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A robust method to quantify pharmaceuticals for the United Nations endorsed Global Estuaries Monitoring (GEM) Programme☆

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ABSTRACT

Following human and animal consumption, pharmaceuticals often remain incompletely metabolized, entering aquatic ecosystems via sources like wastewater discharges, landfill leachates, and aquaculture farms, thereby eventually reaching the ocean through estuaries. This influx poses a significant threat to marine ecosystems. The Global Estuaries Monitoring (GEM) Programme, endorsed by the United Nations Decade of Ocean Science for Sustainable Development (2021–2030), aims to establish a standardized method for monitoring pharmaceuticals in the world's estuaries. This study was performed for simultaneously quantifying 65 pharmaceuticals in small-volume of water samples. The findings revealed that using 20 mL water yielded optimal recoveries between 60 % and 130 %. The influence of pH, salinity and sample matrix on the performance of the method was minimal. 45–50 pharmaceuticals remained stable over a seven-day storage period at both 4 °C and 25 °C. This cost-effective and user-friendly method paves the way for the GEM Programme to monitor pharmaceuticals in over 100 estuaries worldwide.

1. Introduction

Pharmaceuticals, including human medicines and veterinary drugs, are extensively used for the treatment and prevention of diseases in both humans and animals. They encompass a wide range of classes, such as antibiotics, analgesics, antidepressants, antifungals, β-blockers, diuretics, non-steroidal anti-inflammatory drugs (NSAIDs), opioid pain medication, and stimulants (Adedipe et al., 2024). The global pharmaceutical market has experienced significant growth, generating USD 1482.4 million in 2021, with projections to reach USD 2067 million by 2028 (https://www.fnfresearch.com/news/global-pharmaceutical-market). However, due to the widespread use of pharmaceuticals, certain environmental challenges have arisen, including antimicrobial resistance triggered by elevated levels of antibiotics, and toxic effects of various bioactive drug residues to aquatic organisms (Chaves et al., 2022; Zhang et al., 2021). The release of pharmaceuticals into the environment occurs through various pathways, including manufacturing emissions, incomplete absorption by humans or animals, improper disposal of unused drugs, and limited removal capacity in conventional wastewater treatment processes (Wu et al., 2020). Consequently, pharmaceuticals are ubiquitously present in natural environments, posing potential risks to living organisms and human health (Beek et al., 2016; Li, 2014; Wilkinson et al., 2022; Zuccato et al., 2005). Even at ng/L – μg/L levels, pharmaceutical residues can cause irreversible harm to aquatic organisms and human health. Reported effects include the selection of antibiotic resistance by antimicrobial compounds (Larsson and Flach, 2022; Zainab et al., 2020), behavior impairment of wild populations of European perch (*Perca fluviatilis*) after exposure to oxazepam (Brodin et al., 2013), and a 95 % decrease in the population of the Oriental white-backed vulture (*Gyps bengalensis*) due

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to their consumption of diclofenac-treated livestock (Oaks et al., 2004). To address these issues, numerous regulatory bodies, international organizations (e.g., European Union and Organisation for Economic Co-operation and Development), and governmental entities are enforcing guidelines and strategies to control the discharge of pharmaceuticals in wastewater treatment facilities (WWTPs) and the surrounding environment, aiming for efficient management of pharmaceutical pollution (OECD, 2020; EU, 2018; Helwig et al., 2024). Hence, it is crucial to determine the contamination levels and spatiotemporal distributions of pharmaceuticals in the environment, which can serve as a basis for developing pollution control and management measures.

Previous research on pharmaceutical monitoring has primarily focused on freshwater environments, particularly in river systems located in North America, Europe, and China (Bu et al., 2013; Letsinger et al., 2019; Ojemaye and Petrik, 2019). Although a global river monitoring study on pharmaceuticals was published in 2022 (Wilkinson et al., 2022), estuarine environments have received considerably less attention. Given the ecological and economic importance of estuaries, it is highly beneficial to assess the levels of pharmaceutical pollution in these systems on a global scale.

To address this gap, the Global Estuaries Monitoring (GEM) Programme (https://www.globalestuaries.org/) was proposed and endorsed by the United Nations Decade of Ocean Science for Sustainable Development (2021–2030) in June 2021. The United Nations Ocean Decade emphasizes the need for scientific results to be translated into management actions aimed at improving the environmental quality and health of the oceans. Through the GEM Programme, a global network of collaborators has been established to monitor chemical contaminants of emerging concern in major urbanized estuaries worldwide. The primary objectives of the GEM Programme are to characterize the global pollution situation in estuaries, share data on estuarine pollution, and co-design solutions for pollution reduction with partners and stakeholders, ultimately making global estuaries cleaner and safer for all. The first phase of the GEM Programme focuses on investigating pharmaceutical residue concentrations in over 100 estuaries worldwide.

However, the monitoring methods used for freshwater environments may not be suitable for estuarine systems, given the potential influence of factors like salinity, pH, and concentrations of suspended solids on the performance of analytical methods (Fonseca et al., 2021; Kotke et al., 2019). The pH of the sample plays a crucial role in the extraction efficiency of pharmaceuticals (Zhang and Zhou, 2007), which becomes more complex when dealing with samples with a broad range of salinities (Chatwin, 1976). Furthermore, the large sampling volumes typically required (up to 1–2 L) pose logistical challenges and substantial costs for large-scale global sampling campaigns.

Therefore, the primary objective of this study is to develop a robust method for sampling estuarine waters on a global scale and detecting a wide range of pharmaceuticals in these samples. The specific aims of the study include optimizing a solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for accurately detecting and quantifying typical pharmaceuticals in estuarine waters. Additionally, the study seeks to determine the optimal sampling volume for estuarine water samples and evaluate the impacts of water pH, salinity, matrix types, storage time and temperature on the stability and reliability of this methodology. By achieving these objectives, this study will establish a dependable and standardized method for monitoring pharmaceuticals in estuaries worldwide through the GEM Programme. Moreover, the study will also serve as an example of development of standard methods for future studies on the occurrence, distribution, and potential risks of other contaminants of emerging concern at a global-scale.

