Profiling Pharmaceutical Residues in South African Aquatic Ecosystems: A Dual Approach Using Grab Sampling and Passive Sampling Techniques

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DECLARATION

I declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any university.

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Date: 27-05-2025

MANUSCRIPTS

This thesis is based on the following manuscripts in which the candidate is the principal author:

Manuscript 1: Profiling diverse sources of pollution of urban rivers using target and nontarget analysis: A case of a heavily polluted river in South Africa

Sabetha Makoma Mapetla, Nondumiso Mofokeng, Heidi Richards, Kuria Ndungu, Luke Chimuka

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Manuscript 2: Investigating the occurrence of pharmaceuticals in urban estuarine water using grab and passive sampling approaches with UHPLC Q-Orbitrap MS analysis – A case of Durban Marina, South Africa

Sabetha Mapetla, Nondumiso Mofokeng, Heidi Richards, Brent Newman, Kuria Ndungu, Luke Chimuka

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Manuscript 3: The transport and distribution of pharmaceutical contaminants in the Crocodile River system, South Africa

Sabetha Mapetla, Nondumiso Mofokeng, Heidi Richards, Kuria Ndungu, Luke Chimuka

CONFERENCE OUTPUTS

Investigating the impact of desalination and mainland pollutants on marine life and coastal ecosystems of South Africa using a combination of passive samplers and ecotoxicology approaches (44th national SACI convention 2023)

Coupling solid phase extraction with passive sampling for the targeted analysis of pharmaceuticals in urban rivers – The case of Hennop's River (CHROMSA postgraduate workshop 2024)

ABSTRACT

Pharmaceutical pollution in aquatic ecosystems is a growing environmental concern due to its persistence as a result of the continuous output of pharmaceuticals, their potential to bioaccumulate, and the ecotoxicological risks they pose. This study employed a dual sampling approach, that is grab sampling and passive sampling to assess the occurrence, transport, and distribution of pharmaceutical contaminants in South African freshwater and estuarine systems. Ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-Q-Orbitrap MS) was used to analyse pharmaceutical residues across three case studies focusing on urban rivers and estuarine waters.

Chapters 1 and 2 provide a comprehensive review of the existing literature related to pharmaceutical residues in aquatic environments, with a particular focus on the South African context. Chapter 1 provides an overview of pharmaceutical pollution in aquatic ecosystems, highlighting its emergence as a critical environmental issue in South Africa. It discusses the sources, persistence, and ecological impacts of pharmaceutical contaminants, emphasizing their ability to remain biologically active at trace and ultra-trace concentrations. The chapter highlights the importance of effective sampling strategies in monitoring these pollutants, comparing traditional grab sampling with passive sampling techniques. It reviews the limitations of current approaches and identifies knowledge gaps, particularly in South African estuarine systems. The chapter concludes by advocating for an integrated sampling methodology to improve the accuracy and reliability of pharmaceutical pollution assessments, thereby informing better environmental management and policy development. Chapter 2 begins by exploring the global and local occurrence of pharmaceutical contaminants, outlining their sources, pathways, and environmental persistence. The chapter then examines various sampling techniques employed for monitoring these contaminants, specifically highlighting the use of grab sampling and passive sampling methods such as the Chemcatcher device. The strengths, limitations, and comparative efficiencies of these techniques are discussed. Analytical techniques for detecting pharmaceutical residues, especially high-resolution methods UHPLC-MS, are also reviewed. Finally, the chapter addresses the regulatory landscape, potential ecological and identifies existing knowledge gaps that justify the need for further research. This review lays the groundwork for the study's objectives by contextualizing the environmental threat posed by pharmaceutical pollutants and the methodologies suitable for their detection and monitoring.

Chapter 4 explored pharmaceutical pollution in the Hennops River and Hartbeespoort Dam, a heavily polluted system influenced by wastewater treatment plant (WWTP) effluents, agricultural run-off and untreated sewage discharges. In the study, a UHPLC-Q-Orbitrap MS method was developed and optimized for the analysis of carbamazepine, dexamethasone, etilefrine, methocarbamol, nevirapine and venlafaxine. Method optimization yielded SPE recoveries ranging from 55.18 – 80.52 % and 55.73 – 83.92 % for ultrapure and river water, respectively. Method LODs and LOQs ranged from 0.15 – 2.03 ng L^{-1} and 0.49 – 6.75 ng L^{-1}, respectively. A Chemcatcher passive sampler was calibrated for the uptake of the target pharmaceutical compounds. Five of the six pharmaceutical compounds showed good linearity ($R^2 > 0.97$) for the duration of the calibration experiment. Etilefrine, however, was only linear until day eight. As such, the sampling rate for etilefrine could not be determined. For the five compounds that showed good linearity over the 16-day calibration experiment, sampling rates ranging from 0.360 (dexamethasone) to 0.447 (carbamazepine) L day^{-1} were obtained. Applying the sampling rates, time-weighted average (TWA) concentrations in the ranges 0.50 (methocarbamol) – 17.87 (dexamethasone) µg L^{-1} were obtained. These were compared to SPE extract concentrations, which ranged from <LOD (venlafaxine) – 84.71 (dexamethasone) µg L^{-1}. Although the SPE maximum extract concentrations were generally higher than Chemcatcher maximum TWA concentrations, Chemcatcher demonstrated enhanced sensitivity, and in some cases captured TWA concentrations that were up to five times higher than SPE. In suspect screening, 25 pesticides and 61 pharmaceutical compounds were detected with the detection frequency higher for Chemcatcher compared to SPE extracts.

