Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

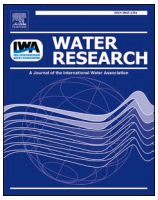
Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Water Research 238 (2023) 120023

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres



Stability and WBE biomarkers possibility of 17 antiviral drugs in sewage and gravity sewers

Jiaqi Wen [a,b], Lei Duan [a,b], Bin Wang [a,b], Qian Dong [a], Yanchen Liu [a], Jun Huang [a,b], Gang Yu [a,b,c,*]

[a] State Key Joint Laboratory of Environmental Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China
[b] Beijing Key Laboratory for Emerging Organic Contaminants Control, Beijing Laboratory for Environmental Frontier Technologies, China
[c] Advanced Interdisciplinary Institute of Environment and Ecology, Beijing Normal University at Zhuhai, 519087, China

ARTICLE INFO

Keywords:
Antiviral drugs
Stability
Sewer sediments
Wastewater-based epidemiology
Flow velocity

ABSTRACT

Wastewater-based epidemiology (WBE) is a promising technique for monitoring the rapidly increasing use of antiviral drugs during the COVID-19 pandemic. It is essential to evaluate the in-sewer stability of antiviral drugs in order to determine appropriate biomarkers. This study developed an analytical method for quantification of 17 typical antiviral drugs, and investigated the stability of target compounds in sewer through 4 laboratory-scale gravity sewer reactors. Nine antiviral drugs (lamivudine, acyclovir, amantadine, favipiravir, nevirapine, oseltamivir, ganciclovir, emtricitabine and telbivudine) were observed to be stable and recommended as appropriate biomarkers for WBE. As for the other 8 unstable drugs (abacavir, arbidol, ribavirin, zidovudine, ritonavir, lopinavir, remdesivir and efavirenz), their attenuation was driven by adsorption, biodegradation and diffusion. Moreover, reaction kinetics revealed that the effects of sediments and biofilms were regarded to be independent in gravity sewers, and the rate constants of removal by biofilms was directly proportional to the ratio of surface area against wastewater volume. The study highlighted the potential importance of flow velocity for compound stability, since an increased flow velocity significantly accelerated the removal of unstable biomarkers. In addition, a framework for graded evaluation of biomarker stability was proposed to provide reference for researchers to select suitable WBE biomarkers. Compared with current classification method, this framework considered the influences of residence time and different removal mechanisms, which additionally screened four antiviral drugs as viable WBE biomarkers. This is the first study to report the stability of antiviral drugs in gravity sewers.

1. Introduction

Antiviral drugs are a category of pharmaceuticals used to treat viral infections, such as influenza, hepatitis, herpes, and acquired immunodeficiency syndrome (AIDs) (Clercq, 2007; Yao et al., 2021). It was estimated that over 240 million people worldwide were affected by chronic hepatitis B virus infection (Schweitzer et al., 2015), and China has a 6.1% weighted prevalence of hepatitis B surface antigen (HBsAg) adjusted for people (Razavi-Shearer et al., 2018). More than 36 million people worldwide are living with HIV infection (Pandey and Galvani, 2019). Ncube et al. (2018) estimated that about 21.78 tons of antiviral drugs were consumed daily around the world. Meanwhile, with the prolonged global pandemic of COVID-19, a large number of anti-influenza drugs have been used continuously in clinical treatment (Kuroda et al., 2021). The preference of purchasing medicines online or at the pharmacy rather than from hospitals also makes the estimation of consumption of antiviral drugs from prescription data or hospital pharmacy sales more inaccurate (Hu et al., 2022). Therefore, it is extremely important to systematically monitor the consumption information of antiviral drugs in the ongoing COVID-19 pandemic.

Wastewater-based epidemiology (WBE) is an objective, timely and convenient monitoring tool and is developed to back-estimate the regional consumption of illicit drugs and pharmaceuticals by analyzing human-excreted compounds in municipal wastewater influent (Daughton, 2001a, b, 2018). WBE has been successfully used to estimate illicit drug abuse (Lancaster et al., 2019; Thomas et al., 2012; Zheng et al., 2021), the consumption of alcohol and tobacco (Reid et al., 2011; Wang et al., 2021; Zheng et al., 2020), the usage of common pharmaceuticals

* Corresponding author.
E-mail address: gangyu@bnu.edu.cn (G. Yu).

https://doi.org/10.1016/j.watres.2023.120023
Received 20 December 2022; Received in revised form 31 March 2023; Accepted 28 April 2023
Available online 30 April 2023
0043-1354/© 2023 Elsevier Ltd. All rights reserved.

Table 1
Pretreatment groups of sewage stability batch test.

Pretreatment	Corresponding processes
Filtration+Sterilization	Hydrolysis
Sterilization	Hydrolysis + Adsorption by SS
Filtration	Hydrolysis + Biodegradation
Filtration+Acidized	Altered Hydrolysis + Biodegradation
Raw sewage	Hydrolysis + Adsorption by SS + Biodegradation
Filtration+LowT(4℃)	In-sample Stability
Ultrapure Water	Blank Control

(Duan et al., 2022; Gao et al., 2016; Tomsone et al., 2022; Xu et al., 2022a) and the prevalence of hepatitis B (Hou et al., 2020). Currently, WBE is also being developed to apply to monitor SARS-CoV-2 (Acosta et al., 2022; Li et al., 2022; Medema et al., 2020; Xu et al., 2022b). At present, the potential of antiviral drugs in WBE estimation needs to be further explored, which requires the support of biomarker stability results in sewers.

As WBE relies on the quantification of specific biomarkers in wastewater, it requires biomarkers to remain stable or undergo predictable removal in sewers (Shimko et al., 2022). If the concentration of unstable biomarkers in the influent of wastewater treatment plants is directly used, the drug consumption will be significantly underestimated. In the urban sewer system, gravity sewer pipes are the main component (Hvitved-Jacobsen et al., 2013). According to the structural and hydraulic conditions in pipes and the existence of suspended solid in wastewater, sediments and biofilms are widespread in gravity sewer pipes, which can cause significant drug removal (Cheng et al., 2022; Hvitved-Jacobsen et al., 2013). Many studies focused on the effect of biofilms on various biomarkers (Ahmed et al., 2021; He et al., 2021; O'Brien et al., 2019). Li et al. (2020) firstly reported the stability of illicit drugs and pharmaceuticals in sewer sediments and proposed a rate constant to describe the transformation processes. Up to now, only a few pilot-scale simulations (Gao et al., 2019; Li et al., 2019) focused on the effects of sediments on biomarkers in gravity sewers. In addition, the sewage flow velocity, another vital operation parameter in gravity sewers, varies considerably in different pipeline conditions or at different seasons (Carrera et al., 2015). Increased flow velocities can reduce the thickness of diffusive boundary layer at the sediment surface by directly changing the shear stress, thereby affecting the mass transfer of biomarkers in sewers. Under this circumstance, the stability of biomarkers might be significantly altered. However, to our best knowledge, no studies have focused on the effect of flow velocity on the biomarker stability.

In this study, we utilized four laboratory gravity sewer reactors to explore the influence of sediments, biofilms and flow velocity on antiviral drugs. The aims of this study include: i) examine the stability of 17 typical antiviral drugs in raw sewage and realistic gravity sewer conditions; ii) verify the rationality of current kinetic model describing biomarkers removal in sewer; iii) explore the effect of sewage flow velocity on stability of biomarkers preliminarily. Additionally, a biomarker stability assessment framework considering residence time and removal mechanism was presented to help researchers screening for suitable biomarkers. These findings would improve the understanding of biomarkers removal processes in gravity sewers, thereby reducing uncertainties in back estimation of antiviral drugs in future WBE studies.

2. Materials and methods

2.1. Chemicals and reagents

The information and properties of 17 target antiviral drugs and 3 other compounds used in this study are listed in Table S1 in the Supplementary Material. The standards of the 20 compounds were purchased from First Standard (China). The isotope-labeled internal standards of acyclovir-d_4, ribavirin-$^{13}C_5$, amantadine-d_{15} and zidovudine-$^{13}C,d_3$ were also acquired from First Standard (China). Ritonavir-d_6 and lamivudine-$^{13}C_1,d_2$ were obtained from Toronto Research Chemicals (Canada). Methanol and formic acid of HPLC grade were purchased from Fisher Scientific (USA) and Sigma-Aldrich (USA), respectively.

