization (WHO) declared COVID-19 a global pandemic on March 11, 2020 (Cucinotta and Vanelli, 2020), which created great disruption and uncertainty worldwide. The new reality of realities including working from home, a shutdown of schools, and economic breakdown created fear, worry, and stress leading to mental health problems (Mental health and COVID-19, 2020). Mental health has been an important issue and conditions related to poor mental health is one of the top ten leading causes of burden (Global, regional, and national burden of 12 mental disorders in 2004 and countries and territories, 1990–2019, 2022) this decade (2010–2020). The cost of poor mental health is estimated in trillions worldwide and projected to be $6 trillion by 2030 (Health, 2020). In the United States, >50 % of the people will be diagnosed with a mental health-related illness at one point in their life (Kessler et al., 2007), leading to probable proximity of medicinal treatments. Medications and prescriptions are recommended for various mental health conditions such as depression and anxiety and comprise about $32 billion per year globally (Global Medicines Use in 2020, 2020). One of the most common problems with these drugs is their potential for dependency and misuse. A recent cross-sectional study in England provided evidence of unnecessary prescription of dependency-forming mental health-related drugs such as antidepressants, benzodiazepines, and Z-drugs (Davies et al., 2022).

Although Z-drugs (zaleplon, zolpidem, and zopiclone are collectively known as “Z-drugs”) were originally prescribed as safe alternatives to benzodiazepines, the evidence for potential abuse and dependence of has been established (Hajak et al., 2003; Iversen, 2013; Victorri-Vigneau et al., 2014). The pharmacokinetic profiles have been reported to minimize the possibility of adverse-effects similar to those produced by benzodiazepines, such as dependence and withdrawal (“National Institute for Clinical Excellence (NICE),” 2004). However, using Z-drugs through injection or higher dose increase the risk of misuse (Voderholzer et al., 2001). Benzodiazepines are another class of primarily prescribed mental health drugs misused in the United States. Some of the common benzodiazepines are alprazolam, oxazepam, diazepam, and bromazepam. This group of drugs is the short-term choice of pharmacotherapy of anxiety and has a very high potential for misuse. Diazepam is the most prescribed substance of the benzodiazepine group (Calcaterra and Barrow, 2014).

In the United States, benzodiazepines and Z-drugs are used as anxiolytics and hypnotics and are popular among women and white adults with increasing misuse among young adults (Peng et al., 2021). In addition, ketamine was initially used as an anesthetic and sedative in the early 1960s to replace phencyclidine (Walsh et al., 2022). Recent studies and reports (Aan het Rot et al., 2012; Berman et al., 2000; Serafini et al., 2014) on its antidepressants effects have made this drug a popular drug in medicine and clinical studies. However, ketamine has been noted as a “club drug,” and it has been found to be misused in the US, UK, continental Europe, and Asia (Kalsi et al., 2011; Li et al., 2011). In the last two decades, these drugs have been found to have a compounding effect when used in combination with other recreational substances (Tardelli et al., 2022). There have been multidrug intoxications leading to death involving ketamine in the United States (Lankena and Clatts, 2005). Contrary to the United States and Europe, information on consumption of these types of substance is minimal despite the higher number of overdose deaths in Mexico.

Wastewater-based epidemiology (WBE) is a complementary technique that assesses the presence of chemical or biological agents in sewage to provide information on the overall health status of the community. WBE generates near real-time data in a specific population level, which can be used to assess benzodiazepines and other illicit drugs or illicit drugs mimicking dynamic substances like new psychoactive substances to minimize public risks (Bade et al., 2022). In 2005, it was first used as a complementary approach for monitoring cocaine use (Zuccato et al., 2005), and has expanded throughout Europe (Archer et al., 2018; Baker et al., 2014a; Baz-Lomba et al., 2016; Lindberg et al., 2005; Ort et al., 2014; Postigo et al., 2011; van Nuijs et al., 2011; Zuccato et al., 2008) Asia (Kim et al., 2015; Lai et al., 2013), and Australia and New Zealand (Kumar et al., 2019; Lai et al., 2016; Tscharke et al., 2016). The emergence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has brought remarkable attention to this technique lately as many universities, governmental and commercial ventures are using it to measure virus genomes as an early warning system and complementary method for assessing community infections (Adhikari and Halden, 2022; Daughton, 2020; Sims and Kasprzyk-Hordern, 2020).