Chapter 5 investigated the occurrence of pharmaceutical compounds in the Durban Marina estuary using both sampling approaches. The study was expanded to nine pharmaceutical compounds with the addition of lopinavir, metformin and trimethoprim, however, these compounds were not included in the laboratory calibration of the Chemcatcher sampler in seawater. In the Chemcatcher calibration experiment, five compounds showed good linearity ($R^2 > 0.981$). The exception was etilefrine, which was linear only until day eight. The seawater laboratory calibration experiment yielded sampling rates from 0.34 (dexamethasone) – 0.42 (carbamazepine & venlafaxine) L day^{-1}. The analytical method was optimized and SPE recoveries were in the ranges 74.82–99.45% in seawater, and 72.24–103.42% in ultrapure water. Method LODs and LOQs were found to range from 0.15 to 2.18, and 0.50 to 7.27 ng L^{-1}, for venlafaxine and methocarbamol, respectively. Chemcatcher consistently shows a higher detection frequency (88.89%) across all sampling sites, whereas SPE detection frequencies

varied more across sites (55.56 – 77.78%). Extract concentration sums per site were found to be higher in Chemcatcher (up to 2574.52 ng L^{-1} at S2) than SPE (up to 43.81 ngL^{-1} at S1). Applying the experimental sampling rates, TWA concentrations up to 290.18 ng L^{-1} (dexamethasone) at S5 were determined. These were compared to the grab water sample concentrations in the sampling medium, which reached a maximum of 0.1594 ng L^{-1} (nevirapine) at S2. Passive samplers revealed the persistent presence of these contaminants, while grab sampling showed fluctuating concentrations. The study also highlighted the influence of tidal dynamics on pharmaceutical distribution, emphasising the limitations of grab sampling in estuarine environments.

Chapter 6 expanded on Chapter 4 by examining the transport and distribution of the nine target pharmaceutical compounds in the Crocodile River system after it exits Hartbeespoort Dam, using the dual sampling approach. The Crocodile River system is a critical water source, which serves mostly agricultural, domestic and industrial activities. The study identified significant pharmaceutical loads, with Chemcatcher consistently demonstrating a higher detection frequency, reaching 100% at S2 and S3, while SPE exhibited greater variability, with detection frequencies ranging from 44.44% (S5) to 88.89% (S2 & S3). As with detection frequency, pharmaceutical concentration sums per site in the extracts were found to be higher with Chemcatcher (up to 1191.05 ng L^{-1} at S2), confirming passive sampling advantages in long-term monitoring. The major contributors to Chemcatcher concentrations were found to be methocarbamol and etilefrine. S3 and S5 exhibited the lowest concentration sums (447.05 ng L^{-1} and 388.60 ng L^{-1}, respectively), despite S3 having the highest detection frequency. Time-weighted average (TWA) concentrations confirmed methocarbamol as the most persistent compound (68.34 ng L^{-1} at S1), followed by trimethoprim (29.52 ng L^{-1} at S3) and lopinavir (30.13 ng L^{-1} at S1). Contaminant transport was influenced by anthropogenic inputs, dilution, and tributary contributions, particularly from the Gwatlhe, Hex and Elands Rivers, which increased contaminant loads at S7 (518.10 ng L^{-1}). The transport and distribution of pharmaceutical contaminants in the Crocodile River system exhibited spatial variability influenced by multiple factors, including anthropogenic inputs, hydrological processes, and the complexity of tributary networks.

Overall, this research highlights the widespread occurrence of pharmaceuticals in South African aquatic environments and emphasises the importance of incorporating passive sampling to capturing low-concentration contamination trends. The study advocates for

integrating passive sampling into routine monitoring programs to enhance the detection, management, and mitigation of pharmaceutical pollution.

DEDICATION

This work is dedicated to me.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
API	Active pharmaceutical ingredients
ARV	Antiretroviral
CFU	Colony forming unit
D	Detected
DA	Dalton
DTG	Diffusive in thin
GC	Gas chromatography
HIV	Human immune deficiency virus
HRMS	High resolution mass spectrometry
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MPT	Microporous polyethylene tube
MS	Mass spectrometry
m/z	Mass to charge ratio
NA	Not analysed
ND	Not detected
NSAID	Non-steroidal anti-inflammatory drugs
OCP	Organochlorine pesticides
PAH	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyl
POCIS	Polar organic chemical integrative sampler
QA	Quality assurance
QC	Quality control
PSU	Practical salinity unit
Rs	Sampling rate
SPE	Solid-phase extraction
SPMD	Semi-permeable membrane devices
t	time
UHPLC	Ultra-high pressure liquid chromatography
WBL	Water boundary layer
WWTP	Wastewater treatment plant

LIST OF SYMBOLS

μ	micro
g	gram
L	litre
n	nano
s	second
m	metre

CHAPTER 1 INTRODUCTION

1.1 Introduction

Water pollution is a global environmental challenge, with pharmaceutical contaminants emerging as a significant threat to aquatic ecosystems. Pharmaceutical compounds, including antibiotics, hormones, painkillers, antidepressants, and other medicinal drugs, enter water bodies through wastewater discharge, agricultural runoff, hospital effluent, and improper disposal of expired medications (Ang et al., 2021; Bahlmann et al., 2014; Martínez-Piernas et al., 2021; Munzhelele et al., 2024; Vaudreuil et al., 2022; Wang et al., 2021). Unlike conventional pollutants, pharmaceuticals are designed to be biologically active at low concentrations, making their presence in aquatic environments particularly concerning. Many of these compounds also exhibit varied environmental half-lives, which range from minutes to several months. This allow them to persist in water bodies and exert prolonged ecological effects (Phillips et al., 2010; Rogowska and Zimmermann, 2022; Vaudreuil et al., 2022).

The accurate assessment of contaminants in any environmental system relies on the initial step of sampling, as this process fundamentally determines the accuracy and reliability of subsequent results. In the case of aquatic ecosystems, in which complex and dynamic processes influence contaminant distribution, the importance of applying suitable sampling methods cannot be overstated. The assessment of pharmaceutical pollution, which is a growing concern is no exception. Effective sampling techniques are critical for capturing representative data that reflects the true extent of contamination. Traditional methods such as grab sampling and more advanced approaches like passive sampling each have unique advantages and limitations (Cristóvão et al., 2021). As such, understanding the strengths, weaknesses, and environmental suitability of these techniques is crucial for designing reliable pharmaceutical pollution monitoring programs. Selecting an appropriate sampling method not only improves the accuracy of pharmaceutical pollution assessments but also aids in developing effective mitigation strategies to protect aquatic ecosystems.