2. Materials and methods

2.1. Target pharmaceuticals and reagents

Table S1 provides detailed information on the target compounds and internal standards used in this study. The 65 target compounds encompassed a wide range of pharmaceutical categories, including 21 antibiotics, eight antihistamines, seven antidepressants, six NSAIDs, three lipid regulators, three stimulants, two antimicrobials, two antiepileptics, two antihyperglycemics, two benzodiazepines, two β-blockers, one anesthetic, one antifungal, one calcium channel blocker, one diuretic, one insect repellent, one opioid, and one artificial sweetener. The selection of these pharmaceuticals was based on their high detection frequencies, concentrations, and ecological risks, as indicated by previous reports (Beek et al., 2016; Fabbri and Franzellitti, 2016; Liu et al., 2020; Wilkinson et al., 2022). Supplementary information in Table S1 provides further details on the procurement sources, as well as the physical and chemical properties of these target compound standards.

For the analysis, specific chemical reagents were employed. Ammonium acetate (HPLC grade) was supplied by Sigma-Aldrich (St. Louis, MO, USA), while formic acid (LC-MS grade) was purchased from Honeywell (Seelze, Germany). Sodium chloride was procured from J&K (Beijing, China), and methanol (HPLC grade) was obtained from Merck (Darmstadt, Germany). Ultrapure water was generated using the Milli-Q® IQ 7003 ultrapure and pure water purification system (Bedford, MA, USA), while distilled water was purchased from Watson (Hong Kong, China).

2.2. Sample pretreatment

To adjust the pH values of the water samples, formic acid was initially employed. Subsequently, each sample (10 mL, 20 mL, or 50 mL) was mixed with a 2-ng internal standard mixture (20 μL, 100 ng/mL). SPE was performed on the samples using a hydrophilic-lipophilic balance (HLB) cartridge (3 mL, 60 mg, Waters, Milford, MA, USA). The HLB cartridge was conditioned with 5 mL of methanol and 5 mL of acidified ultrapure water. Following the conditioning step, the water sample was loaded onto the cartridge and allowed to pass through naturally. The cartridge was then washed with 5 mL of ultrapure water and subsequently dried under vacuum for approximately 15–20 min. The retained target compounds were eluted using 5 mL of methanol containing 0.02 % (*v*/v) formic acid, and the eluent was gently evaporated under a stream of nitrogen at a temperature of 35 °C until complete dryness was achieved. Finally, the resulting sample was dissolved in 100 μL of methanol and filtered through a 0.22 μm polytetrafluoroethylene (PTFE) filter.

2.3. LC-MS/MS analysis

The detection of target pharmaceuticals was carried out using an Agilent 1290 Infinity ultra-high performance liquid chromatography (UHPLC) system (Palo Alto, CA, USA), coupled with a SCIEX QTRAP 5500 tandem mass spectrometer (Woodlands, Singapore). Separation of the target compounds was achieved using a 100 mm Agilent ZORBAX Eclipse Plus C18 column (2.1 mm internal diameter, 1.8 μm particle diameter, Palo Alto, CA, USA), equipped with a solvent smoother (Ampel, Shanghai, China), and a 5 mm Agilent ZORBAX Eclipse Plus C18 guard column (2.1 mm internal diameter, 1.8 μm particle diameter, Palo Alto, CA, USA). The mass spectrometry system was operated in multiple reaction monitoring (MRM) mode, with both positive and negative electrospray ionizations (ESI) employed. For each compound, two MRM transition ions were optimized and monitored, with the higher intensity ion used for quantitation and the other for confirmation (Table S2).

In the positive ESI mode, mobile phase A consisted of HPLC-grade water with 0.02 % (*v*/v) formic acid, while mobile phase B consisted

of methanol with 0.02 % (v/v) formic acid. In the negative ESI mode, mobile phase A contained HPLC-grade water with 5 mM ammonium acetate, and mobile phase B consisted of methanol with 5 mM ammonium acetate. The flow rate for both positive and negative ESI was set at 0.2 mL/min. The column temperature was maintained at 40 °C, and the autosampler temperature was set at 6 °C. Precursors, product ions, declustering potential (DP), collision energy (CE), collision cell exit potential (CXP), and retention time for each compound can be found in Table S2. The HPLC gradients and source-dependent parameters for positive and negative ESI are provided in Table S3.

2.4. Method optimization

A comprehensive series of experiments was conducted to evaluate the accuracy of the method for analyzing small-volume samples (10 mL, 20 mL, and 50 mL) and to investigate the impact of various factors, including water pH, salinity, matrix, and storage conditions. The experimental optimization process is visually represented in Fig. 1. In order to establish a reliable baseline, the target concentration of each of the 65 spiked pharmaceuticals in all experimental groups was uniformly set to 100 ng/L, aligning with the commonly observed concentrations of pharmaceuticals in water (Wilkinson et al., 2022). To explore the influence of water pH, the samples were intentionally acidified using formic acid to achieve pH values of 3, 4, and 5, while samples without acidification, maintaining a pH of 7, were included as the control group. Salinity effects were examined by introducing artificial sea salt into ultrapure water, resulting in salinities of 15 and 30 PSU, respectively. Control samples without addition of salt (0 PSU) were also prepared. To assess the impact of the water matrix, samples were collected from both the Shing Mun River and the Shing Mun River Estuary in Hong Kong, representing distinct matrices of river water and seawater, respectively. Furthermore, the effects of storage time and temperature were investigated by subjecting the samples to storage at either 4 °C or 25 °C, shielding them from light for a period of seven days. This duration corresponds to the maximum delivery time typically required by express companies for global sample transportation. The performance of different sampling volumes under the influence of all these factors was thoroughly examined. Each experimental group was executed in triplicate to ensure statistical reliability. The acceptable range for the recovery of the target compounds was determined as 60 % to 130 %, and deviations in detected concentrations between experimental groups within 20 % were considered acceptable in terms of accuracy (Martinez Piernas et al., 2021; Wilkinson et al., 2019).