Medicaid is a federal program that provides health coverage to low-income people in the United States (Medicaid, 2022). The Medicaid database provides information on drug utilization based on prescription volume. Nevertheless, drug misuse is not only present among people who have access to a Medicaid prescription and its consumption includes other medical programs or no prescribed consumption. This is the first study using the U.S. Medicaid drug prescription database to evaluate drug utilization trends determined by WBE before and during a pandemic event.

However, the use of WBE in the United States has seen limited use, focusing mainly on parents and metabolites of opioids and cannabinoids (Banta-Green et al., 2009; Bijlsma et al., 2020; Bishop et al., 2020a; Gerrity et al., 2011; Gushgari et al., 2019a; Heuett et al., 2015; Rushing and Burgard, 2019; Subedi and Kannan, 2014). There are very few findings reporting the incidence of mental health drugs such as benzodiazepines and antidepressants in wastewater in the United States (Bishop et al., 2020a; Montgomery et al., 2021; Ng et al., 2020; Oliveira et al., 2015; Skees et al., 2018; Stamper et al., 2017). There is only one study reporting the detection of zolpidem in the United States in hospital effluent (Oliveira et al., 2015). To the author’s knowledge, there has been no wastewater study on zaleplon in the United States and Mexico. This is also the first study to use the Medicaid data on mental health drugs utilization and compare it with WBE to cross-validate the drug consumption trend in the United States. The goal of the present study was to (i) understand the occurrence of ketamine, benzodiazepines, and Z-drugs in wastewater in the United States and Mexico, (ii) compare their uses before and during the pandemic, and (iii) assess the consumption trend
between prescription volume obtained from Medicaid data and wastewater generated data.

2. Methods and materials

2.1. Standards and reagents

All the native and labeled standards were purchased from Sigma Aldrich (Milwaukee, WI) and Cerilliant (Round Rock, TX). The target analytes in this study were ketamine, zolpidem, zaleplon, clonazepam, alprazolam, temazepam, lorazepam, diazepam and its exclusive metabolite nordiazepam. All the deuterated compounds include ketamine-d₄, zolpidem-d₄, zaleplon-d₄, clonazepam-d₄, alprazolam-d₅, lorazepam-d₄, temazepam-d₃, diazepam-d₆ and nordiazepam-d₅. All the structures and drug classes are presented in Table S1. LC-MS-grade (99 %) methanol, acetic acid, and water were obtained from Fluka (Milwaukee, WI), and LC-MS-grade acetone was purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, Mo). All the native and isotopically labeled compounds were prepared in methanol.

2.2. Wastewater samples

Both grab and composite wastewater samples were collected from ten different states (Fig. 1) from the US and 14 states from Mexico between July 2020 to October 2020. Details on the average flow data and population served are presented in Table S2. All the samples from the United States were either time proportional or flow-proportional. Due to confidentiality agreements with municipalities, the exact location of the facilities is not revealed here. However, the samples from Mexico included University colleges across Mexico and wastewater treatment plants. These wastewater samples (500 mL to 2 L) were collected in acid-washed bottles and transferred to high-density polyethylene sample bottles placed on ice in coolers and transported to the Biodesign Center for Environmental Health Engineering. After collection, the samples were immediately processed, and the resultant organic extracts were stored at −20 °C until further analysis. For sampling in Mexico, wastewater sample collection, storage and shipment procedure is previously published (Sosa-Hernández et al., 2022). Samples were shipped with express (next-day) service to the Biodesign Center for Environmental Health Engineering.

2.3. Sample processing and analysis

Wastewater samples were analyzed by following the methods described with some modifications (Gushgari et al., 2019b). First, all the raw wastewater samples were processed in duplicate and spiked with a mixture of deuterated compounds at a 5 ng/mL concentration. Then, the spiked raw wastewater influent samples (100 mL) were loaded onto Oasis HLB 150 mg solid-phase extraction (SPE) cartridges (Waters, Milford, MA), which were first conditioned with 5 mL of methanol followed by water using automated extraction (Dionex AutoTrace 280; Sunnyvale, CA, USA) at a rate of 1.5 mL/min. Next, the cartridges were washed with 5 mL of water and dried with nitrogen for 10 min. Next, the elution of analytes was performed using a 50:50 mixture of acetone and methanol with 0.5 % formic acid to 2 mL. Finally, 100 μL of the final extract was blown down under a gentle stream of nitrogen and reconstituted in 30:70 (v/v) methanol and water for further analysis.