2.2. Stability of antiviral drugs in sewage

Raw sewage was collected from a municipal sewer on the campus of Tsinghua University (Beijing, China), containing 237–348 mg of chemical oxygen demand (COD)/L, 34–44 mg of NH_3-N/L, 0.42–0.51 g of suspended solid (SS)/L, 1.7–4.9 mg of H_2S-S/L. As shown in Table 1, six different pretreatment groups were designed to investigate the contribution of different processes and in-sample stability at a low temperature (4℃). Sterilization was carried out at a temperature of 121℃ and a pressure of 0.12 MPa for 20 min (Lin et al., 2010; Liu et al., 2022a) and filtration was performed with 0.45 μm glass fiber filters (Whatman, UK).

Except for lamivudine (~1 μg/L), raw sewage didn't contain other target antiviral drugs (Table S6). In each reactor, we added 60μL 2 mg/L mixed standard solution into 40 ml sewage, resulting in an initial concentration of 3 μg/L for each drug. Every pretreatment group contained three parallel reactors. Except for the low temperature group, all groups were incubated at 25℃ and stirred at 160 rpm for 24 h in dark. Sewage samples were collected at multiple time points (0, 0.5, 1, 2, 4, 8, 12 and 24 h), filtered, spiked with internal standards (20 μg/L) and then analyzed by direct injection.

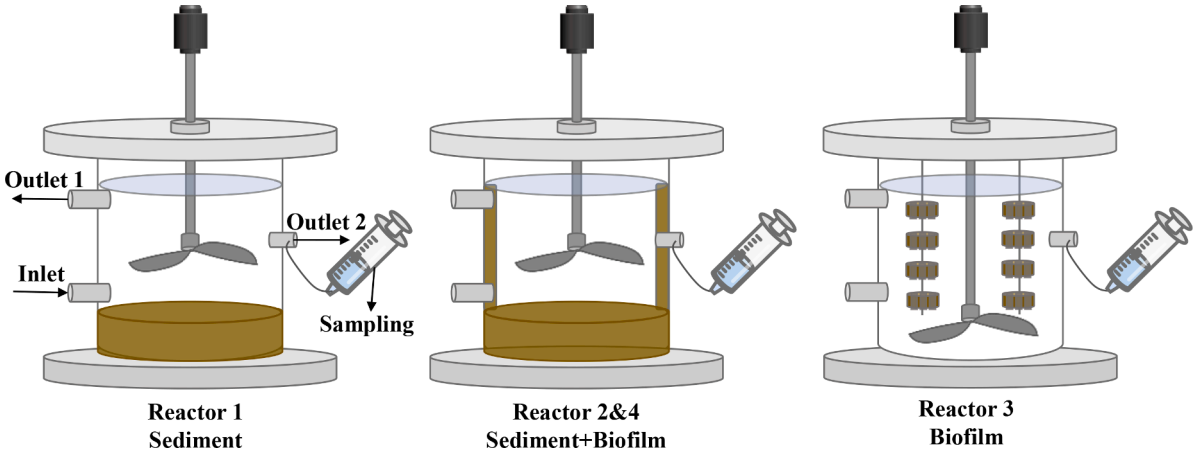


Fig. 1. Structures of gravity sewer reactors used in this study.

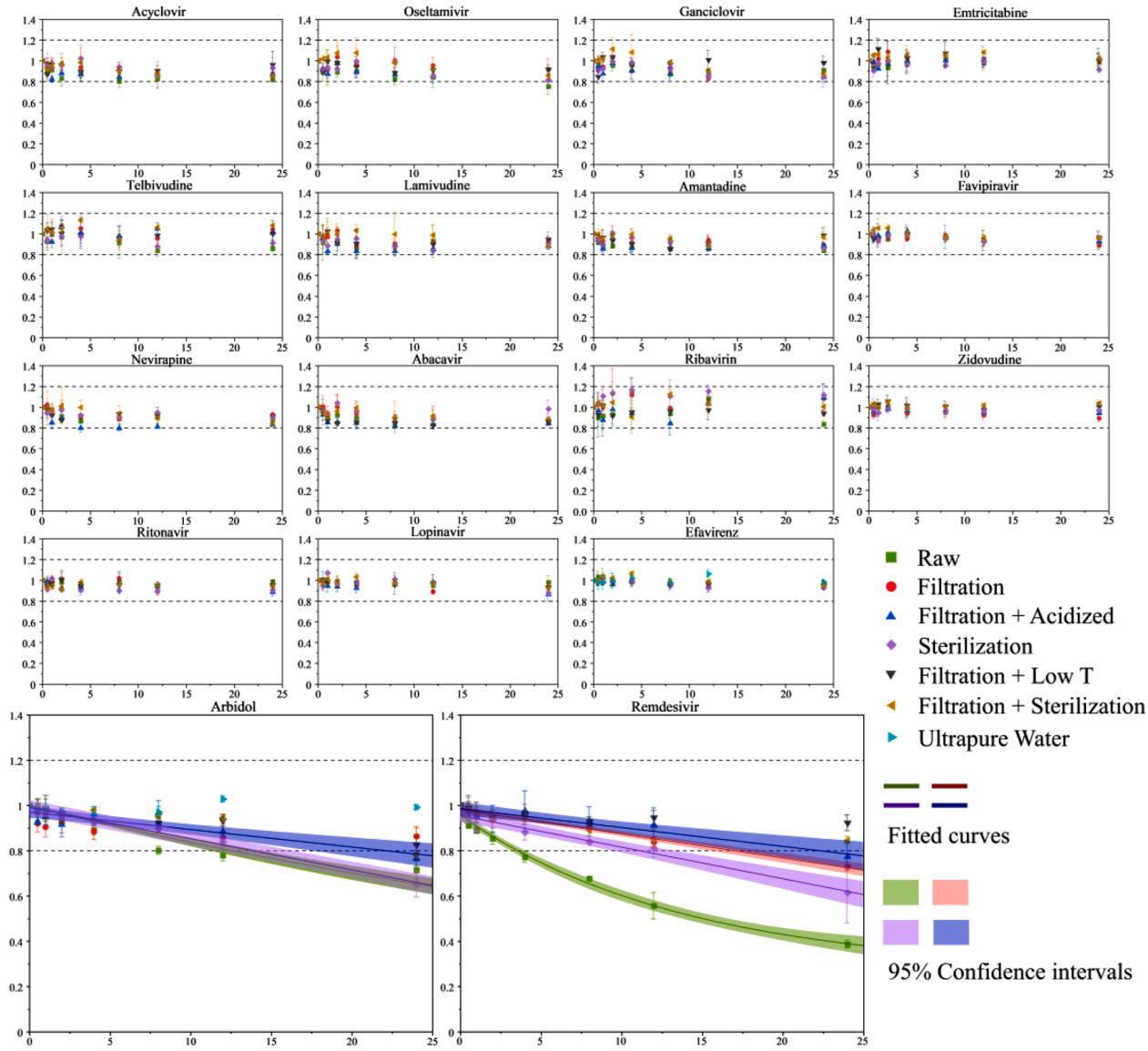


Fig. 2. Variations of 17 antiviral drugs under different sewage pretreatment conditions. (X-axis: time (hours) after spiking; Y-axis: ratio of concentration compared to $t = 0$).

2.3. Laboratory gravity sewer reactor

According to previous studies (Li et al., 2020, 2018), we employed four gravity sewer reactors (Fig. 1) to investigate the stability of antiviral drugs in gravity sewers. The laboratory reactors had an effective volume of 1.8 L (14 cm in diameter and 12 cm in depth) (Zuo et al., 2021). Sediment samples with an initial depth of approximately 5 cm were added into reactor 1 and reactor 2. The biofilm systems in reactor 2 and 3 were established on inner wall and surface of carriers, respectively, by sewage cultivation. The resulting biofilm-area to wastewater-volume (A/V) ratio was 28.6 m^2/m^3 in reactor 2 and 55 m^2/m^3 in reactor 3. In addition, reactor 4 was a replica of reactor 2.

The reactors were fed with the same fresh sewage as Section 2.2 via the inlet on the sidewall by a peristaltic pump and the effluent was drained through the outlet 1. The average wastewater retention time was 12 h. Reactor 1, 2 and 3 were operated at a stirring speed of 24 rpm while reactor 4 was operated at 54 rpm, resulting in shear velocities of 0.07 and 0.17 m/s, respectively. The detailed conversion equations were obtained from previous studies and were listed in the SI, Section 1 (Liu et al., 2016; Zuo et al., 2021). All four reactors have been in stable operation for over 300 days. During the whole operation, the biofilms attached on inner walls of reactor 1,3 and stirrer were removed each week.