The established and optimized method was further applied and validated in Kai Tak River and its estuary in Hong Kong. Water samples were collected from upstream to the estuary in March 2023 (Table S4), with two replicates taken at each sampling location using the standardized sampling kit developed in this study (Figure S1).

2.5. Quality assurance and quality control

To ensure the rigorism of the sample collection, pretreatment, and analysis process, a field blank control sample and a spiked quality control sample were included for each sampling campaign. These control samples played a crucial role in evaluating potential contamination, degradation, or transformation of the target compounds at each stage of the process that could compromise the accuracy and reliability of the results. The field blank control sample consisted of ultrapure water, serving as a reference for assessing any background contamination. Furthermore, the spiked quality control sample was also prepared by spiking all target pharmaceuticals into ultrapure water at a concentration of 100 ng/L.

To ensure consistent instrumental performance throughout the analytical run, instrumental quality control samples were regularly analyzed. These samples encompassed a laboratory blank control and a calibration quality control. The laboratory blank control, composed of pure methanol, helped identify any potential contamination originating from the laboratory environment or the analytical instrumentation itself. The calibration quality control, on the other hand, comprised all target pharmaceuticals and internal standards spiked in methanol at a concentration of 20 ng/mL. These instrumental quality control samples were analyzed once every 12 injections of the samples, serving as benchmarks to verify the stability and reliability of the instrument and the analytical method.

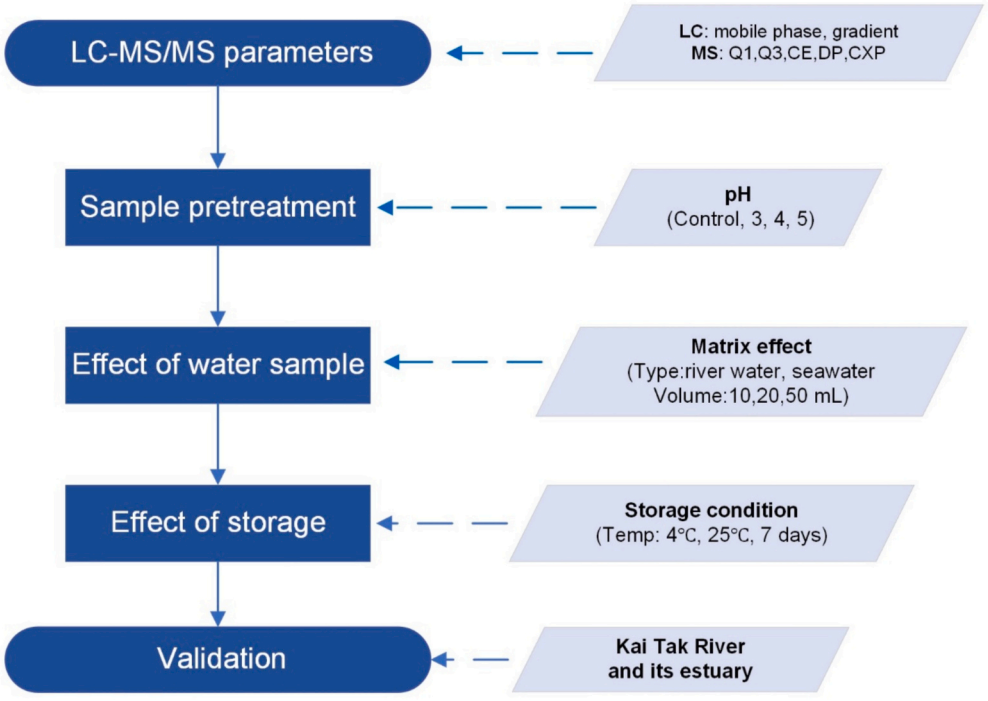

Fig. 1. Schematic experiment diagram of this study.

The limit of detection (LOD) and limit of quantification (LOQ) for each compound were determined based on the lowest detection concentrations of the spiked target pharmaceuticals, achieving signal-to-noise (S/N) ratios of 3 and 6, respectively. The specific values for the LOD and LOQ of each compound can be found in Table S5.

2.6. Data analysis

Data analysis was conducted using Excel 2021 (Microsoft, Redmond, WA, USA) and IBM SPSS Statistics version 27.0.1 (Armonk, NY, USA), while graphing was performed using Origin 2023 (OriginLab Corporation, Northampton, MA, USA). The recovery of each target pharmaceutical was calculated according to Eq. (1).

$$Recovery\ (\%) = \frac{C_e}{C_s} \times 100 \quad (1)$$

where C_e is the detected concentration of each pharmaceutical in the spiked water samples undergoing the treatment processes, and C_s is the detected concentration of each pharmaceutical in the spiked solution (i.e., 10 ng/mL spiked solution for 10 mL samples, 20 ng/mL spiked solution for 20 mL samples, and 50 ng/mL spiked solution for 50 mL samples).

The matrix effect (*ME*) for each target pharmaceutical was calculated using Eq. (2).

$$ME = \frac{C_{sp} - C_{bl}}{C_0} \quad (2)$$

where C_{sp}, C_{bl}, and C_0 denotes the detected concentration of each target pharmaceutical in spiked matrix solution, blank matrix solution, and the spiked solution, respectively. *ME* values falling within the range of 0.8 to 1.2 are considered to have a low impact. Values ranging between 0.5 and 0.8, as well as those between 1.2 and 1.5, are classified as having a moderate matrix effect. Values below 0.5 or above 1.5 are indicative of a strong matrix effect (Martinez Piernas et al., 2018).