Liquid chromatography was performed using a Shimadzu Nexera™ XR ultra-high-pressure LC (Shimadzu Corporation, Kyoto, Japan) on a short 5.0 × 5.0 mm, 4.6 μm particle size Raptor™ ARC-18 column (Restek, PA, USA) housed in an EXP® Direct Connect holder. Mass spectrometric analyses were performed using an LCMS-8060 (Shimadzu Corporation, Kyoto, Japan). A sample injection volume of 5 μL was used at an optimal flow rate of 0.5 mL/min. Water with 0.1 % formic acid was used as mobile phase A and methanol and acetonitrile (50:50) with 0.1 % formic acid as an organic modifier by volume was used as mobile phase B. Detailed gradient information is available in previously published instrumentation section (Ng et al., 2020) and Supplement information (S1).

2.4. Quality assurance/quality control

For each compound, a minimum 7-point calibration curve (0.1 ng/L to 50,000 ng/L) with a minimum coefficient of determination of r² ≥ 0.99 was constructed and concentrations were calculated using the isotope dilution method. Method blanks (deionized water) and instruments blanks (30:70 methanol and water) were included throughout the instrument run. Method detection limits were calculated following the US Environmental Protection Agency (USEPA, 2016) revised protocol as previously established (Sapowit et al., 2016b). No analyte-specific peaks were detected in the blank samples during the analysis. MDLs ranged between 6 and 139 ng/L (Table S3). MDL for diazepam was relatively higher. Precision was calculated using previously published methods using Relative Percentage

![Fig. 1. Community wastewater sampling locations (n = 70) across 10 states of the US and 14 states of Mexico with raw wastewater (n = 376) collected over four months (July–October 2020).](attachment:image)
Differences (RPD) (Driver et al., 2020) and RPDs duplicates were within 20% for all analytes, except in 5% of cases where RPDs were between 20 and 30%. Interday and intraday variation was assessed by analyzing a set of standards multiples times a day for three different days. Matrix effect was assessed by comparing the peak area of analytes spiked in post-extraction ultrapure water at two concentration levels (1 μg/L and 5 μg/L) to the analytical standard prepared in LC-grade water. There was an insignificant difference (<10%) between the peak area of analytes spiked in post extraction ultrapure water and peak area of the analytical standard of the same concentration validating the robustness of the method.

All the analytes were quantified using the isotopic dilution method, where analyte responses (native peak area) were proportioned to the responses of corresponding isotope-labeled standards. Two different ions were used for the quantification (most abundant) and identification (second most abundant) of the MRM transitions (Table S4). Other important quality control such as retention time compatibility (±0.2 min of the retention time of the standard compound under the same conditions), qualifier to quantifier ion ratio of 30% of the ion ratio of analytes in standard was necessary as mentioned in previously published method performance criteria (Kumar et al., 2022).

2.5. Calculations

2.5.1. Estimation of mass loadings and per-capita mass consumption

The mass loadings of mental health drugs of study (benzodiazepines, ketamine and Z-drugs) were calculated from the influent wastewater flow and corresponding concentration using Eq. (1):

\[ \text{Mass Load} \left( \frac{\text{mg}}{\text{day}} \right) = \text{Influent Concentration} \left( \frac{\text{ng}}{L} \right) \times \text{Influent WW Flow} \left( \frac{\text{L}}{\text{day}} \right) \times \left( \frac{1 \text{ mg}}{1,000,000 \text{ ng}} \right) \]

(1)

The estimated per-capita normalized consumption was calculated using Eq. (2):

\[ \text{Drug Consumption} \left( \frac{\text{mg}}{\text{day} \times 1000 \text{ persons}} \right) = \frac{\text{Mass Load} \left( \frac{\text{mg}}{\text{day}} \right)}{\left( \frac{1000}{\text{Population}} \right) \times \text{Correction Factor}} \]

(2)

The correction factor for each analyte studied is presented in Table S5. The correction factor was taken from the literature for most of the compounds and calculated for temazepam and diazepam.