One such sampling approach is grab-sampling. It involves the collection of discrete water samples at specific points and time. This method is straightforward, cost-effective, and widely used in water quality monitoring campaigns. It provides a snapshot of contamination levels, which is valuable for detecting episodic pollution events. However, grab sampling is labour intensive, particularly when dealing with large volumes of water or multiple sampling sites.

Additionally, it may not capture temporal variations in contaminant concentrations, leading to an incomplete representation of pollution levels (Ma et al., 2009).

Grab sampling has been extensively used in South African rivers for water quality monitoring, often focusing on parameters such as nutrient levels, microbial contaminants, and heavy metals (Adams et al., 2020; Atangana and Oberholster, 2021; Hoorzook et al., 2021). It has also been used to assess pharmaceutical pollution in aquatic ecosystems of South Africa (Agunbiade and Moodley, 2015; Horn et al., 2020; Monapathi et al., 2021). However, its application to pharmaceutical pollution has limitations. Given the episodic nature of pharmaceutical discharges, such as those from WWTP or stormwater runoff, grab sampling alone may fail to provide an accurate picture of contamination levels. Additionally, when investigating pollution levels in water bodies such as the marine ecosystem, where dilution may lead to contaminants going undetected due to instrumentation detection limits, grab sampling may not be ideal (Zhu et al., 2019). These limitations highlight the importance of complementing grab sampling with other techniques to improve data reliability.

Passive sampling, in contrast, offers a time-integrated approach to contaminant monitoring. It relies on the natural diffusion or partitioning of chemicals into a sampler over an extended deployment period. For this reason, it provides a more comprehensive view of pollution levels (Vrana et al., 2005). This technique is particularly advantageous for detecting trace-level contaminants and accounting for temporal fluctuations in pollution levels. Passive samplers are also cost-effective in the long term, as they require fewer site visits and minimal maintenance. Despite these advantages, passive sampling has limitations. These include sensitivity to environmental variables such as water flow, fouling, and the need for careful calibration (Caban, Lis and Stepnowski, 2022; Wang et al., 2022).

Passive samplers have been increasingly deployed in South African rivers and estuaries to monitor a wide range of pollutants, including pesticides and heavy metals (Amdany et al., 2014; Rimayi et al., 2019; Motsoane et al., 2020; Khulu et al., 2022). Their application to pharmaceutical monitoring, however, remains underexplored. By integrating passive sampling into existing monitoring studies, the detection of pharmaceuticals can be enhanced, particularly those present in trace concentrations that may elude conventional methods such as grab sampling.

Pharmaceutical contaminants in South African rivers and estuaries present a significant and growing environmental challenge. Rivers serve as critical pathways, transporting pharmaceutical residues from urban centres, agricultural fields, and industrial sites into estuarine and subsequently the marine environments. The high variability of river flow, coupled with periodic droughts, high tidal movements in estuaries, and floods in South Africa, further complicates the monitoring of pharmaceutical pollution (Dube, Nhamo and Chikodzi, 2022). These contaminants encompass a wide range of pharmaceutical classes, including antibiotics, anti-inflammatory drugs, analgesics, hormones, antiretrovirals (ARVs), and psychotropic substances, intended for human and veterinary use (Manickum and John, 2014; Gumbi et al., 2017; Faleye et al., 2019; Kamika et al., 2021). Once introduced into the environment, some of these compounds can persist due to their chemical stability and bioactivity (Yoshioka and Aso, 2007). Their presence disrupts aquatic ecosystems by altering microbial community structures, impacting the health and behaviour of non-target organisms, and exacerbating the global issue of antibiotic resistance (Vermeirssen et al., 2005; Watanabe et al., 2016).

South Africa's rivers, such as the Vaal, Crocodile, Umgeni, and Hennop's, are particularly vulnerable to pharmaceutical pollution (Wepener et al., 2011; Agunbiade and Moodley, 2014; Arukwe et al., 2016; James and Simatele, 2024). These systems receive substantial contaminant loads as a result of inefficient or outdated WWTP infrastructure, rapid urbanization, and agricultural runoff consisting of pharmaceutical residues, and their metabolites among other contaminants. For instance, the Crocodile River downstream of Hartbeespoort Dam is heavily influenced by inputs from urban, agricultural and industrial areas, while the Hennop's River is impacted by untreated sewage discharges (Rimayi et al., 2018). The pollution strain on South African rivers is amplified by country's variable hydrological patterns, which include prolonged droughts and flash floods, which influence the transport, dilution, and concentration of pollutants (Bangira et al., 2015; Botai et al., 2016).

Similarly, estuarine environments, such as False Bay in the Western Cape (Ojemaye and Petrik, 2022), Swartkops Estuary in the Eastern Cape (Gyedu-Ababio, 2011) and industrial hubs like the Durban Harbour in KwaZulu Natal, function as sinks for contaminants carried by rivers (Ngubane et al., 2019). These estuaries are highly complex systems, where fluctuations in salinity, tidal mixing, and seasonal changes create dynamic pathways for contaminant dispersion and transformation. The interaction of fresh and saline water further complicates the behaviour and fate of pharmaceuticals, influencing their bioavailability and potential toxicity

(Letsinger et al., 2019). The Durban Harbour, for example receives a combination of industrial and WWTP discharges, urban runoff, and shipping activities contribute to the contamination levels, making it a critical area for pharmaceutical pollution monitoring (Forbes and Demetriades, 2008).

Despite the ecological and socio-economic importance of these aquatic systems, research on pharmaceutical pollution in South African estuaries remains limited (Munzhelele et al., 2024). Most existing studies focus on rivers and broader water quality parameters, leaving critical gaps in understanding the occurrence, distribution, and ecological impacts of pharmaceuticals (Ngubane et al., 2019; Weideman, Perold and Ryan, 2020; Gani et al., 2021). Furthermore, current research primarily relies on grab sampling, which has inherent limitations in capturing fluctuating contamination levels (Ngubane et al., 2019; Ojemaye and Petrik, 2022; Netshithothole and Madikizela, 2024; Newman et al., 2024). These gaps hinder the development of effective water management policies and the implementation of targeted mitigation strategies to address pharmaceutical contamination in marine ecosystems.