2.4. Batch test to assess stability of antiviral drugs in gravity sewer reactors

A batch test was designed to explore the stability of antiviral drugs in gravity sewers. Before each test, all reactors were drained completely and extra bioactive parts, such as biofilm attached on stirrer, were removed. Raw sewage was collected to determine the background concentration. Then, mixed stock solution was added into sewage to obtain a spiked concentration of at least 3 μg/L. Rhodamine B was added together to indicate the adsorption of unbiodegradable hydrophobic compounds. The mixed sewage was slowly poured into reactors, ensuring that biofilms and sediments were undisturbed. After that the stirrers were turned on. The whole experiment was conducted under dark conditions. Each test was conducted in triplicate. During the experimental period, liquid samples were taken at 0, 0.5, 1, 2, 4, 8, 12, 24 h. Two artificial sweeteners (acesulfame and sucralose) existing in raw sewage were measured together as reference of variation of stable compounds. The concentrations of sulfate and sulfide in reactor 2 and 3

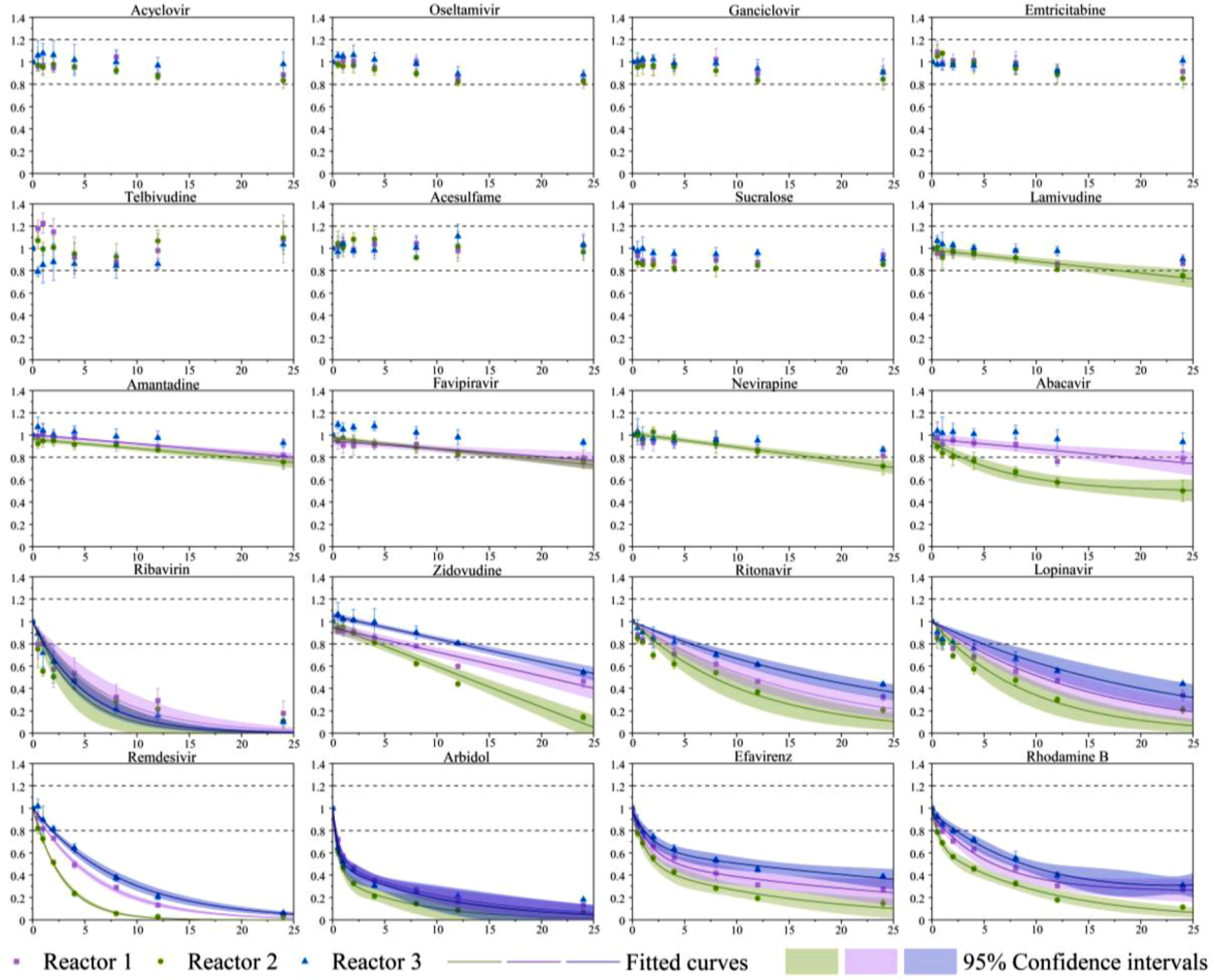


Fig. 3. Regression results for variation of investigated antiviral drugs in sediment (R1), sediment + biofilm (R2) and biofilm (R3) sewer reactors. (X-axis: time (hours) after spiking; Y-axis: ratio of concentration compared to $t = 0$).

were determined in the first 12 h. The analytical methods of sewage parameters are listed in SI, Section 2.

2.5. Analytical methods

2.5.1. Pretreatment and analytical method of target compounds

In each sampling, 400μl liquid sample was added to a vial containing 100μl methanol. After spiking 10 ng isotope internal standard, the mixed solution was filtered by 0.22 μm PTFE microporous membrane. 10μL of filtered sample was injected for analysis using a UPLC system (Shimadzu, Japan) coupled to a tandem mass spectrometer (Sciex 5500 QTrap, America). The chromatographic separation was performed on a Kinetex 2.6 μm Biphenyl 100 × 3.0 mm column (Phenomenex, America) at 40°C and the flow rate was 0.4 mL/min. The mobile phase gradient, mass spectrometry parameters and QA/QC information are listed in the SI (Table S3, S4, S5). Data was quantified by MultiQuant 3.0.3. A ten-point calibration curve was used (0.05–50 μg/L) for quantification. For every 15 samples, a calibration point, a spiked sewage sample and a method blank were analyzed to confirm instrument status and method performance.

2.5.2. Analytical method of microbial community of sediments

Microbial DNA was extracted from the samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). The DNA concentration and purity were determined with NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). The V4 region of the bacterial 16S rRNA gene were amplified with primer pairs 515F (5′-GTGCCAGCMGCCGCGG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The detailed analysis methods are described in the SI Section 3.

2.6. Statistical analysis

At time 0, the concentrations of each biomarker were used as the initial concentration (C_0). Zero-order kinetics, first-order kinetics and exponential two-phase kinetics were applied to fit the degradation of biomarkers (SI Section 4) and concentrations below LOQ were excluded. If correlation value (R^2) of zero or first order kinetics was over 0.8, the model with a higher R^2 would be used. The two-phase kinetics was selected only if it was the only adequate model (R^2>0.8). Attenuation of certain biomarker in different sewer reactors was fitted using the same model to generate comparable data. The straight line in Fig. 4 was fitted by the least squares method based on relative residuals (Hendricks, 1931). More details are shown in the SI section 5. Statistical analysis and data visualization were implemented by Origin 2021 and R version 4.2.1.

3. Results and discussion

3.1. Stability of antiviral drugs in sewage

This study firstly investigated the stability of target antiviral drugs in raw sewage. The loss of nonvolatile drugs in sewage can be attributed to the combination of hydrolysis, adsorption and biodegradation (Liu et al., 2022a). The 24-hour concentration changes of 17 antiviral drugs under different pretreatments are shown in Fig. 2. Considering the influence of matrix effect, measurement error and concentration range of two stable sweeteners (section 3.2.3), we referred to the criteria adopted by (Ahmed et al., 2021) for selecting stable biomarkers:

a **Highly stable biomarkers:** 0–20% removal in aqueous phase within 24 h;
b **Moderately stable biomarkers:** 20–50% removal within 24 h;
c **Unstable biomarkers:** >50% removal within 24 h;

The results showed that, except for arbidol (umifenovir) and remdesivir, most antiviral drugs were highly stable in sewage within 24 h. For arbidol, apparent removal was found in both raw and sterilized sewage but it was suppressed by filtration, indicating that its removal was mainly due to particle adsorption. A high log K_{ow} value (Table S1) made it more inclined to be adsorbed by organic matter. Ul'yanovskii et al. (2022) also found the accumulation of arbidol and its transformation products in biological sludge at wastewater treatment plants and bottom sediments of reservoirs. As for remdesivir, both sterilization and filtration lessened its attenuation. The sum of reduction percentages of the two pretreatment groups well explained the variation in raw sewage and acidification had no extra effect compared to filtration, indicating that remdesivir may be simultaneously adsorbed and biodegraded in raw sewage. It was reported that intravenous remdesivir will be rapidly hydrolyzed to its metabolite by liver esterases (Zhang et al., 2022). Although no study has reported the biodegradation process of remdesivir in wastewater, we speculated that its biotransformation may be attributed to the potential esterase activity present in sewage (Fischer et al., 2013).