3. Results and discussion

3.1. Optimization of LC-MS/MS conditions

In this study, the MRM transitions were carefully optimized to obtain the quantifier and qualifier ions for the 65 target pharmaceuticals. The CE, DP, and CXP values utilized for the optimization are summarized in Table S2. To enhance chromatographic separation and detection sensitivity, different compositions of mobile phase A (HPLC-grade water) and mobile phase B (methanol) were employed, incorporating various additives, for the analysis of calibration samples with a concentration of 5 ng/mL. The results revealed that the addition of 0.02 % (*v*/v) formic acid in the mobile phases achieved the most satisfactory separation performance in terms of both peak shape and intensity for the positive ESI mode (Figure S2). This finding aligns with previous studies, such as the work conducted by (Lee et al., 2024). For the negative ESI mode, the mobile phases containing 5 mM ammonium acetate exhibited the best performance compared to other concentrations, such as 0.5 mM or 2 mM ammonium acetate. Ammonium acetate was found to enhance the intensity and stability of the target signal by facilitating the generation of the necessary adducts (Kostiainen and Kauppila, 2009). Extraction ion chromatograms (XIC) depicting the 65 pharmaceuticals are presented in Figure S3.

For the selection of internal standards, priority was given to the same substance labeled with isotopes (Table S2). In cases where isotopically labeled targets were unavailable, internal standards were selected based on the following criteria: (1) possessing similar physicochemical properties and chemical structures; (2) exhibiting comparable retention times; and (3) demonstrating comparable extraction recoveries. Using the optimized LC-MS/MS method, the LODs and LOQs for the 65 target pharmaceuticals in this study ranged from 0.02 to 17.9 ng/L and from 0.03 to 35.7 ng/L, respectively (Table S5).

3.2. Effects of water pH and salinity

The recoveries of 48 pharmaceuticals (no pH adjustment), 50 pharmaceuticals (pH adjust to 3), 43 pharmaceuticals (pH adjust to 4), and 51 pharmaceuticals (pH adjust to 5) were found to fall within the acceptable range of 60 % to 130 % under different pH conditions (Fig. 2). Additionally, the results of the one-way ANOVA analysis indicated that there were no significant differences in the recoveries of most target pharmaceuticals across the four treatment groups ($p > 0.05$, Table S6). Some compounds, such as triamterene, diethyltoluamide, lidocaine, nicotine, propranolol, and atenolol, exhibited lower recoveries, which could be attributed to their strong sorption to the adsorbent and difficulty in elution during the SPE process within the corresponding pH window (Anderson et al., 2005). Moreover, the high recoveries (>130 %) of some substances may be attributed to signal fluctuations in the mass spectrometer. Additionally, it might be possible that substances with similar physical and chemical properties to the target compounds were retained in the processed samples, thereby interfering with the detection of the target compounds. However, it is worth noting that acidic extraction proved to be efficient in recovering the majority of the target pharmaceuticals.

Regarding salinity, the results showed that 49, 46, and 48 pharmaceuticals exhibited acceptable recoveries (60 % – 130 %) in samples with 0, 15, and 30 PSU salinities, respectively (Fig. 3). Notably, significant differences in recoveries were observed among samples with different salinities ($p \leq 0.05$, Table S6). Most pharmaceuticals demonstrated lower recoveries at 30 PSU, with an average difference of 13.8 ± 25.9 % between 0 and 30 PSU (Fig. 3). This phenomenon suggests that salinity may have an impact on the detection of target compounds, but the overall effect of salinity on the recovery was limited and considered acceptable.

3.3. Effects of water matrices and volumes

This study also examined the recoveries of target pharmaceuticals in samples with river water (Dissolved oxygen (DO) = 9.90 mg/L, pH = 8.42, salinity = 0.38 PSU) and seawater (DO = 8.41 mg/L, pH = 7.94, salinity = 36.37 PSU) as matrices. The results demonstrated that the river water matrix had a low impact on 42, 39, and 34 pharmaceuticals in 10 mL, 20 mL, and 50 mL water samples, respectively (Fig. 4, Table S7). The quantity of pharmaceuticals showing minimal matrix effects ($0.8 \leq ME \leq 1.2$) and those with low-to-moderate impact levels ($0.5 \leq ME \leq 1.5$) diminished with an increase in sample volume (Fig. 4a). This finding suggests that using smaller volumes, such as 10 mL or 20 mL, yields improved results in reducing the matrix effect in river water samples. Nonetheless, the seawater matrix did not significantly impact the recoveries of target pharmaceuticals. This observation can be attributed to the lower concentration of dissolved organic matter present in seawater (Bialk Bielinska et al., 2016). Consequently, the developed method is applicable for the detection of most target pharmaceuticals in both river water and seawater samples.

3.4. Effects of storage conditions

After a seven-day storage period, it was observed that the target pharmaceuticals displayed greater stability when stored at 4 °C compared to 25 °C for all tested sample volumes (Fig. 5). For instance, triclosan, an antimicrobial agent, exhibited lower stability at 25 °C, likely due to an increased degradation rate at higher temperatures. Considering the matrix effect, sample volumes of 10 mL and 20 mL were found to be preferable over 50 mL. If a consistent storage temperature of 4 °C could be maintained, the preference would be for 10 mL. However, considering potential temperature fluctuations during long-distance