It is therefore essential to advance pharmaceutical pollution investigations by integrating passive sampling with grab sampling. This combined approach allows for distinguishing between transient spikes and persistent contamination, enhances the detection of contaminants across a wide concentration range, and ensures data accuracy, ultimately strengthening confidence in findings. Such studies will provide valuable insights into the transport and fate of pharmaceuticals, their interactions with environmental variables, and their effects on aquatic life.

REFERENCES

Adams, J. *et al.* (2020) 'Nutrient enrichment as a threat to the ecological resilience and health of South African microtidal estuaries', *Afr. J. Aquat. Sci.*, 45(1–2), 23–40. doi.org/10.2989/16085914.2019.1677212.

Agunbiade, F.O. and Moodley, B. (2014) 'Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa', *Environ. Monit. Assess.*, 186(11), 7273–7291. doi.org/10.1007/s10661-014-3926-z.

Agunbiade, F.O. and Moodley, B. (2015) 'Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, Kwazulu-Natal, South Africa', *Environ. Toxicol. Chem.*, 35(1), 36–46. doi.org/10.1002/etc.3144.

Amdany, R. *et al.* (2014) 'Assessment of bioavailable fraction of POPS in surface water bodies in Johannesburg City, South Africa, using passive samplers: an initial assessment', *Environ. Monit. Assess.*, 186(9), 5639–5653. doi.org/10.1007/s10661-014-3809-3.

Arukwe, A. *et al.* (2016) 'Developmental alterations and endocrine-disruptive responses in farmed Nile crocodiles (Crocodylus niloticus) exposed to contaminants from the Crocodile River, South Africa', *Aquat. Toxicol.*, 173, 83–93. doi.org/10.1016/j.aquatox.2015.12.027.

Atangana, E. and Oberholster, P.J. (2021) 'Using heavy metal pollution indices to assess water quality of surface and groundwater on catchment levels in South Africa', *J. Afr. Earth Sci.*, 182, p. 104254. doi.org/10.1016/j.jafrearsci.2021.104254.

Bangira, T. *et al.* (2015) 'Investigating flash floods potential areas using ASCAT and TRMM satellites in the Western Cape Province, South Africa', *Geocarto Int.*, 30(7), 737–754. doi.org/10.1080/10106049.2014.997302.

Botai, C. *et al.* (2016) 'Characteristics of Droughts in South Africa: A Case Study of Free State and North West Provinces', *Water*, 8(10), 439. Available at: https://doi.org/10.3390/w8100439.

Caban, M. *et al*. (2022) 'Limitations of Integrative Passive Samplers as a Tool for the Quantification of Pharmaceuticals in the Environment – A Critical Review with the Latest Innovations', *Crit. Rev. Anal. Chem.*, 52(6), pp. 1386–1407. doi.org/10.1080/10408347.2021.1881755.

Cristóvão, M.B. *et al.* (2021) 'Detection of anticancer drugs in wastewater effluents: Grab versus passive sampling', *Sci. Total Environ.*, 786, 147477. doi.org/10.1016/j.scitotenv.2021.147477.

Dube, K., *et al* (2022) 'Flooding trends and their impacts on coastal communities of Western Cape Province, South Africa', *GeoJournal*, 87(S4), 453–468. doi.org/10.1007/s10708-021-10460-z.

Faleye, A.C. *et al.* (2019) 'Concentration and reduction of antibiotic residues in selected wastewater treatment plants and receiving waterbodies in Durban, South Africa', *Sci. Total Environ.*, 678, 10–20. doi.org/10.1016/j.scitotenv.2019.04.410.

Forbes, A.T. and Demetriades, N.T. (2008) *Estuaries of Durban, KwaZulu-Natal, South Africa. Report for the Environmental Management Department, eThekwini Municipality*. Durban.

Gani, K.M. *et al.* (2021) 'Emerging contaminants in South African water environment- a critical review of their occurrence, sources and ecotoxicological risks', *Chemosphere*, 269, 128737. doi.org/10.1016/j.chemosphere.2020.128737.

Gumbi, B.P. *et al.* (2017) 'Assessment of nonsteroidal anti-inflammatory drugs by ultrasonic-assisted extraction and GC-MS in Mgeni and Msunduzi river sediments, KwaZulu-Natal, South Africa', *Environ. Sci. Pollut. Res.*, 24(24), 20015–20028. doi.org/10.1007/s11356-017-9653-6.

Gyedu-Ababio, T.K. (2011) 'Pollution Status of Two River Estuaries in the Eastern Cape, South Africa, based on Benthic Meiofauna Analyses', *J. Water Resource Prot.*, 03(07), 473–486. doi.org/10.4236/jwarp.2011.37057.

Häder, D.P. *et al.* (2020) 'Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications', *Sci. Total Environ.*, 713, 136586. doi.org/10.1016/j.scitotenv.2020.136586.

Hoorzook, K.B. *et al.* (2021) 'Soul of the Jukskei River: The Extent of Bacterial Contamination in the Jukskei River in Gauteng Province, South Africa'Int. J. Environ. Res. Public Health, 18(16), 8537. doi.org/10.3390/ijerph18168537.

Horn, S. *et al.* (2020) 'Impact of Potential COVID-19 Treatment on South African Water Sources Already Threatened by Pharmaceutical Pollution', *Environ. Toxicol. Chem.*, 39(7), 1305–1306. doi.org/10.1002/etc.4734.

James, L.N. and Simatele, M.D. (2024) 'Bystanders or active participants? Mobilising meaningful participation in River Basin management: lessons from the Gauteng Province, South Africa', *Int. J. River Basin Manag.*, 1–15. doi.org/10.1080/15715124.2024.2417405.