3.2. Stability of antiviral drugs in gravity sewer reactors

3.2.1. Physicochemical and biological properties of sewer sediments and biofilms

The bulk density of sediment was 1.45 g/cm^3. The total solids (TS) and volatile solids (VS) contents were 69.3% and 3.1% (wet weight), respectively, which were comparable to previous studies (Liu et al., 2015; Zuo et al., 2021). The wastewater pH in all reactors ranged at 7.2–7.7 during the batch test. Furthermore, the changes of sulfur species were commonly used to indicate biological activity in sewers (Zuo et al., 2020a). The sulfate concentration changes in reactor 2 and 3 were shown in Figure S1. The linear regression results of the first 4 h showed that the maximum sulfate reduction rates of reactor 2 and 3 were 2.24 mgS/L/h and 2.40 mgS/L/h, respectively. The results were comparable with previous results of sediments and biofilms in laboratory gravity sewer (Ahmed et al., 2021; He et al., 2021; Li et al., 2020; Liu et al., 2016). Because of the presence of overhead air, sulfide concentration in solution exhibited weak linearity.

Additionally, to ensure representation and consistency of sediments across reactors, we analyzed the microbial community of surface sediments in reactor 1 and 2 with two biological replicates. The dominant phyla in reactor sediments were *Proteobacteria, Halobacterota, Firmicutes, Chloroflexi* and *Bacteroidota* (Figure S2), which were similar to sewer sediments in previous studies (Shi et al., 2018; Zuo et al., 2021, 2020b). Combined with the genus-level structure (Figure S3), it further illustrated the similarity of sediments between two reactors and the presence of methanogens and sulfate-reducing bacteria (Mohanakrishnan et al., 2009). Overall, the gravity sewer reactors mimicked a representative condition of real sewers.

3.2.2. Effects of sediments and biofilms on antiviral drugs

In this section, we conducted simulations to observe the variation of antiviral drugs in three gravity sewer reactors with sediments and biofilms. The results showed that investigated compounds exhibited various removal patterns in reactor 1, 2 and 3 (Fig. 3). In 24 h, 5 antiviral drugs (acyclovir, oseltamivir, ganciclovir, emtricitabine, telbivudine) and 2 sweeteners (acesulfame and sucralose) were highly stable (<20% removal). Five drugs (lamivudine, amantadine, favipiravir, nevirapine and abacavir) were classified as moderately stable. Among them, lamivudine, amantadine, favipiravir and nevirapine were stable in the first 12 h, but followed by continuous decreases with an overall removal of 24–28% by 24 h in reactor 2. Considering that the median sewer retention time was estimated to be much less than 12 h (3.3 h) (Kapo et al., 2017), these four antiviral drugs can be considered stable in gravity sewer in the back estimation of WBE. The other eight compounds, including ribavirin, zidovudine, ritonavir, arbidol, lopinavir, remdesivir, efavirenz and rhodamine B, experienced significant concentration decreases (>50%) in gravity sewer reactors and were identified as unstable biomarkers.

Most unstable biomarkers, except for ribavirin and zidovudine, have relatively high log K_{ow} values (Table S1), which made them more inclined to be absorbed by sludge or sediments (Nannou et al., 2020). Moreover, the variations of these antivirals, such as efavirenz, lopinavir and arbidol, in three reactors were highly consistent with the adsorption-controlled rhodamine B. This illustrated both the relevant effects of adsorption and the indicative role of rhodamine B for hydrophobic biomarkers. In all reactors, the concentration variation of rhodamine B followed a two-phase kinetic model. At the beginning, the first rapid phase may represent the diffusion process of biomarkers from the water phase to sediment or biofilm surfaces. Because of the concentration gradient between water and sediment phases, the spiked compounds diffused into sediments and were diluted by mixing with pore water in the porous sediments (Kunkel and Radke, 2008; Li et al., 2020, 2015). Subsequently, adsorption played a major role in the second phase.

On the other hand, the hydrophilic unstable antivirals, including abacavir, zidovudine and ribavirin, were probably dominated by biodegradation. Compared to other studies, the half-life of abacavir in reactor 2 (24 h) fell between the values observed in wetland water (80 h) and activated sludge (0.44 h), while the half-life of zidovudine in the three reactors (12–25 h) was shorter than that observed in activated sludge (54.4 h) (Funke et al., 2016; Prasse et al., 2015). So far, no study has measured the biodegradation rate of ribavirin, but its removal rate in this study was higher than that observed in moving bed biofilm reactor and the anoxic-anaerobic-anoxic-oxic process (Liu et al., 2022b). These differences to some extent reflect the uncertainty of laboratory simulations. In future, pilot or field scale studies (Li et al., 2018) and calibration studies based on environmental monitoring and prescription data (Kannan et al., 2023) are needed to validate the stability results and provide more reliable correction factors for WBE estimation of unstable biomarkers.

3.3. Verification of the kinetic model of WBE biomarkers removal in sewer

A kinetic model dividing the concentration decreases of biomarkers into abiotic and biotic processes has been widely used to describe the concentration changes of biomarkers with time in the sewer reactors (Li et al., 2020, 2019, 2018; Li et al., 2021; McCall et al., 2016; Ramin et al., 2018). The widely used first-order and zero-order kinetic equations can be respectively described as follows:

$$C = C_0 \cdot e^{-k_{total} \cdot t} = C_0 \cdot e^{-(k_{ww}+k_{sd}+k_{bio}) \cdot t} = C_0 \cdot e^{-\left(k_{ww}+k_{sd}+k_{bio}^{'} \cdot \frac{A}{V}\right) \cdot t} \quad (1)$$

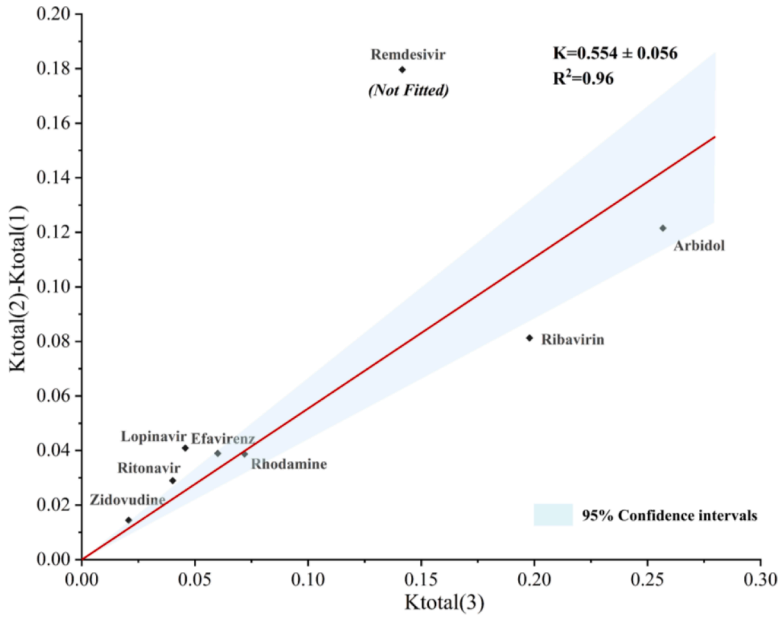


Fig. 4. Cross-origin linear regression results of $k_{reactor2}$−$k_{reactor1}$ and $k_{reactor3}$.

$$C = -\left(k_{ww,0} + k_{sd,\,0} + k_{bio,0}\right)\cdot t + C_0 = -\left(k_{ww,0} + k_{sd,\,0} + k'_{bio,0}\cdot\frac{A}{V}\right)\cdot t + C_0 \tag{2}$$