2.6. Prescription data mining

The prescription data on alprazolam, clonazepam, diazepam, ketamine, lorazepam, nordiazepam, temazepam, zaleplon, and zolpidem were obtained from the data.medicaid.gov database for the years 2019 to 2021, which focused on their prescription volumes. Data on metformin was also extracted as a comparison chemical to see the changes in other drugs. Metformin was chosen as a baseline drug implying a drug of lifestyle with little/no suggested cases of misuse and abuse to evaluate the comparison in the pandemic. Data was collected for chemicals mentioned above and their brand-name counterparts (Table S6) using the Medicaid State Drug Utilization Database. The total number of prescriptions and Medicaid enrollee data was also extracted to calculate the number of prescriptions per 10,000 Medicaid enrollees. The pre-pandemic data was considered from the first quarter of 2019 to the first quarter of 2020, and the pandemic data was considered from the second quarter of 2020 to the first quarter of 2021. At the time of writing, second-quarter data from 2021 was not available in the database.

2.7. Statistical analysis

All the statistical tests were performed in the R software v.4.1.1. The normality of the datasets was determined using the Shapiro-Wilk test, skewness and kurtosis z-values for normality. Statistical differences were first determined by the Kruskal-Wallis rank-sum test, which ranks the data and tests for differences in the distributions of each chemical between states (wastewater study) and quarters (prescription data). When the Kruskal-Wallis rank-sum test was found to be significant (p < 0.05), the Dunn post hoc test for multiple comparisons was performed to determine significant differences between states (p < 0.05) with Bonferroni correction.

3. Results and discussion

3.1. Concentrations in USA and Mexican wastewater

All the mental health drugs (nine) were detected in the wastewater samples either in the United States or Mexico for the sampling period. The average concentration of drugs in the community wastewater in the US ranged from 6 and 248 ng/L, respectively (Table S7). Of nine analytes, six were detected in the US except alprazolam, diazepam, and its metabolite nordiazepam (Fig. 2). Ketamine was detected in seven states, including Arizona, Florida, Kentucky, New Jersey, New Mexico, Texas, and Washington. The ranking of the drugs on a concentration basis from highest to lowest (ng/L) in the US follows lorazepam (248 ± 90), temazepam (118 ± 22), ketamine (82 ± 11), clonazepam (53 ± 18), zolpidem (12 ± 2) and zaleplon (6 ± 1).

The average concentration of ketamine (ng/L) was 82 ± 11 in the US wastewater. Ketamine was detected above MDL only once in Florida and New Mexico. Zolpidem was detected (<MDL) in Alaska, Florida, New Jersey, and Washington. The highest concentration of zolpidem (67 ng/L) was detected in Arizona. To the author’s knowledge, zaleplon was detected for the first time in the USA wastewater with an average concentration of 6 ng/L. Zaleplon was detected only in Washington state out of 10 states. Zolpidem was detected in 5 states (Washington, New Jersey, Florida, Alaska, and Arizona) with single detection in New Jersey (10 ng/L) and Florida (7 ng/L). Clonazepam was detected in two states (Washington, Arizona), and lorazepam was detected in three states (Washington, Alaska, Arizona), with single detection in Alaska (42 ng/L). Temazepam was detected across all ten states (Washington, Texas, New Jersey, Kansas, New Mexico, New Jersey, Florida, Alaska, Arizona, Kentucky) with single detection in Texas (40 ng/L), Florida (188 ng/L), and Alaska (280 ng/L).

The average concentration of drugs in Mexico ranged from 8 to 490 ng/L (Table S7). All the analytes were detected in Mexico except zolpidem. The concentration (ng/L) ranking from high to low follows: diazepam (490), ketamine (166 ± 12), lorazepam (155 ± 70), nordiazepam (80 ± 30), alprazolam (56), temazepam (54 ± 10), clonazepam (36 ± 10) and zaleplon (8 ± 1). Diazepam was detected in higher concentrations (490 ng/L) in the hospital effluent, followed by ketamine (166 ng/L) and lorazepam (155 ng/L). There was only a single detection of diazepam and alprazolam in Mexico. Alprazolam is commonly prescribed benzodiazepine and its absence in the wastewater influent could be due to the low number of students in the University campuses, suggesting no misuse in the communities in this study.