Kamika, I. *et al.* (2021) 'The occurrence of opioid compounds in wastewater treatment plants and their receiving water bodies in Gauteng province, South Africa', *Environ. Pollut.*, 290, 118048. doi.org/10.1016/j.envpol.2021.118048.

Khulu, S. *et al.* (2022) 'Development and application of a membrane assisted solvent extraction-molecularly imprinted polymer based passive sampler for monitoring of selected pharmaceuticals in surface water', *Water Res.*, 225, 119145. doi.org/10.1016/j.watres.2022.119145.

Letsinger, S. *et al.* (2019) 'Spatial and temporal occurrence of pharmaceuticals in UK estuaries', *Sci. Total Environ.*, 678, 74–84. doi.org/10.1016/j.scitotenv.2019.04.182.

Ma, J.S. *et al.* (2009) 'Sampling Issues in Urban Runoff Monitoring Programs: Composite versus Grab', *Environ. Eng.*, 135(3), 118–127. doi.org/10.1061/(ASCE)0733-9372(2009)135:3(118).

Manickum, T. and John, W. (2014) 'Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa)', *Sci. Total Environ.*, 468–469, 584–597. doi.org/10.1016/j.scitotenv.2013.08.041.

Monapathi, M.E. *et al.* (2021) 'Pharmaceutical Pollution: Azole Antifungal Drugs and Resistance of Opportunistic Pathogenic Yeasts in Wastewater and Environmental Water', *Appl. environ. soil sci.*, 2021, 1–11. doi.org/10.1155/2021/9985398.

Motsoane, N. *et al.* (2020) 'Technical development and optimisation of a passive sampler based on polymer inclusion membrane for uptake of copper, nickel, cobalt and cadmium in surface waters', *Environ. Technol. Innov.*, 19, 100939. doi.org/10.1016/j.eti.2020.100939.

Munzhelele, E.P. *et al.* (2024) 'Pharmaceutical Contaminants in Wastewater and Receiving Water Bodies of South Africa: A Review of Sources, Pathways, Occurrence, Effects, and Geographical Distribution', *Water*, 16(6), 796. doi.org/10.3390/w16060796.

Netshithothole, R. and Madikizela, L.M. (2024) 'Occurrence of Selected Pharmaceuticals in the East London Coastline Encompassing Major Rivers, Estuaries, and Seawater in the Eastern Cape Province of South Africa', *ACS Meas. Sci. Au.*, 4(3), 283–293. doi.org/10.1021/acsmeasuresciau.4c00004.

Newman, B.K. *et al.* (2024) 'Occurrence and potential hazard posed by pharmaceutically active compounds in coastal waters in Cape Town, South Africa', *Sci. Total Environ.*, 949, 174800. doi.org/10.1016/j.scitotenv.2024.174800.

Ngubane, N.P. *et al.* (2019) 'Determination of naproxen, diclofenac and ibuprofen in Umgeni estuary and seawater: A case of northern Durban in KwaZulu–Natal Province of South Africa', *Reg. Stud. Mar. Sci.*, 29, 100675. doi.org/10.1016/j.rsma.2019.100675.

Ojemaye, C.Y. and Petrik, L. (2022) 'Pharmaceuticals and Personal Care Products in the Marine Environment Around False Bay, Cape Town, South Africa: Occurrence and Risk-Assessment Study', *Environ. Toxicol. Chem.*, 41(3), 614–634. doi.org/10.1002/etc.5053.

Phillips, P.J. *et al.* (2010) 'Pharmaceutical Formulation Facilities as Sources of Opioids and Other Pharmaceuticals to Wastewater Treatment Plant Effluents', *Environ. Sci. Technol.*, 44(13), 4910–4916. doi.org/10.1021/es100356f.

Rimayi, C. *et al.* (2018) 'Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa', *Sci. Total Environ.*, 627, 1008–1017. doi.org/10.1016/j.scitotenv.2018.01.263.

Rimayi, C. *et al.* (2019) 'Use of the Chemcatcher® passive sampler and time-of-flight mass spectrometry to screen for emerging pollutants in rivers in Gauteng Province of South Africa', *Environ. Monit. Assess.*, 191(6), 388. doi.org/10.1007/s10661-019-7515-z.

Rogowska, J. and Zimmermann, A. (2022) 'Household Pharmaceutical Waste Disposal as a Global Problem—A Review', *Int. J. Environ. Res. Public Health*, 19(23), 15798. doi.org/10.3390/ijerph192315798.

Vaudreuil, M.A. *et al.* (2022) 'Pharmaceutical pollution of hospital effluents and municipal wastewaters of Eastern Canada', *Sci. Total Environ.*, 846, 157353. doi.org/10.1016/j.scitotenv.2022.157353.

Vermeirssen, E.L.M. *et al.* (2005) 'Characterization of Environmental Estrogens in River Water Using a Three Pronged Approach: Active and Passive Water Sampling and the Analysis of Accumulated Estrogens in the Bile of Caged Fish', *Environ. Sci. Technol.*, 39(21), 8191–8198. doi.org/10.1021/es050818q.

Vrana, B. *et al.* (2005) 'Passive sampling techniques for monitoring pollutants in water', *TrAC, Trends Anal. Chem.*, 24(10), 845–868. doi.org/10.1016/j.trac.2005.06.006.

Wang, P. *et al.* (2022) 'Effects of biofouling on the uptake of perfluorinated alkyl acids by organic-diffusive gradients in thin films passive samplers', *Environ. Sci.: Process. Impacts.*, 24(2), 242–251. doi.org/10.1039/D1EM00436K.

Watanabe, H. *et al.* (2016) 'Chronic toxicity of an environmentally relevant mixture of pharmaceuticals to three aquatic organisms (alga, daphnid, and fish)', *Environ. Toxicol. Chem.*, 35(4), 996–1006. doi.org/10.1002/etc.3285.

Weideman, E.A. *et al* (2020) 'Limited long-distance transport of plastic pollution by the Orange-Vaal River system, South Africa', *Sci. Total Environ.*, 727, 138653. doi.org/10.1016/j.scitotenv.2020.138653.