Where k_{total} (h^{-1}) represents the total removal rate in aqueous phase of gravity sewer reactor. k_{ww} (h^{-1}) is used to describe the conversion of biomarkers in raw sewage. k_{sd} (h^{-1}) indicates the effect of sediments. k_{bio} (h^{-1}) and k'_{bio} ($m\cdot h^{-1}$) stand for the biotransformation by biofilms without or with the normalization with respect to the A/V ratio (m^{-1}), respectively. $k_{ww,0}$, $k_{sd,\,0}$, $k_{bio,0}$ and $k'_{bio,0}$ represent the corresponding parameters in zero-order kinetics. According to the kinetic model, the removal of biomarkers in three reactors of this study can be described as eq (3)-(5) separately:

$$k^{\,Total}_{\,Reactor-1} = k_{ww} + k_{sd} \tag{3}$$

$$k^{\,Total}_{\,Reactor-2} = k_{ww} + k_{sd} + k'_{bio}\cdot\left(\frac{A}{V}\right)_{Reactor-2} \tag{4}$$

$$k^{\,Total}_{\,Reactor-3} = k_{ww} + k'_{bio}\cdot\left(\frac{A}{V}\right)_{Reactor-3} \tag{5}$$

The rate difference between reactor 1 and 2 is caused by the sewer biofilm (3)-(4):

$$k^{\,Total}_{\,Reactor-2} - k^{\,Total}_{\,Reactor-1} = k'_{bio}\cdot\left(\frac{A}{V}\right)_{Reactor-2} \tag{6}$$

For stable or moderately stable biomarkers in raw sewage, k_{ww} is small or equal to 0. Then, the rate constants of three reactors will satisfy a linear relationship:

$$k^{\,Total}_{\,Reactor-2} - k^{\,Total}_{\,Reactor-1} = \mathbf{K}\cdot k^{\,Total}_{\,Reactor-3} \tag{7}$$

Where the constant **K** is the ratio of the A/V ratio of reactor 2 and reactor 3:

$$\mathbf{K} = \left(\frac{A}{V}\right)_{Reactor-2} \Big/ \left(\frac{A}{V}\right)_{Reactor-3} \tag{8}$$

To verify the rationality of kinetic model, the rate constants of 7 unstable antiviral drugs and rhodamine B in sewer reactors were fitted according to the relationship of Eq. (6) (Fig. 4). A least squares method based on relative residuals was used to average the contributions of each point. For compounds merely fitting the two-phase kinetic model, such as rhodamine B, an adjusted first-order kinetics was used to make rate constants comparable (SI Seaction2). All relevant data used in fitting are listed in Table 2. The rate constants of other curves in Fig. 3 can be found in Table S7.

As shown in Fig. 4, except for remdesivir, all compounds passed a cross-origin straight line with a high correlation (R^2=0.96). The slope value ($K = 0.554\pm0.056$) was basically consistent with the actual A/V ratio of the two reactors ($K_{theoretic}$=0.52), indicating that the kinetic model is suitable for gravity sewer reactors containing both sediments and biofilms. However, remdesivir was the only unstable biomarker in raw sewage (>50% attenuation), and even if the influence of k_{ww} was considered, its rate constant still cannot satisfy the linearity. As discussed in Section 3.1, the removal of remdesivir in raw sewage was

Table 2
Regression results of unstable compounds in sewer reactors.

Biomarkers	Reactor 1			Reactor 2			Reactor 3		
	R^2	Fitting model	k	R^2	Fitting model	k	R^2	Fitting model	k
Ribavirin	0.85	First-order	0.15	0.75	First-order	0.23	0.97	First-order	0.20
Zidovudine	0.95	Zero-order	0.022	0.98	Zero-order	0.036	0.98	Zero-order	0.021
Ritonavir	0.91	First-order	0.061	0.88	First-order	0.090	0.94	First-order	0.040
Arbidol	0.89	First-order	0.24	0.91	First-order	0.36	0.84	First-order	0.26
Lopinavir	0.81	First-order	0.068	0.89	First-order	0.11	0.81	First-order	0.046
Remdesivir	0.99	First-order	0.20	0.99	First-order	0.35	0.99	First-order	0.12
Efavirenz	0.97	First-order	0.088	0.95	First-order	0.13	0.93	First-order	0.060
Rhodamine B	0.98	First-order	0.090	0.96	First-order	0.13	0.98	First-order	0.072

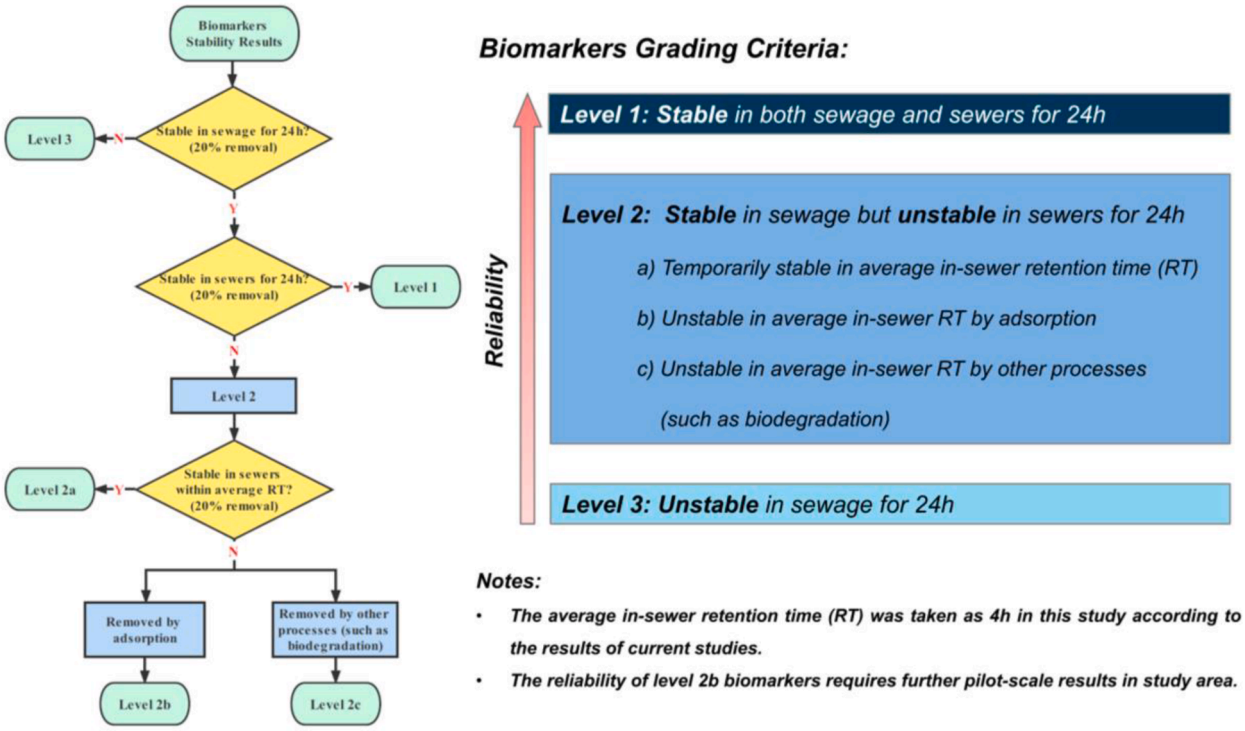


Fig. 5. Framework to evaluate the stability of biomarkers.

contributed by hydrolysis, adsorption of SS and biodegradation. This suggested that, for some biomarkers, the effect of sewage probably cannot be simply reflected by k_{ww} in a gravity sewer reactor. Generally, the influence of sediments and biofilms on biomarkers satisfies the additive relationship of independent effects. Even in the presence of sediment, the removal rate produced by biofilm was still proportional to the A/V value. In consequence, the current kinetic model effectively describes the removal of biomarkers in gravity sewer reactors.

3.4. The effect of flow velocity on stability of WBE biomarkers

In this section, the effect of sewage flow velocities in gravity sewers on stability of biomarkers was preliminarily investigated through setting a high stirring speed (54 rpm) in reactor 4. Flow velocities can change the shear stress in sewers and then affect the mass transfer resistance of substrates (e.g., sulfide, sulfate, organic matter and dosed chemicals) (Jiang et al., 2010; Shypanski et al., 2018). The variations of investigated compounds in reactor 2 and 4 were shown in Figure S4. Interestingly, for highly or moderately stable antiviral drugs, an increased flow velocity did not alter their stability. This was consistent with the variations of two stable benchmarks, acesulfame and sucralose. If varying flow velocities under natural conditions don't alter the stability of in-sewer stable biomarkers, the uncertainty in their WBE estimations would be effectively reduced.