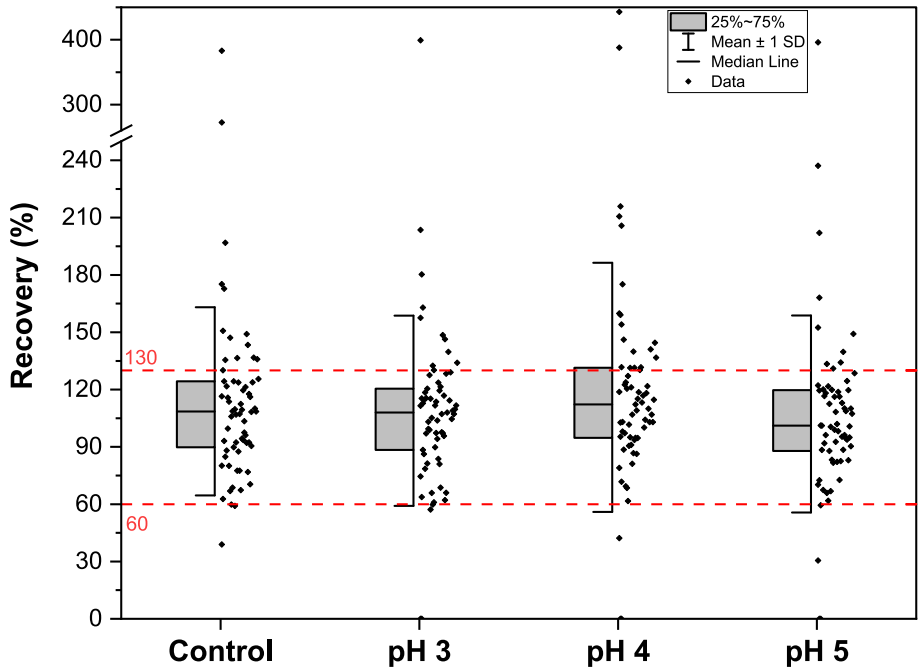

Fig. 2. Recoveries of 65 target pharmaceuticals under different water pH conditions: Control indicated no adjustment of pH.

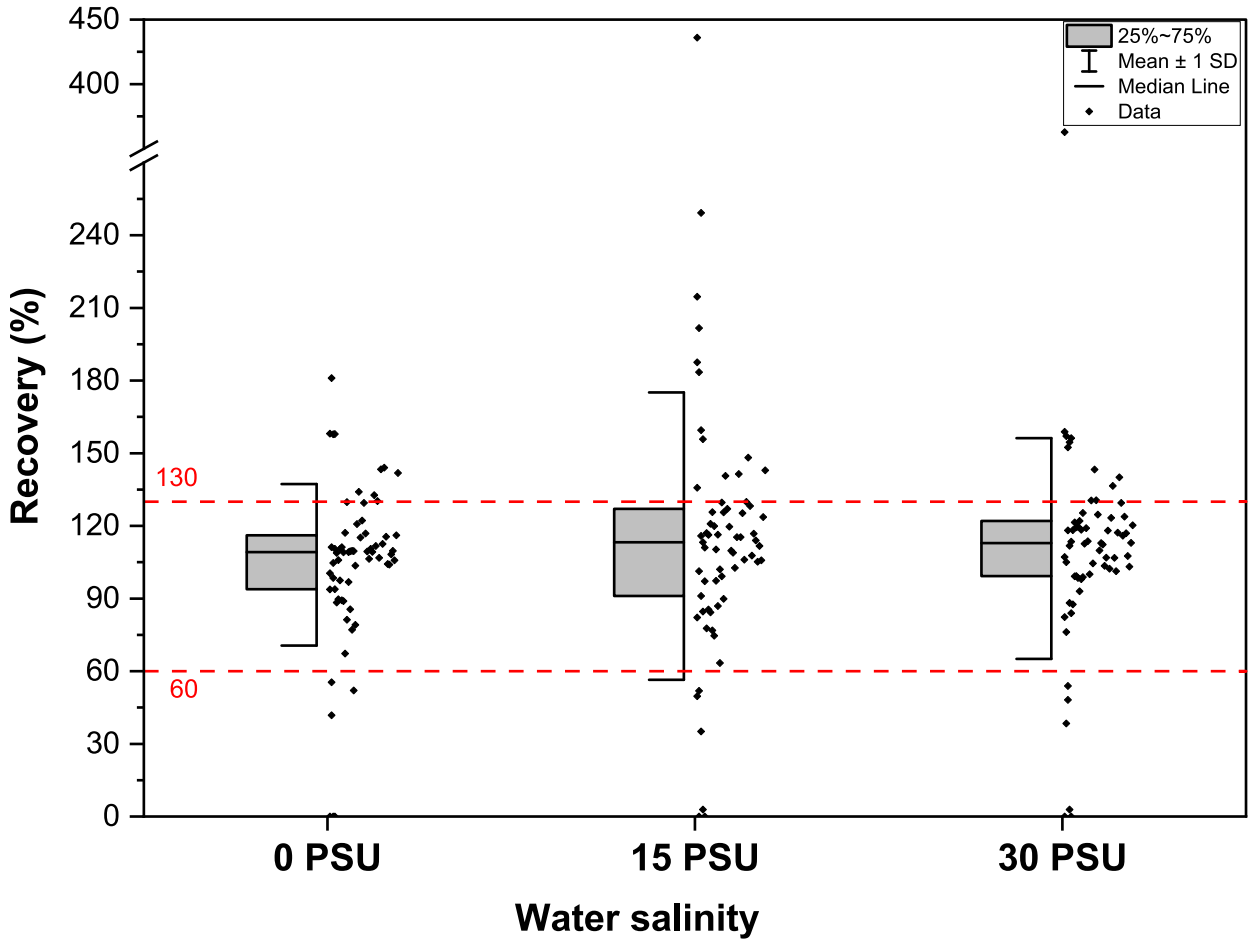

Fig. 3. Recoveries of 65 target pharmaceuticals under different water salinity conditions.