Wepener, V. *et al.* (2011) 'An assessment of the influence of multiple stressors on the Vaal River, South Africa', *Phys. Chem. Earth*, 36(14–15), 949–962. doi.org/10.1016/j.pce.2011.07.075.

Yoshioka, S. and Aso, Y. (2007) 'Correlations between Molecular Mobility and Chemical Stability During Storage of Amorphous Pharmaceuticals', *J. Pharm. Sci.*, 96(5), 960–981. doi.org/10.1002/jps.20926.

Zhu, J. *et al.* (2019) 'Dilution characteristics of riverine input contaminants in the Seto Inland Sea', *Mar. Pollut. Bull.*, 141, 91–103. doi.org/10.1016/j.marpolbul.2019.02.029.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

The first report on the presence of pharmaceutical compounds in the aquatic environment was in the 1970's (Tabak and Bunch, 1970). This came about as a leap in technological and instrumental advancements in the simultaneous detection and quantification of these compounds in complex aqueous matrices was made (Majors, 1980; Zubillaga and Maerker, 1990). As it became evident that WWTP do not completely remove these compounds prior to effluent release, their environmental fate emerged as a critical area of study (Waleng and Nomngongo, 2022). Pharmaceutical compounds are a diverse group of complex molecules used in both veterinary and human medicine. They serve as therapeutic and diagnostic agents for a host of illnesses and diseases (Boxall *et al.*, 2012). Due to their broad nature, this class of compounds is categorized into subclasses based on their chemical properties. The most common subclass comprises of organic pharmaceutical compounds, which can further be divided into small and large molecule drugs (Bergeron and Tripp, 2020).

Small molecule drugs are synthetic compounds which comprise of active pharmaceutical ingredients (APIs) that typically have molecular weights up to 900 daltons. These drugs are usually administered orally and diffuse rapidly through cell membranes to reach intracellular sites of action. They constitute over 90% of pharmaceuticals on the market, including widely used medications such as aspirin, insulin, and antihistamines (Rao, Kabir and Mohamed, 2010). In contract, large pharmaceutical compounds are biological in origin. That is, they are made up of proteins comprised of thousands of amino acids. These drugs target specific genotype or protein receptors as their mode of action (Chakrabarti, Wylie and Schuster, 1989). Unlike small molecule drugs, these are derived from natural sources. Advances in biotechnology have made it possible to isolate these compounds, and through retrosynthesis, they can be synthetically reproduced (Feher and Schmidt, 2003). Examples of this class of pharmaceutical compounds include hormonal regulators such as insulin, which can be administered orally or through injections (Ameller *et al.*, 2004). This study focuses on the persistence of selected organic small molecule drugs from varying therapeutic classes in South African rivers and estuaries.

2.2 Properties of pharmaceutical compounds

Pharmaceutical compounds vary greatly in physiochemical properties due to the different functional groups they contain. These functional groups dictate the functions they are intended

for. These compounds are characterized by relatively high molecular weights, typically ranging from 100 to 1000 DA. The diversity of this group of compounds can be attributed to their multifunctionality as they are composed of a single or a combination of multiple function groups which include amines (primary, secondary, or tertiary), carboxylic acids, alcohols, polycyclic, aromatic/aliphatic, conjugated systems to only name a few (Kolář *et al.*, 2002).

Due to the ionizability of most pharmaceutical compounds, they undergo equilibrium processes which are highly dependent on the pH of the medium they are present in. This is because pharmaceuticals can either be acidic or basic, and can be present as cations, anions, or as compounds bearing both a positive and negative charge (Reinholds *et al.*, 2017). Very few pharmaceuticals are present as zwitterions, that is as compounds bearing both a positive and negative charge with a net charge of zero (Mazzenga and Berner, 1991). As such, their ionizability plays a key role in their fate in the environment. That is, anionic pharmaceuticals will have high solubility factors in surface waters, whereas cationic pharmaceuticals will undergo ionic complexation with minerals and clay which are found in soils and sediments (Xu *et al.*, 2021).

Another important parameter when looking at the persistence of pharmaceutical compounds in the environment is their octanol-water partition factor. This parameter can be generally defined as the distribution of a chemical substance between a biphasic system that consists of oil and water. This is very important as it gives an understanding on the hydrophilicity and lipophilicity of chemical compounds. This becomes useful in the profiling of pharmaceuticals in the environment as their distribution in surface water, soil, sediment, sludge and lipids of biota can be predicted (Chmiel *et al.*, 2019). This is especially important in risk assessment studies that look at the bioaccumulation of pharmaceutical compounds in aquatic species and their subsequent toxicity (Yamamoto *et al.*, 2009).

The half-lives of pharmaceutical compounds are important in predicting the fate these compounds. The elimination rate of a drug can be defined as the duration of time required for the concentration of the drug to be reduced to half of its initial dose in the body. The half-life of pharmaceuticals gives an understanding on the how much of a particular drug will be excreted from the body, which is useful in gaining knowledge of how most drugs end up in WWTPs. This is also important in pharmaceutical bioaccumulation studies in aquatic species. Because of the broad range of pharmaceutical compounds, their properties also vary greatly.

This is no exception for their elimination rates. The half-lives of pharmaceuticals can be as short as one hour as is the case for lidocaine, or as long as 18 days, as is the case for leflunomide (Teschner and Burst, 2010; Martell *et al.*, 2017).

2.3 Fate and transport of pharmaceuticals in the environment

Understanding the physicochemical properties of pharmaceutical compounds is essential for evaluating their environmental fate. Three key factors in this process are their ability to partition into lipids, bioavailability, and adsorption into environmental matrices (Róg, Girych and Bunker, 2021; Nyamba *et al.*, 2024). However, before assessing their behaviour in the environment, it is necessary to understand their sources.

Pharmaceutical compounds enter the environment through various pathways. The primary source of pharmaceuticals in aquatic environments is secretion post-human consumption, while disposal from the manufacturing industry represents a secondary source (Larsson, de Pedro and Paxeus, 2007; Abdel Hady *et al.*, 2021).