For the 8 unstable compounds in sewer reactors, except for remdesivir, a higher flow velocity significantly increased their removal rates. Notably, the attenuation of indicator rhodamine B changed from two-phase kinetics to first-order kinetics, indicating that its adsorption was significantly accelerated. That's probably because a higher flow velocity facilitated its diffusion from the water phase to sediment and biofilm parts. The variation of rhodamine B to some extent explained why the removal of unstable hydrophobic antivirals (arbidol, ritonavir, lopinavir, and efavirenz) was expedited. For biomarkers determined by biodegradation, a higher velocity probably accelerated their removal (e.g. zidovudine, ribavirin) but might have no significant effect (e.g. remdesivir, abacavir), primarily depending on whether their removal was controlled by mass transfer. In addition, another study reported that an increased shear velocity significantly changed the abundance of specific microbial phyla in different layers of sediments, which might also affect some biodegradation processes (Zuo et al., 2021).

Overall, the results of this study indicate that flow velocity is a potential factor affecting biomarker stability. Whereas, this finding is uncertain due to the variable conditions of actual pipeline networks in different locations as well as the influence of pipeline intersections on flow velocity (Carrera et al., 2015). Therefore, we recommend pilot-scale or field pipeline studies to further investigate the effect of flow velocity and establish correlation factors for WBE studies.

3.5. WBE biomarker possibility—a grading criterion

Previous studies on stability of biomarkers usually classified them by apparent removal rates in laboratory-scale reactors (Ahmed et al., 2021; Choi et al., 2020; He et al., 2021), as was the previous discussion in this article (Section 3.1). However, current methods do not pay enough attention to the important role of in-sewer residence time and the influence of different effects such as adsorption and biodegradation. Considering the variation of in-sewer residence time in different regions, it is difficult to directly determine whether those moderately stable compounds are suitable biomarkers. For example, abacavir was an inappropriate biomarker in this study, but the other four moderately stable biomarkers are suitable for most circumstances. Therefore, based on current standards, this study proposed a framework for the assessment of biomarker stability. The stability results were graded into three levels as shown in Fig. 5.

As shown in Fig. 5, biomarkers are classified into 3 levels by whether stable in sewage or sewers within 24 h. Biomarkers that are stable in both sewage and sewers are classified as Level 1, those that are stable in the sewage but not in sewers are Level 2, and those that are unstable in sewage are Level 3. For biomarkers of level 1, they are always stable and are recommended as suitable candidates for WBE. Level 2 biomarkers are further divided into three sub-levels (a, b, and c) to reflect the influence of in-sewer residence time and different effects. Generally, the

Table 3
Grading results of target antiviral drugs in this study.

Level	Biomarkers				
Level 1	Acyclovir	Oseltamivir	Ganciclovir	Emtricitabine	Telbivudine
Level 2a	Lamivudine	Amantadine	Favipiravir	Nevirapine	
Level 2b	Ritonavir	Lopinavir	Efavirenz		
Level 2c	Abacavir	Ribavirin	Zidovudine		
Level 3	Arbidol	Remdesivir			

in-sewer residence time determine the stability of most biomarkers. In other words, if the target compounds keep temporarily stable within the residence time, they remain a viable option for back estimation. Therefore, these temporarily stable biomarkers are identified as level 2a, which is a potential class of biomarker candidates second only to reliable level 1 ones. According to the reports by Kapo et al. (2017) and Ort et al. (2014) on sewer conditions of America and Europe, respectively, the average in-sewer residence time (RT) is assigned as 4 h in this study, and it can be variable in other studies depending on the actual situation in the study area. Then, for the biomarkers unstable (>20% removal) in the average retention time, those totally affected by adsorption are classified as level 2b, and those undergoing other processes such as biodegradation are recognized as level 2c. This classification is to reflect the special effects of adsorption. It should be noted that adsorption might gradually weaken or even approach equilibrium with the increase of adsorption quantity. Therefore, for some chronic disease medicines (such as hepatitis B, hypertension, etc.), their adsorption in actual sewers might be much less than the estimation of the laboratory simulation. These level 2b compounds would also be viable biomarkers under some circumstances. Finally, compounds at level 2c and level 3 are highly unpredictable in sewage and sewers, so we recognize them as unsuitable biomarkers from the perspective of in-sewer stability. The grading framework can not only help researchers screen appropriate biomarkers, but also provide readers with a direct reference for the reliability of back estimation results by different biomarkers.

According to this framework, the 17 antiviral drugs investigated in this article could be graded and the results were shown in Table 3. Five antivirals were classified into level 1 and considered as the most reliable biomarkers. Four moderately stable drugs were categorized as level 2a and regarded as potential biomarker choices. Three other antivirals identified as level 2b ones could also be considered under certain conditions after careful evaluation. The other 5 level 2c and level 3 drugs were the least recommended biomarker choices in this study. Compared with the standard in Section 3.1, which can only directly identify five suitable biomarkers, this framework solved the classification problem of moderately stable biomarkers and finally recommended a total of 9 antiviral drugs for WBE estimation. These biomarkers can be used in regional WBE studies to estimate community consumption of drugs for influenza, hepatitis B, herpes, and HIV, providing valuable information for public health.

In addition, although not included in this study, metabolites as a category of potential biomarkers should also meet this framework. The metabolites' information of the 17 target antivirals was summarized in Table S9. It can be seen that most targets are eliminated mainly as parent or glucuronide conjugates that can be totally reconverted to the parent form(Gao et al., 2017). However, there are still some metabolites that are the main form of excretion, such as oseltamivir carboxylate, T705M1 and 8-OH-efavirenz. This is a limitation of the study and further experiments are needed to reveal the stability of these metabolites.

4. Conclusions

This study examined the stability of 17 common antiviral drugs in both sewage and gravity sewers with the presence of sediments and biofilms under two typical average flow velocities. The results showed that arbidol and remdesivir were significantly removed in untreated sewage and the stability of investigated biomarkers varied in gravity sewer reactors due to adsorption, biodegradation and diffusion mechanisms. A kinetic model using sediment reaction rate k_{sd} and biofilm rate normalized to A/V ratio k'_{bio} were found to be suitable for gravity sewers. An increased flow velocity might not affect the stability of stable biomarkers, but probably accelerated the removal processes of unstable biomarkers. This study proposed a grading framework to comprehensively evaluate the biomarker stability. Under this framework, a total of 9 antiviral drugs, including 5 level 1 antivirals (acyclovir, oseltamivir, ganciclovir, emtricitabine and telbivudine) and 4 level 2a antivirals (lamivudine, amantadine, favipiravir and nevirapine), were recommended as appropriate WBE biomarkers from the perspective of in-sewer stability.

CRediT authorship contribution statement

Jiaqi Wen: Methodology, Data curation, Investigation, Writing – original draft. **Lei Duan:** Methodology, Investigation, Writing – review & editing. **Bin Wang:** Writing – review & editing. **Qian Dong:** Investigation. **Yanchen Liu:** Methodology, Writing – review & editing. **Jun Huang:** . **Gang Yu:** Methodology, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

Acknowledgements

This work was financially supported by the Major Project of National Natural Science Foundation of China (52091544) and the Research Fund, Vanke School of Public Health, Tsinghua University.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.120023.

References

Acosta, N., Bautista, M.A., Waddell, B.J., McCalder, J., Beaudet, A.B., Man, L., Pradhan, P., Sedaghat, N., Papparis, C., Bacanu, A., Hollman, J., Krusina, A., Southern, D.A., Williamson, T., Li, C., Bhatnagar, S., Murphy, S., Chen, J., Kuzma, D., Clark, R., Meddings, J., Hu, J., Cabaj, J.L., Conly, J.M., Dai, X., Lu, X., Chekouo, T., Ruecker, N.J., Achari, G., Ryan, M.C., Frankowski, K., Hubert, C.R.J., Parkins, M.D., 2022. Longitudinal SARS-CoV-2 RNA wastewater monitoring across a range of scales correlates with total and regional COVID-19 burden in a well-defined urban population. Water Res. 220, 118611.

Ahmed, F., Li, J., O'Brien, J.W., Tscharke, B.J., Samanipour, S., Thai, P.K., Yuan, Z., Mueller, J.F., Thomas, K.V., 2021. In-sewer stability of selected analgesics and their metabolites. Water Res. 204, 117647.