The popularity of ketamine as antidepressants and drugs of abuse, as previously reported from surveys and studies, justifies the presence of ketamine in wastewater in both the US and Mexico (Lankenau et al., 2010; Milani et al., 2021). However, both ketamine and diazepam were not detected in an international study previously in the United States (Arizona) and Mexico (Monterrey) in 2019 (Ng et al., 2020). The absence of this drug in the result of the previous study could be due to the fact that the samples in Mexico for that particular study were grab samples, and this new study involved the combination of both grab and composite samples. Hence, the concentration of ketamine and diazepam could have been below the limit of detection, or their absence could be due to the sampling procedure (grab). Another study conducted between 2017 and 2018.
collecting wastewater samples from 15 different treatment plants in Mexico reported the first detection of ketamine in Mexico wastewater (Cruz-Cruz et al., 2021). The concentration profile of most drugs had higher variability due to the difference in the population catchments. There were hospitals, university campuses, residential areas, commercial areas in rural and urban areas. The single detection of diazepam in Mexico was from the hospital sample, and the study previously conducted lacked the hospital samples (Ng et al., 2020).

Previously, lorazepam, diazepam, and alprazolam had been detected in the United States at a mean concentration of 18, 6, and 26 ng/L, respectively, in New York wastewater (Subedi and Kannan, 2015). The average concentration in this study was higher for lorazepam, which could be due to the composite samples from hospitals and samples taken closer to the residential areas. Recently, ketamine (36 ng/L), temazepam (34.1 ng/L) were reported in wastewater, whereas alprazolam, diazepam, nordiazepam, and zolpidem were not detected in the Western United States (Bishop et al., 2020a).

Ketamine has been detected in many parts of the world using the WBE, including the US, UK, Czech, Mainland China, Greece, Canada, Sweden, Australia, Spain, Belgium, South Korea, Croatia, Slovakia, Malaysia, Hongkong, and Taiwan since 2008 as tabulated by Du et al. (Du et al., 2020). The concentration of ketamine across these countries varies from 4 to 1175 ng/L. The highest reported concentration was found in China in 2012 (Khan et al., 2014).

### 3.2. Estimated per capita consumption in the USA

The per capita consumption of the drugs in the US wastewater was estimated using Eq. (2) as explained in the methodology section and expressed in mg/day/1000 people. Out of nine drugs, the per capita consumption was calculated for only six drugs, including ketamine, temazepam, clonazepam, lorazepam, zaleplon, and zolpidem (Fig. 3). The estimated mass consumption was higher in Arizona for ketamine and temazepam, whereas the concentration was higher in Washington for clonazepam and lorazepam. The per capita consumption of Z-drugs (zaleplon and zolpidem) was calculated only in Washington due to the limited detection. Temazepam is a minor metabolite of diazepam (Baker et al., 2014a) and it was not considered for the excretion factor to calculate the prevalence of diazepam. Temazepam is predominately excreted as parent compound and hence it was used itself for calculating its mass loadings.

The overall average estimated consumption (mg/day/1000 people) of these drugs in the US follows the ranking from higher to lower as clonazepam (1110 ± 270), zolpidem (644 ± 280), zaleplon (332 ± 3), lorazepam (170 ± 100), ketamine (81 ± 7) and temazepam (62 ± 25). There was a single detection of clonazepam in Arizona, as mentioned previously. The average estimated consumption of ketamine (mg/day/1000 people) in Arizona was (109 ± 21), which was higher than in the other four states (Kentucky, Washington, New Jersey, Texas, and Washington). The average estimated consumption of temazepam (mg/day/1000 people) in Arizona was (62 ± 16) higher than other five states (Kansas, Kentucky, New Jersey, New Mexico, and Washington). Most of the drugs were detected in Arizona and Washington compared to other states in this study.

A recent study demonstrated that the excretion factors for ketamine were not appropriate due to the limited pharmacokinetic data, and a revised excretion factor of 20% was recommended for the consumption estimation (Du et al., 2020). This was incorporated in this study, and hence the comparison between the consumption estimates of ketamine was only
compared with the study using a 20% excretion factor. In Hanoi, consumption of ketamine was found 32 ± 12 mg/day/1000 people and 38 ± 14 mg/day/1000 people across two sites, approximately three-fold lower than in the US. In the western United States, the mass loading of the ketamine was 108 mg/day/1000 people in site A which is close to the average consumption found across five states in a recent study (Bishop et al., 2020a). However, the same study did not report the mass loadings of diazepam, nordiazepam, temazepam, and zolpidem. We were unable to obtain the population data, flow data from hospitals and some communities in Mexico, and hence the estimated mass loadings of those drugs have not been presented in this study.