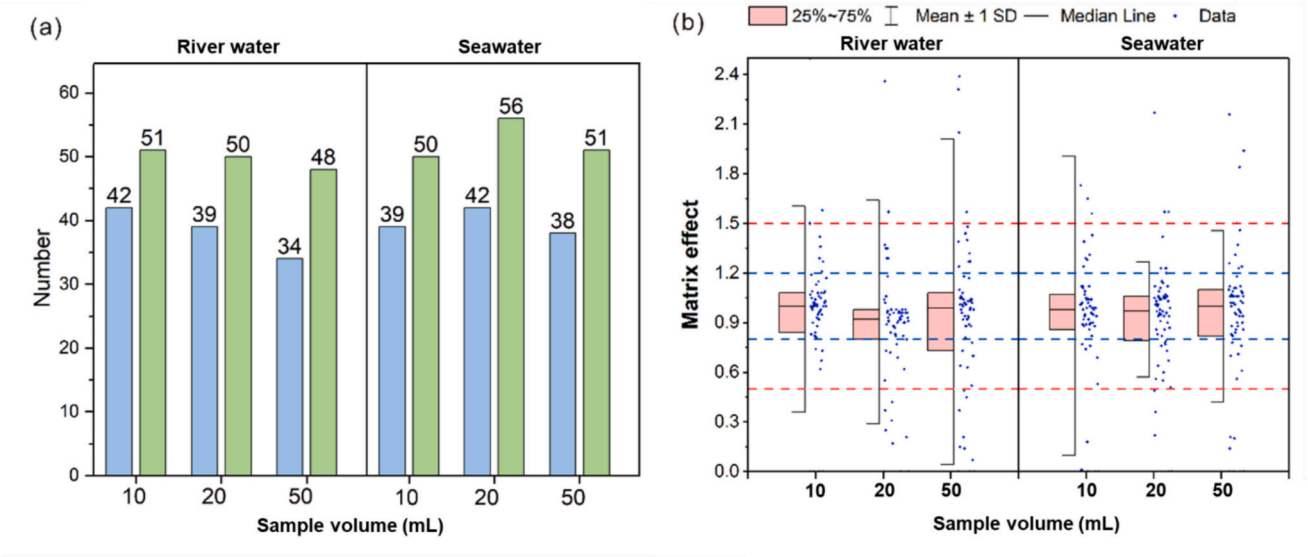

Fig. 4. Matrix effect (*ME*) in river water and seawater: (a) Number of pharmaceuticals within ±20 % accuracies (blue) and within ±50 % accuracies (green) in different water matrix types and sample volumes; (b) Half box chart displaying matrix effect values (*ME*) on different target pharmaceuticals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

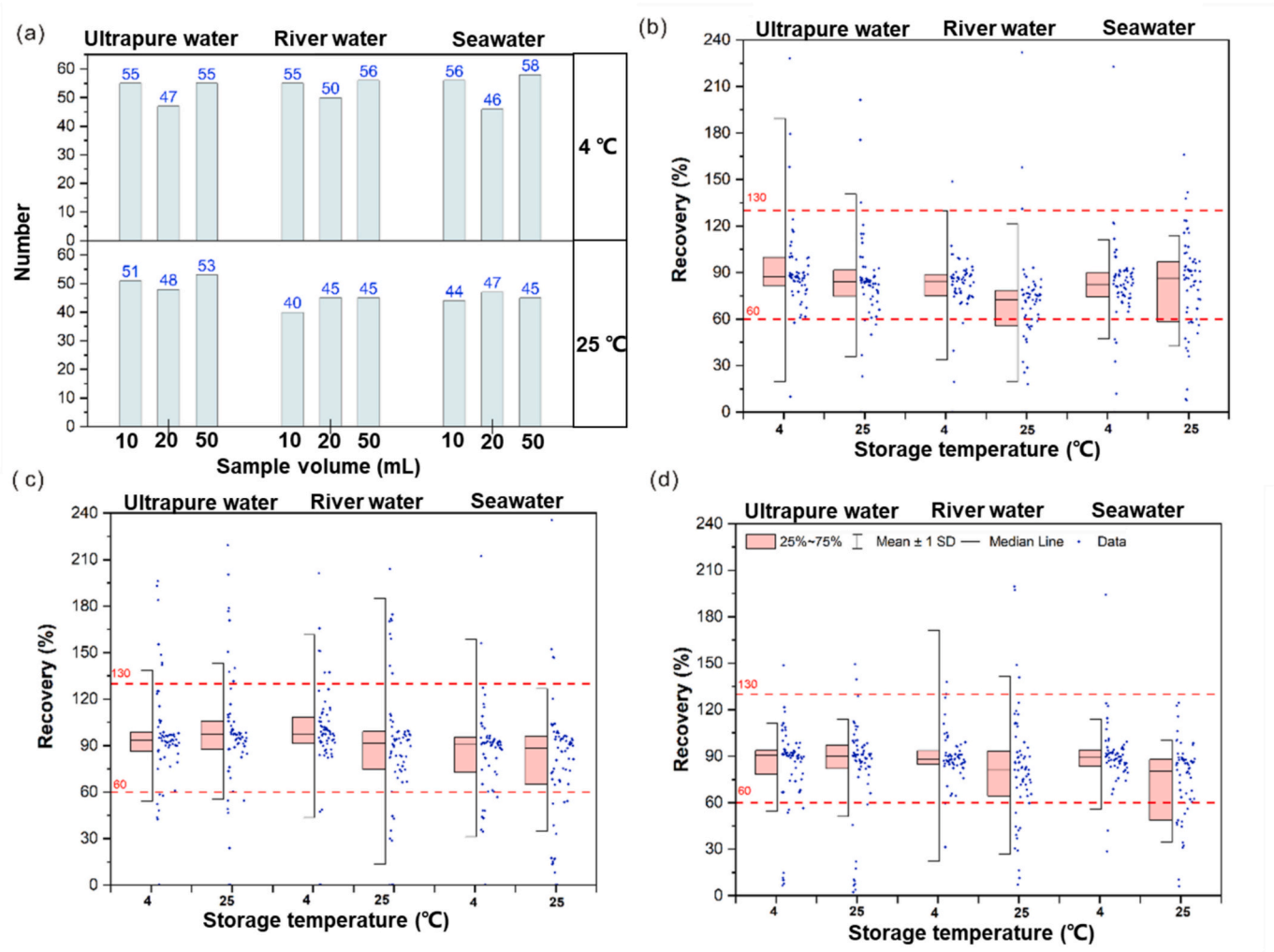

Fig. 5. Recoveries of target pharmaceuticals stored at 4 °C and 25 °C for 7 days: (a) Number of pharmaceuticals with acceptable recoveries (60–130 %), (b – d) Half box charts displaying storage condition effects on pharmaceuticals in (b) 10 mL, (c) 20 mL, and (d) 50 mL water samples.