Carrera, L., Springer, F., Lipeme-Kouyi, G., Buffiere, P., 2015. A review of sulfide emissions in sewer networks: overall approach and systemic modelling. Water Sci. Technol. 73 (6), 1231–1242.
Cheng, Z., Dong, Q., Yuan, Z., Huang, X., Liu, Y., 2022. Fate characteristics, exposure risk, and control strategy of typical antibiotics in Chinese sewerage system: a review. Environ. Int. 167, 107396.
Choi, P.M., Li, J., Gao, J., O'Brien, J.W., Thomas, K.V., Thai, P.K., Jiang, G., Mueller, J.F., 2020. Considerations for assessing stability of wastewater-based epidemiology biomarkers using biofilm-free and sewer reactor tests. Sci. Total Environ. 709, 136228.
Clercq, E.D., 2007. Three decades of antiviral drugs. Nat. Rev. Drug Discov. 6 (12), 941-941.
Daughton, C.G., 2001a. Pharmaceuticals and Care Products in the Environment. American Chemical Society, pp. 348–364.
Daughton, C.G., 2001b. Pharmaceuticals and Care Products in the Environment. American Chemical Society, pp. 2–38.
Daughton, C.G., 2018. Monitoring wastewater for assessing community health: sewage Chemical-Information Mining (SCIM). Sci. Total Environ. 619-620, 748–764.
Duan, L., Zhang, Y., Wang, B., Yu, G., Gao, J., Cagnetta, G., Huang, C., Zhai, N., 2022. Wastewater surveillance for 168 pharmaceuticals and metabolites in a WWTP: occurrence, temporal variations and feasibility of metabolic biomarkers for intake estimation. Water Res. 216, 118321.
Fischer, K., Wolff, B., Emmerling, C., 2013. Inter and intra plant variability of enzyme profiles including various phosphoesterases and sulfatase of six wastewater treatment plants. Bioresour. Technol. 137, 209–219.
Funke, J., Prasse, C., Ternes, T.A., 2016. Identification of transformation products of antiviral drugs formed during biological wastewater treatment and their occurrence in the urban water cycle. Water Res. 98, 75–83.
Gao, J., Banks, A., Li, J., Jiang, G., Lai, F.Y., Mueller, J.F., Thai, P.K., 2017. Evaluation of in-sewer transformation of selected illicit drugs and pharmaceutical biomarkers. Sci. Total Environ. 609, 1172–1181.
Gao, J., Li, J., Jiang, G., Shypanski, A.H., Nieradzik, L.M., Yuan, Z., Mueller, J.F., Ort, C., Thai, P.K., 2019. Systematic evaluation of biomarker stability in pilot scale sewer pipes. Water Res. 151, 447–455.
Gao, J., O'Brien, J., Du, P., Li, X., Ort, C., Mueller, J.F., Thai, P.K., 2016. Measuring selected PPCPs in wastewater to estimate the population in different cities in China. Sci. Total Environ. 568, 164–170.
He, C., Li, J., Jiang, G., Chen, S., Niel, C., Yuan, Z., Mueller, J.F., Thai, P., 2021. Transformation of phthalates and their metabolites in wastewater under different sewer conditions. Water Res. 190, 116754.
Hendricks, W.A., 1931. The use of the relative residual in the application of the method of least squares. Ann. Math. Stat. 2 (4), 458–478.
Hou, C., Hua, Z., Xu, P., Xu, H., Wang, Y., Liao, J., Di, B., 2020. Estimating the prevalence of hepatitis B by wastewater-based epidemiology in 19 cities in China. Sci. Total Environ. 740, 139696.
Hu, Y., Wei, X., Zhu, Q., Li, L., Liao, C., Jiang, G., 2022. COVID-19 pandemic impacts on humans taking antibiotics in China. Environ. Sci. Technol. 56 (12), 8338–8349.
Hvitved-Jacobsen, T., Vollertsen, J., Nielsen, A.H., 2013. Sewer Processes: Microbial and Chemical Process Engineering of Sewer Networks. CRC Press.
Jiang, G., Gutierrez, O., Sharma, K.R., Yuan, Z., 2010. Effects of nitrite concentration and exposure time on sulfide and methane production in sewer systems. Water Res. 44 (14), 4241–4251.
Kannan, A., Sims, N., Hold, A.J., Jagadeesan, K., Standerwick, R., Barden, R., Kasprzyk-Hordern, B., 2023. The burden of city's pain treatment – A longitudinal one year study of two cities via wastewater-based epidemiology. Water Res. 229, 119391.
Kapo, K.E., Paschka, M., Vamshi, R., Sebasky, M., McDonough, K., 2017. Estimation of U. S. sewer residence time distributions for national-scale risk assessment of down-the-drain chemicals. Sci. Total Environ. 603-604, 445–452.
Kunkel, U., Radke, M., 2008. Biodegradation of acidic pharmaceuticals in bed sediments: insight from a laboratory experiment. Environ. Sci. Technol. 42 (19), 7273–7279.
Kuroda, K., Li, C., Dhangar, K., Kumar, M., 2021. Predicted occurrence, ecotoxicological risk and environmentally acquired resistance of antiviral drugs associated with COVID-19 in environmental waters. Sci. Total Environ. 776, 145740.
Lancaster, K., Rhodes, T., valentine, k., Ritter, A, 2019. A 'promising tool'? A critical review of the social and ethico-political effects of wastewater analysis in the context of illicit drug epidemiology and drug policy. Curr. Opin. Environ. Sci. Health 9, 85–90.
Li, J., Ahmed, W., Metcalfe, S., Smith, W.J.M., Tscharke, B., Lynch, P., Sherman, P., Vo, P.H.N., Kaserzon, S.L., Simpson, S.L., McCarthy, D.T., Thomas, K.V., Mueller, J. F., Thai, P., 2022. Monitoring of SARS-CoV-2 in sewersheds with low COVID-19 cases using a passive sampling technique. Water Res. 218, 118481.
Li, J., Gao, J., Thai, P.K., Mueller, J.F., Yuan, Z., Jiang, G., 2020. Transformation of illicit drugs and pharmaceuticals in sewer sediments. Environ. Sci. Technol. 54 (20), 13056–13065.
Li, J., Gao, J., Thai, P.K., Shypanski, A., Nieradzik, L., Mueller, J.F., Yuan, Z., Jiang, G., 2019. Experimental investigation and modeling of the transformation of illicit drugs in a pilot-scale sewer system. Environ. Sci. Technol. 53 (8), 4556–4565.
Li, J., Gao, J., Thai, P.K., Sun, X., Mueller, J.F., Yuan, Z., Jiang, G., 2018. Stability of illicit drugs as biomarkers in sewers: from lab to reality. Environ. Sci. Technol. 52 (3), 1561–1570.
Li, J., Gao, J., Zheng, Q., Thai, P.K., Duan, H., Mueller, J.F., Yuan, Z., Jiang, G., 2021. Effects of pH, temperature, suspended solids, and biological activity on transformation of illicit drug and pharmaceutical biomarkers in sewers. Environ. Sci. Technol. 55 (13), 8771–8782.
Li, Z., Sobek, A., Radke, M., 2015. Flume experiments to investigate the environmental fate of pharmaceuticals and their transformation products in streams. Environ. Sci. Technol. 49 (10), 6009–6017.
Lin, A.Y.-C., Lin, C.-A., Tung, H.-H., Chary, N.S., 2010. Potential for biodegradation and sorption of acetaminophen, caffeine, propranolol and acebutolol in lab-scale aqueous environments. J. Hazard. Mater. 183 (1), 242–250.
Liu, A., Lin, W., Ming, R., Guan, W., Wang, X., Hu, N., Ren, Y., 2022a. Stability of 28 typical prescription drugs in sewer systems and interaction with the biofilm bacterial community. J. Hazard. Mater. 436, 129142.
Liu, Q., Feng, X., Chen, N., Shen, F., Zhang, H., Wang, S., Sheng, Z., Li, J., 2022b. Occurrence and risk assessment of typical PPCPs and biodegradation pathway of ribavirin in wastewater treatment plants. Environ. Sci. Ecotechnol. 11, 100184.
Liu, Y., Ni, B.-J., Ganigué, R., Werner, U., Sharma, K.R., Yuan, Z., 2015. Sulfide and methane production in sewer sediments. Water Res. 70, 350–359.
Liu, Y., Tugtas, A.E., Sharma, K.R., Ni, B.-J., Yuan, Z., 2016. Sulfide and methane production in sewer sediments: field survey and model evaluation. Water Res. 89, 142–150.
McCall, A.-K., Scheidegger, A., Madry, M.M., Steuer, A.E., Weissbrodt, D.G., Vanrolleghem, P.A., Kraemer, T., Morgenroth, E., Ort, C., 2016. Influence of different sewer biofilms on transformation rates of drugs. Environ. Sci. Technol. 50 (24), 13351–13360.
Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ. Sci. Technol. Lett. 7 (7), 511–516.
Mohanakrishnan, J., Gutierrez, O., Sharma, K.R., Guisasola, A., Werner, U., Meyer, R.L., Keller, J., Yuan, Z., 2009. Impact of nitrate addition on biofilm properties and activities in rising main sewers. Water Res. 43 (17), 4225–4237.
Nannou, C., Ofrydopoulou, A., Evgenidou, E., Heath, D., Heath, E., Lambropoulou, D., 2020. Antiviral drugs in aquatic environment and wastewater treatment plants: a review on occurrence, fate, removal and ecotoxicity. Sci. Total Environ. 699, 134322.
Ncube, S., Madikizela, L.M., Chimuka, L., Nindi, M.M., 2018. Environmental fate and ecotoxicological effects of antiretrovirals: a current global status and future perspectives. Water Res. 145, 231–247.
O'Brien, J.W., Choi, P.M., Li, J., Thai, P.K., Jiang, G., Tscharke, B.J., Mueller, J.F., Thomas, K.V., 2019. Evaluating the stability of three oxidative stress biomarkers under sewer conditions and potential impact for use in wastewater-based epidemiology. Water Res. 166, 115068.
Ort, C., van Nuijs, A.L.N., Berset, J.-.D., Bijlsma, L., Castiglioni, S., Covaci, A., de Voogt, P., Emke, E., Fatta-Kassinos, D., Griffiths, P., Hernández, F., González-Mariño, I., Grabic, R., Kasprzyk-Hordern, B., Mastroianni, N., Meierjohann, A., Nefau, T., Östman, M., Pico, Y., Racamonde, I., Reid, M., Slobodnik, J., Terzic, S., Thomaidis, N., Thomas, K.V., 2014. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. Addiction 109 (8), 1338–1352.
Pandey, A., Galvani, A.P., 2019. The global burden of HIV and prospects for control. Lancet Hiv 6 (12), E809–E811.
Prasse, C., Wenk, J., Jasper, J.T., Ternes, T.A., Sedlak, D.L., 2015. Co-occurrence of photochemical and microbiological transformation processes in open-water unit process wetlands. Environ. Sci. Technol. 49 (24), 14136–14145.
Ramin, P., Polesel, F., Brock, A.L., Plósz, B.G., 2018. The impact of temperature on the transformation of illicit drug biomarkers in wastewater. Sci. Total Environ. 644, 1612–1616.
Razavi-Shearer, D., Gamkrelidze, I., Nguyen, M.H., Chen, D.-.S., Van Damme, P., Abbas, Z., Abdulla, M., Abou Rached, A., Adda, D., Aho, I., 2018. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol. Hepatol. 3 (6), 383–403.
Reid, M.J., Langford, K.H., Mørland, J., Thomas, K.V., 2011. Analysis and interpretation of specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative measurement of regional alcohol consumption. Alcoholism 35 (9), 1593–1599.
Schweitzer, A., Horn, J., Mikolajczyk, R.T., Krause, G., Ott, J.J., 2015. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet 386 (10003), 1546–1555.
Shi, X., Ngo, H.H., Sang, L., Jin, P., Wang, X.C., Wang, G., 2018. Functional evaluation of pollutant transformation in sediment from combined sewer system. Environ. Pollut. 238, 85–93.
Shimko, K.M., O'Brien, J.W., Li, J., Tscharke, B.J., Brooker, L., Thai, P.K., Choi, P.M., Samanipour, S., Thomas, K.V., 2022. In-sewer stability assessment of anabolic steroids and selective androgen receptor modulators. Environ. Sci. Technol. 56 (3), 1627–1638.
Shypanski, A.H., Yuan, Z., Sharma, K., 2018. Influence of pressure main pumping frequency on sulfide formation rates in sanitary sewers. Environ. Sci. 4 (3), 403–410.
Thomas, K.V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic, R., Hernández, F., Karolak, S., Kasprzyk-Hordern, B., Lindberg, R.H., 2012. Comparing illicit drug use in 19 European cities through sewage analysis. Sci. Total Environ. 432, 432–439.
Tomsone, L.E., Perkons, I., Sukajeva, V., Neilands, R., Kokina, K., Bartkevics, V., Pugajeva, I., 2022. Consumption trends of pharmaceuticals and psychoactive drugs in Latvia determined by the analysis of wastewater. Water Res. 221, 118800.
Ul'yanovskii, N.V., Kosyakov, D.S., Sypalov, S.A., Varsegov, I.S., Shavrina, I.S., Lebedev, A.T., 2022. Antiviral drug Umifenovir (Arbidol) in municipal wastewater during the COVID-19 pandemic: estimated levels and transformation. Sci. Total Environ. 805, 150380.