After human consumption, many APIs are not fully metabolized. For instance, only about 70% of some antibiotics, such as sulfamethoxazole, are metabolized, leaving up to 30% of the drug to be excreted unchanged (Masters *et al.*, 2003). However, the extent of API metabolism varies between drugs. Some pharmaceuticals, such as paracetamol, undergo extensive metabolism, leaving only a minimal fraction excreted unchanged in urine. In adults, approximately 1–4% of an administered dose of paracetamol is excreted unchanged, indicating near-complete metabolism (Allegaert *et al.*, 2015). The incomplete metabolism of APIs results in the excretion of unchanged APIs along with their metabolites into sewage systems. These compounds may react with other chemicals in sewage systems to form new compounds or remain unchanged as they travel to WWTPs (Nguyen *et al.*, 2021).

In WWTPs, influent is treated using a combination of methods such as filtration, chemical oxidation, and biological processes. These treatment processes generate by-products, such as sludge, and biogas in plants using anaerobic processes. The primary output of WWTPs is effluent, which may be discharged into the environment via water bodies (Liang *et al.*, 2022). Although WWTP technologies are effective in removing many contaminants, not all APIs are eliminated prior to effluent discharge (Bisognin *et al.*, 2021). As a result, some pharmaceutical residues persist and enter aquatic ecosystems, potentially impacting the environment. Up to

100 µg L^{-1} of acetaminophen was detected in effluent samples from a WWTP in Puno, Peru. Other drugs detected include clarithromycin, trimethoprim, ciprofloxacin, sulfamethoxazole and azithromycin. Their concentrations ranged from 1.86 to 4.47 µg L^{-1} (Nieto-Juárez *et al.*, 2021).

Hospitals represent another major source of pharmaceuticals in the environment. Effluent from hospitals contains APIs, their metabolites, diagnostic agents, disinfectants, and other contaminants. This waste is often discharged into public sewer networks and treated alongside domestic effluent, making it challenging to monitor pharmaceutical residues specifically from hospital sources. The variability in pharmaceutical outputs from hospitals further complicates monitoring. Factors such as the types of treatments provided, the drugs used, and the volume of pharmaceuticals administered at any given time influence the composition of hospital effluent (Vaudreuil *et al.*, 2022). Sulfamethoxazole, naproxen, diclofenac, ibuprofen, acetaminophen, carbamazepine, iopromide, atenolol, and caffeine were detected in WWTP from 12 hospitals in northern Vietnam (Hoang, Do and Kim, 2024).

The pharmaceutical manufacturing industry also contributes to environmental contamination, albeit as a secondary source compared to post-human consumption. During the production of pharmaceuticals, waste streams containing APIs and their intermediates are generated. These waste streams may arise from equipment cleaning, production errors, or residual substances in discarded materials (Larsson, de Pedro and Paxeus, 2007). Although many manufacturing facilities implement strict waste management protocols and use advanced treatment technologies, such as high-temperature incineration and chemical neutralization, improper disposal practices or inadequate treatment can result in the release of APIs into the environment (Ang *et al.*, 2021). Discharges from manufacturing plants have been found to contain higher concentrations of certain pharmaceuticals compared to domestic or hospital effluent, especially in regions where regulatory enforcement is weak (Ogunbanwo *et al.*, 2022). In China, up to 34 antibiotics were detected in waste from a pharmaceutical manufacturing plant in concentrations up to 19 µg L^{-1}. These concentrations were found to be higher than those from WWTP effluent (up to 12 µg L^{-1}) in the same area (Wang *et al.*, 2021). These discharges can contribute to localized environmental hotspots, with potential impacts on aquatic ecosystems and the development of antimicrobial resistance (AMR).

2.4 Pharmaceuticals in South African freshwater ecosystems

Pharmaceutical residues in South Africa's freshwater systems have been detected at various concentrations, with antibiotics, analgesics, and hormones among the most commonly reported compounds (Farounbi and Ngqwala, 2020; Vumazonke, Khamanga and Ngqwala, 2020). Studies have shown that antibiotics in particular contribute to the development of AMR in aquatic environments, a critical concern for public health (Ateba *et al.*, 2020). Hormonal contaminants, such as synthetic estrogens, have been associated with endocrine disruption in fish populations, leading to reproductive anomalies and population declines in certain regions (Kroløkke, Bang and Hvidtfeldt, 2024). Additionally, the presence of anti-inflammatory drugs, beta-blockers, and antiretrovirals in surface waters has raised concerns about their long-term effects on aquatic biodiversity and ecosystem stability (Abafe *et al.*, 2018; Desbiolles *et al.*, 2018; Amos Sibeko *et al.*, 2019).

Research on pharmaceutical pollution in freshwater systems in South Africa has been growing, although it remains relatively limited compared to global studies. Nonetheless, several studies and monitoring efforts have highlighted critical hotspots and provided insight into the extent and impacts of pharmaceutical contamination. These hotspots include Vaal River System, Umgeni River Catchment and Hennop's River (Schoeman, 1979; Wepener *et al.*, 2011; Agunbiade and Moodley, 2014).

The Vaal River is a vital water source for domestic, industrial, and agricultural use. It has been a focal point for pharmaceutical pollution studies. The river receives significant wastewater inputs from densely populated urban areas and industrial activities, including pharmaceutical manufacturing (Iloms *et al.*, 2020). Monitoring efforts have detected the presence of antibiotics, anti-inflammatory drugs, and hormonal compounds in both the river and its tributaries. For example, sulphonamides, tetracyclines, and nonsteroidal anti-inflammatory drugs (NSAIDs) have been identified at concentrations that pose risks to aquatic organisms, including fish and invertebrates (Madikizela *et al.*, 2022). In the Klip River, a tributary of Vaal River, 47 pharmaceuticals were detected with targeted analysis and suspect screening. In the study, concentrations up to 0.257, 0.355 and 0.432 $\mu g\, L^{-1}$ for flumequine, oxolinic acid and acetaminophen, respectively, were reported (Madikizela *et al.*, 2022). In another study, the presence of pharmaceutical compounds was investigated in a stream which flows into the Klip River. The stream is contaminated with effluent from a WWTP and leachates from a municipal dumpsite. In water samples from the stream, 15 antibiotics were detected in concentrations up to 0.686 $\mu g\, L^{-1}$ (flumequine) (Ncube *et al.*, 2021).