transportation, the optimal choice shifted to 20 mL. In 20 mL of river water samples, 50 and 45 pharmaceuticals demonstrated acceptable recoveries at 4 °C and 25 °C, respectively. In 20 mL of seawater samples, the numbers were 46 at 4 °C and 47 at 25 °C (Fig. 5). Detailed information can be found in Table S8. It is important to note that the observed recoveries for most target pharmaceuticals remained within acceptable limits (Fig. 5).

3.5. Establishment of the sample collection protocol

Based on the optimized method, a lightweight, safe-to-transport, and user-friendly sampling kit was designed to facilitate the global sampling campaign for the first phase of the GEM Programme (Figure S1). The standard sampling kit weighed 1.15 kg before sampling and 1.54 kg after sampling. Its size was 29.0 × 17.5 × 21.0 cm. Based on the results of the above experiment, the sample volume had been determined to be 20 mL. During the sampling campaign in each estuary, it was recommended to collect duplicate samples at four designated sampling sites, resulting in a total of eight water samples. Additionally, two quality control samples were included, consisting of one field blank control sample and one spiked control sample, as described in Section 2.5. Therefore, each sampling kit was equipped with five ice packs, seven polypropylene syringes (20 mL each, one for each sampling site, one for the field blank control sample, one for the spiked control sample, and one as a backup), seven glass fiber filters (GF/F, 0.7 μm), seven seal bags (for packaging used filters), ten amber glass vials (20 mL) for water sample collection and quality control sample operation, one amber glass vial containing 20 mL ultrapure water for the field blank control sample, one amber glass vial containing 20 mL of mixed spike solution (100 ng/L for each target pharmaceutical) for the spiked quality control sample, and one 700 mL stainless steel bucket with a 10 m nylon cord attached (Figure S1).

In addition to the sampling kit, GEM global collaborators required additional resources during the sampling campaign. These included a sampling tutorial video, a detailed sampling protocol, self-fillable and printed sampling labels, a data log for recording information before and during sampling, and an example of the sampling data log. All these resources can be accessed through the GEM website (https://www.globalestuaries.org/resources).

For each estuary, it was preferable to select four sampling sites as follows: A) upstream of the river (above the urban area), B) downstream of the river (passing through the urban area), C) inner estuary, and D) outer estuary. Whenever possible, sampling should be conducted during the dry season and neap tide periods. The sampling process can be summarized as follows: At each sampling site, the stainless-steel bucket should be rinsed three times with river/estuary water before sampling. In the next step, 20 mL of river/estuary water should be aspirated into the syringe for rinsing, followed by filtering 5 mL of river/estuary water through the GF/F filter for rinsing. The amber glass vials should be rinsed with the filtrate. Duplicate water samples were then collected, with each sample containing 20 mL of the filtrate. The field blank control sample and the spiked quality control sample should be prepared at the first site of each estuary using the same procedures as the sample collection process.

Throughout the sampling process, it was essential to document the sampling time, GPS coordinates, physicochemical parameters of the water sample (including water pH, temperature, salinity, and dissolved oxygen), and detailed descriptions of the surrounding environment, particularly potential discharge points such as stormwater discharge outlets and sewage effluents upstream of the sampling site.