Wang, Z., Zheng, Q., Gartner, C., Chan, G.C.K., Ren, Y., Wang, D., Thai, P.K., 2021. Comparison of tobacco use in a university town and a nearby urban area in China by intensive analysis of wastewater over one year period. Water Res. 206, 117733.
Xu, L., Zang, J., Cong, W., Holton, E., Jiang, L., Sheppard, S.K., Wang, Y., Wang, N., Weeks, J., Fu, C., Jiang, Q., Lambert, H., Kasprzyk-Hordern, B., 2022a. Assessment of community-wide antimicrobials usage in Eastern China using wastewater-based epidemiology. Water Res. 222, 118942.
Xu, X., Deng, Y., Zheng, X., Li, S., Ding, J., Yang, Y., On, H.Y., Yang, R., Chui, H.-.K., Yau, C.I., Tun, H.M., Chin, A.W.H., Poon, L.L.M., Peiris, M., Leung, G.M., Zhang, T., 2022b. Evaluation of RT-qPCR primer-probe sets to inform public health interventions based on COVID-19 sewage tests. Environ. Sci. Technol. 56 (12), 8875–8884.
Yao, L., Chen, Z., Dou, W., Yao, Z., Duan, X., Chen, Z., Zhang, L., Nong, Y., Zhao, J., Ying, G., 2021. Occurrence, removal and mass loads of antiviral drugs in seven wastewater treatment plants with various treatment processes. Water Res. 207, 117803.
Zhang, Q., Melchert, P.W., Markowitz, J.S., 2022. In vitro evaluation of the impact of Covid-19 therapeutic agents on the hydrolysis of the antiviral prodrug remdesivir. Chem. Biol. Interact. 365, 110097.
Zheng, Q., Gartner, C., Tscharke, B.J., O'Brien, J.W., Gao, J., Ahmed, F., Thomas, K.V., Mueller, J.F., Thai, P.K., 2020. Long-term trends in tobacco use assessed by wastewater-based epidemiology and its relationship with consumption of nicotine containing products. Environ. Int. 145, 106088.
Zheng, Q., Ren, Y., Wang, Z., Liu, J., Zhang, Y., Lin, W., Gao, J., Thomas, K.V., Thai, P.K., 2021. Assessing patterns of illicit drug use in a Chinese city by analyzing daily wastewater samples over a one-year period. J. Hazard. Mater. 417, 125999.
Zuo, Z., Song, Y., Ren, D., Li, H., Gao, Y., Yuan, Z., Huang, X., Zheng, M., Liu, Y., 2020a. Control sulfide and methane production in sewers based on free ammonia inactivation. Environ. Int. 143, 105928.
Zuo, Z., Sun, Z., Zhang, Y., Wang, M., Ren, D., Li, S., Yuan, Z., Liu, Y., Huang, X., 2021. In situ exploration of the sulfidogenic process at the water-sediment interface in sewers: mechanism and implications. ACS ES&T Eng. 1 (3), 415–423.
Zuo, Z., Zheng, M., Chang, J., Ren, D., Huang, X., Yuan, Z., Liu, Y., 2020b. Free nitrous acid-based suppression of sulfide production in sewer sediments: in-situ effect mechanism. Sci. Total Environ. 715, 136871.