3.3. Pre-pandemic vs pandemic

Eight wastewater samples from the archive from the Human Health Observatory (HHO) from the Arizona State University Biodesign Center for Environmental Health Engineering were run as the baseline before the pandemic. These eight samples included wastewater samples from Arizona, Illinois, Kentucky, and Mexico in between 2015 and 2019. Wastewater samples from Mexico was a grab samples whereas, other samples were composite flow or time-weighted composite samples. Ketamine and lorazepam were only detected in those wastewater samples before and after the pandemic. Alprazolam was detected at around 60 ng/L in wastewater in Arizona in a Southwestern University before the pandemic (Gushgari et al., 2018), however it was not detected in Arizona during the pandemic in the same location. Alprazolam is a popular drug among students (Reines et al., 2020), and the lockdown and pandemic in the Southwestern University community could have resulted in no detection of alprazolam in the wastewater.

3.4. Prescription data

Most of the prescription volume for traditional benzodiazepines (alprazolam, clonazepam, diazepam, lorazepam and temazepam) and Z-drugs (zaleplon and zolpidem) decreased from first quarter (2019) to first quarter (2021) and was statistically significant (p < 0.05) for alprazolam, clonazepam, and lorazepam. When these substances were found to be changing during the pandemic, a Dunn-test was performed to compare each quarter. During the second quarter of 2020, when WHO announced the pandemic, the prescription volume decreased (p < 0.05) by 11% per 10,000 Medicaid enrollees. There was a 17% decline in the prescription volume of lorazepam during the second quarter of 2020 (p < 0.05) per 10,000 Medicaid enrollees (Fig. S1). The decrease in the lorazepam prescription is due to the shortage of drugs mentioned by the Food and Drug Administration (FDA) (Burry et al., 2020). There was reporting of a drug shortage of clonazepam and alprazolam during the pandemic, which could be due to manufacturing and quality problems and unavailability of raw materials and logistic issues, which was the case for other classes of benzodiazepines (Shukar et al., 2021).

3.5. Study limitations

WBE is a promising technique to understand the drug consumption profile and the behavior of community health. However, there are some shortcomings associated with this technique, including due to unaccounted uncertainties in sampling, in sewer degradation of the drugs and their transformation, stability of these drugs in storage after sample processing, and variable excretion factor reported in the literature. The relationship among these variables drives the fingerprint of the chemical analyses leading to the obtained result. Preanalytical measurement error may occur during in-pipe and holding times. The samples travel time from Mexico to the United States at the Arizona State University took 3 days maximum. Most of the benzodiazepines, ketamine and Z-drugs are found to be stable in wastewater. Some previous works has shown that no acid preservation during the sample preparation show higher stability among benzodiazepines like diazepam, nordiazepam, alprazolam, temazepam, lorazepam and clonazepam (Bade et al., 2020; Baker and Kasprzyk-Hordern, 2011; Jelic et al., 2015; Racamonde et al., 2014). Ketamine, metformin, zaleplon and zolpidem are also relatively stable compounds in wastewater and have <20% transformation in the sewer as demonstrated by various studies in the literature.
This study did not account for adsorption of these compounds to sewer biofilms and may impact the per capita loadings reported in this study. In this study, population estimates were obtained from the census for calculating the per-capita mass loadings in the United States, which could have resulted in an over- or under-estimation of drug consumption rates, depending on the quality of data and the possibility of population estimates being outdated.

Flow proportional composite sampling approach is superior compared to other approaches. However, due to the limitation of the resources, grab sampling approach was used in Mexico and is one of the main limitations of this study.

However, in low-to-middle-income countries (LMICs), access to autosamplers is rare due to financial limitations. However, lack of resources should not limit sampling in these counties. Grab sample data are useful to evaluate the presence of mental health drugs, and this is the first study in Mexico to evaluate benzodiazepines and Z-drugs in educational institutions. Flow data and other necessary parameters were not available for the grab samples and hence an uncertainty analysis could not be performed. Mass loadings could not be computed due to the availability of a limited amount of grab samples only and complete absence of reliable flow data. Thus, only the concentration of these drugs is reported in this study for the Mexico samples to provide information for further studies.