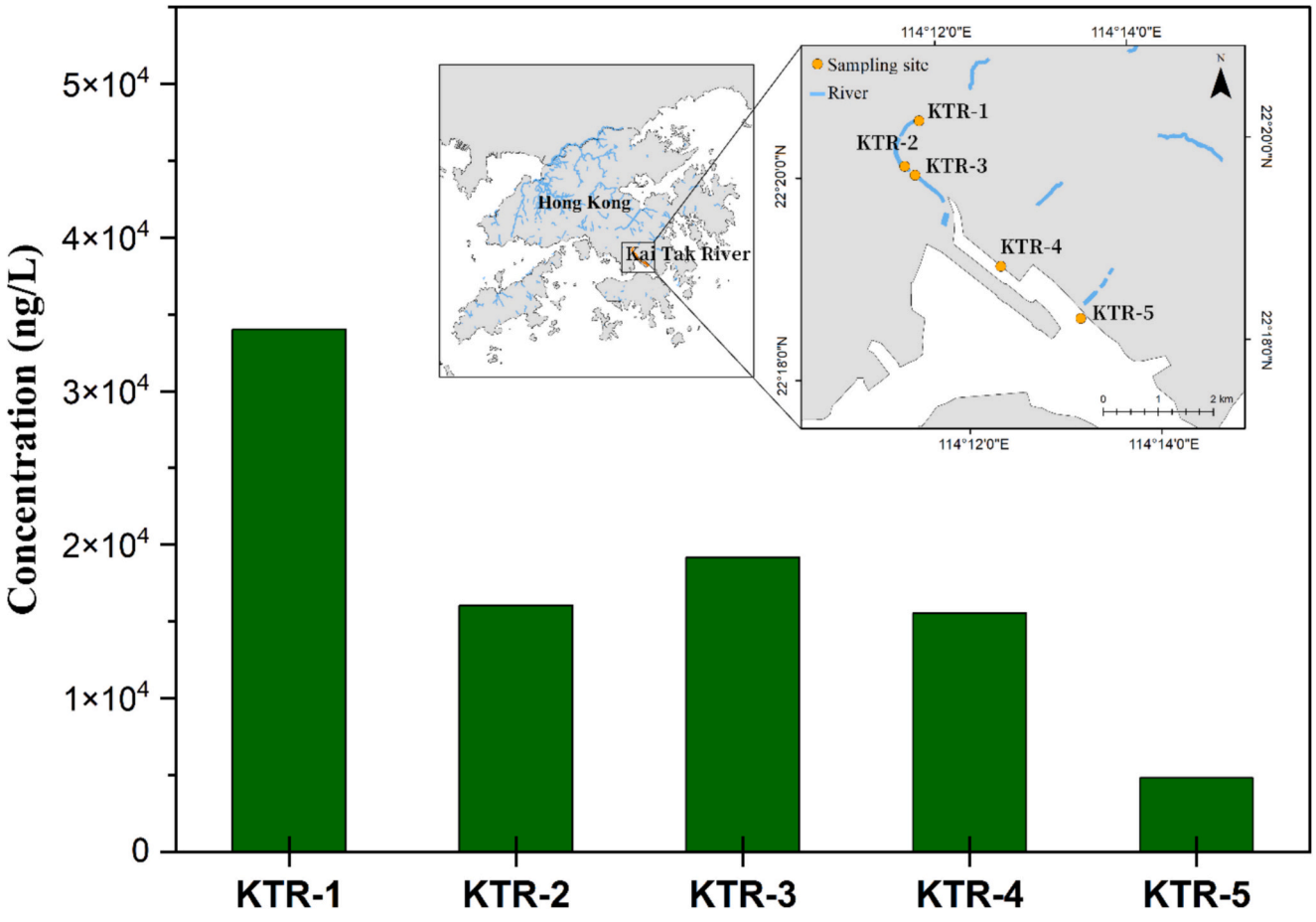

Fig. 6. Cumulative concentrations of 65 target pharmaceuticals in Kai Tak River and its estuary.

The vials containing water samples and quality control samples, as well as the seal bags containing the used GF/F filters, should be properly labeled and sent to the State Key Laboratory of Marine Pollution at City University of Hong Kong for further analysis.

3.6. Method testing in Kai Tak river and its estuary

During the method testing of the method, the water samples were collected across five sampling sites in the Kai Tak River and its estuary, and the target pharmaceuticals were detected (Fig. 6). KTR-1 exhibited the highest cumulative concentration at 34.1 μg/L, followed by KTR-3 at 19.2 μg/L, KTR-2 at 16.1 μg/L, and KTR-4 at 15.5 μg/L. The cumulative concentration at KTR-5, located at the estuary, was the lowest at 4.81 μg/L potentially due to seawater dilution (Fig. 6). Figure S4 illustrates the concentrations of each target at each sampling site. KTR-1 had the highest number of detected pharmaceuticals, with 64 identified targets. KTR-2 and KTR-3 closely followed with 63 detected targets each. Atenolol was not detected in either the river or estuary samples. Sucralose levels consistently remained elevated, ranging from 1.35 μg/L to 23.6 μg/L, indicating a significant influence of human activities (Figure S4). Cimetidine (for treating heartburn, stomach ulcers and reflux disease) was present at a concentration of 1.07 μg/L in KTR-3, whereas other pharmaceuticals were detected at relatively lower levels. The dominant antibiotics detected were sulfamerazine and sulfapyridine, with average concentrations of 0.55 μg/L and 0.49 μg/L, respectively. Furthermore, the concentrations of caffeine and paracetamol increased from the river to the estuary, surpassing 0.50 μg/L and 0.15 μg/L, respectively. When compared to the results obtained in 2016, the current study revealed slightly higher antibiotic concentrations (Deng et al., 2018). These two compounds were also the contaminants with the highest concentrations reported in global rivers (Wilkinson et al., 2022).

To summarize, the optimized small-volume sampling method successfully detected the target pharmaceuticals in the Kai Tak River and its estuary. The method demonstrated cost-effectiveness, robustness, and suitability for the detection and quantification of pharmaceuticals in estuaries. Therefore, it can be confidently implemented in the first phase of the GEM Programme.

4. Conclusions

This study presents a robust small-volume sampling and analysis method that effectively detects 65 pharmaceuticals in water samples from rivers to estuaries. The entire process, encompassing water sample collection, long-distance transport, pretreatment, and analysis, has undergone meticulous optimization and validation. Standardized sampling materials, including the sampling kit, tutorial video, and protocol, have been provided to ensure consistency and reliability in implementation. Our findings indicate that the pH and salinity of water samples have minimal effects on the recoveries of most target pharmaceuticals. The matrix effect is acceptable with a sampling volume of 10 mL or 20 mL for both river water and seawater samples. A sample volume of 20 mL is recommended to ensure accuracy and reliability, particularly after long-distance transportation. With the methodology employed in this study, it is possible to accurately monitor approximately 45–50 pharmaceuticals. However, the monitoring results of the remaining 15–20 pharmaceuticals should be approached with caution and subjected to thorough evaluation and treatment through quality assurance and quality control procedures. This developed method provides a solid foundation for supporting the United Nations endorsed GEM Programme.

CRediT authorship contribution statement

Qiong Luo: Writing – original draft, Methodology, Investigation, Formal analysis. **Chong Chen:** Writing – review & editing, Supervision, Project administration, Investigation, Formal analysis. **Racliffe Weng Seng Lai:** Writing – review & editing, Validation, Methodology. **Shaopeng Xu:** Validation. **Demilade T. Adedipe:** Methodology, Investigation. **Guang-Jie Zhou:** Writing – review & editing, Supervision. **Alistair B.A. Boxall:** Writing – review & editing, Investigation, Conceptualization. **Bryan W. Brooks:** Writing – review & editing, Supervision, Investigation. **Kenneth Mei Yee Leung:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2025.117860.

Data availability

Data will be made available on request.

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