Passive samplers are another sampling approach used in the wastewater studies. Passive autosamplers, like the polar organic chemical integrative samplers (POCIS) are used for illicit drugs and one drug from this study (ketamine). However, even this technique of passive samplers does have three-fold difference in the concentration as reported by recent study (Bishop et al., 2020b). An extensive review on passive samplers has been recently published (Hahn et al., 2021). A spectrum of alternative sampling technologies exist to address the limitations inherent to grab sampling (e.g., Jonsson et al., 2019; Supowit et al., 2016a) and these could be applied in future WBE studies in settings including developing countries. Only ketamine has been detected using POCIS or diffusive gradients in thin-films (DGT) in the Europe and Australia. In the United States, a recent study used POCIS for ketamine, alprazolam, diazepam, nordiazepam and temazepam (Bishop et al., 2020b). However, only a comparison with temazepam between 24 h composite sample was available in that study. A recent study (Zhi et al., 2020) on wastewater effluent dominated stream looked at the occurrence and spatiotemporal of pharmaceuticals including diazepam, nordiazepam, alprazolam, lorazepam, metformin and temazepam. For quantitative studies, most common passive samplers like POCIS suffer from some limitations, mainly relating to the reliability of derived estimations of the sampling rates (Rs) from experimental measurements, as well the lack of a well-developed performance reference compound (PRC) exposure correction method and this depends on site to site requiring multisite calibration. A grab sample may also be sufficient if the occurrence of a compound is to be assessed qualitatively (Ort and Lawrence, 2010) without further getting into mass loadings or per capita as performed in this study.

Pharmacokinetics is an important part of the WBE study, and there are various metabolic pathways and different other benzodiazepines could be excreted as mentioned above. Nordiazepam is a very minor metabolite of diazepam (Baker et al., 2014b) and later transformed to oxazepam (which may come from temazepam). Nordiazepam can come from chlorodiazepoxide and clorazepate, but the excretion rate is as high as 19 % for nordiazepam, which is further metabolized by hydrolysis (FDA, 2010). Temazepam, however is predominately excreted as a parent compound and included in this study. Because of the complex metabolism and transformation of benzodiazepines, parent benzodiazepines with the correction factor were used in this study. Some common benzodiazepines such as temazepam and nordiazepam as a minor metabolite (<5 %) of diazepam were considered for parent compound analysis, whereas 6 other compounds do not have any cross metabolism. In addition, most of the transformation are <16 %, and are acceptable in analytical chemistry and stability experiments (McCall et al., 2016). In addition, the Medicaid database provides data only for low-income populations, which may result in potential bias when extrapolating drug usage rates to the general population.

4. Conclusions

This study measured ketamine concentration in wastewater in the United States, which reflected some of the highest ketamine consumption rates presented in the literature in the United States using WBE. This study shows the first detection of zaleplon and zolpidem in wastewater from the U.S. and Mexico during the pandemic. There was a decrease in the prescription volume of lorazepam (p < 0.05) in the Medicaid database. In contrast, an increasing mass loading per capita of lorazepam was found in wastewater, suggesting the potential increase either in the misuse or in the legal use of lorazepam among patients through private insurance and populations not covered through Medicaid program. The present study highlights the need for monitoring substance use during high-stress events, such as the COVID-19 pandemic, and illustrates the utility of WBE to provide data that cannot be obtained by other means to determine actual drug use, which is the sum of both licit and illicit drug sourcing and consumption.

CRediT authorship contribution statement

Sangeet Adhikari: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Data curation, Writing – original draft, Writing – review & editing. Rahul Kumar: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – review & editing. Erin M. Driver: Resources, Project administration, Investigation, Writing – review & editing. Devin A. Bowes: Resources, Investigation, Writing - review & editing. Keng Tiong Ng: Conceptualization, Project administration, Writing – review & editing. Elda M. Melchor-Martinez: Writing – review & editing. Manuel Martinez-Ruiz: Writing – review & editing. Karina G. Coronado-Apodaca: Writing – review & editing. Ted Smith: Resources, Writing – review & editing. Aruni Bhattacharjee: Resources, Writing – review & editing. Brian J. Piper: Formal analysis, Resources, Writing – review & editing. Kenneth L. McCall: Formal analysis, Resources, Writing – review & editing. Roberto Parra-Saldívar: Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing. Leon P. Barron: Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing. Rolf U. Halden: Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

R.U.H. and E.M.D. are cofounders of AquaVitas, an Arizona State University startup company providing commercial services in wastewater-based epidemiology. R.U.H. also is the founder of OneWaterOneHealth, a nonprofit project of the Arizona State University Foundation. B.J. Piper is part of an osteoarthritis research team supported by Pfizer and Eli Lilly.

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