The Umgeni River, a key water source for the KwaZulu-Natal province, has also been the subject of pharmaceutical pollution studies. The detection of select pharmaceutical compounds including antipyretic, antibiotics and antipsychotic drugs in wastewater, river water and sediments in the system has been reported (Matongo *et al.*, 2015). Wastewater treatment plants discharging into the river have been found to release antibiotics, antiretrovirals, and analgesics into the water. The presence of antiretrovirals, commonly used in South Africa's HIV treatment programs, is particularly concerning, as these compounds may have unanticipated ecological effects. Studies have also highlighted that traditional wastewater treatment processes in this region are ineffective at removing pharmaceuticals, resulting in their accumulation downstream. A WWTP releasing waste into the Umgeni River system was shown to reduce influent concentrations by 43.0–94.2 % before discharge (Agunbiade and Moodley, 2014). Another study showed the presence of the antiretroviral drugs atazanavir, efavirenz, lopinavir and nevirapine in both influent and effluent from WWTPs in KwaZulu Natal that discharge into the Umgeni River (Abafe *et al.*, 2018).

In the Eastern Cape, pharmaceutical pollution reflects the region's socioeconomic and infrastructural disparities. Urban areas, such as East London, have documented pharmaceutical residues in treated effluents discharged into rivers (Netshithothole *et al.*, 2024). Meanwhile, rural communities often rely on untreated surface water, which is vulnerable to pharmaceutical contamination from agricultural runoff and informal waste disposal practices (Netshithothole and Madikizela, 2024). Monitoring efforts in this region have identified antibiotics and endocrine-disrupting compounds as dominant contaminants, particularly in areas with intensive livestock farming. Ten endocrine disrupting hormones detected in river water and wastewater samples from a WWTP in Alice. Concentrations up to 1 μg L^{-1} for the hormone estrone were reported (Farounbi and Ngqwala, 2020).

Several barriers hinder effective monitoring efforts in South Africa. Limited funding and resources restrict the number of water bodies that can be studied, while a lack of uniform sampling protocols leads to inconsistent data. Additionally, the absence of specific regulations governing pharmaceutical pollution hampers the enforcement of pollution control measures. Addressing these challenges will require investment in infrastructure, capacity-building for researchers, and the development of national guidelines for monitoring and mitigating pharmaceutical contamination.

Case studies and monitoring efforts in South Africa have provided valuable insights into the scope and impacts of pharmaceutical pollution in freshwater environments. However, more extensive and systematic research is needed to fully understand the long-term implications and to inform policy decisions aimed at protecting water resources and ecosystems

2.5 Pharmaceuticals in estuarine and marine ecosystems of South Africa

The presence of pharmaceutical compounds in marine environments along South Africa's extensive coastline has become an emerging concern (Ojemaye and Petrik, 2022a). The South Africa's marine ecosystem, which supports diverse ecosystems is increasingly threatened by pharmaceutical pollution. The South African marine ecosystem comprises of salt marshes, mangrove forests, coral reefs, the open ocean, and the deep-sea ocean and estuaries, all of which contribute to the biodiversity and productivity of the marine environment. For the purpose of this study, South African estuaries will be the main focus.

Estuaries are biodiverse ecosystems which play an important role in the health of the marine environment (Reddering and Rust, 1990). Estuaries serve as breeding grounds for a host of aquatic organisms including fish and invertebrate. They aid in carbon storage which helps with climate regulation and serve as a channel for species to move between fresh and saline water (Howe, Rodríguez and Saco, 2009). Although estuaries form a small portion of the marine environment, their importance should not be underestimated. South Africa is home to 290 estuaries, 250 of which are classified as functional. Many of these are now classified as functionally degraded (van Deventer et al., 2025). The current state of South African estuaries can be attributed to stressors which include coastal development, industrial activities, human disturbance, and changes in salinity profiles due to disturbances in river water supply from activities that take place upstream, and wastewater discharge (Ramm, 1990). The deterioration in the quality of estuaries in South Africa has led to a loss of species (Cyrus, 1991).

Studies have detected a range of pharmaceutical compounds in South African marine environments. Among the most common are antibiotics, antidepressants, antiretrovirals, and NSAIDs (Ojemaye and Petrik, 2022). The widespread use of antiretroviral drugs in South Africa’s public health sector has resulted in their detection in marine sediments and water, particularly near urban outfalls (Ncube *et al.*, 2018). Hormonal compounds, such as synthetic

estrogens, have also been identified in coastal zones, raising concerns about endocrine disruption in marine species (Truter, Wyk and Newman, 2015).

Pharmaceuticals in marine environments can have diverse and far-reaching ecological impacts. Antibiotics, for instance, may disrupt marine microbial communities, which play a critical role in nutrient cycling and ecosystem functioning (Ding and He, 2010). The development of antibiotic-resistant bacteria in marine ecosystems is a significant concern, with implications for both marine biodiversity and public health (Kasanah and Hamann, 2004). Marine organisms, including fish, molluscs, and crustaceans, can bioaccumulate pharmaceutical compounds, which may alter their behaviour, reproduction, and physiological functions (Moreno-González *et al.*, 2016). For example, exposure to synthetic hormones has been linked to feminization and reduced fertility in marine species (Álvarez-Muñoz *et al.*, 2015). Similarly, antidepressants and anti-anxiety medications have been shown to affect the feeding and predator avoidance behaviours of marine organisms (Guler and Ford, 2010). These disruptions can cascade through the food web, ultimately affecting ecosystem stability and resilience. Several studies have begun to shed light on pharmaceutical pollution in South Africa's marine environments (Petrik *et al.*, 2017; Ngubane *et al.*, 2019).

2.5.1 Pharmaceutical pollution along the coast of KwaZulu natal

Coastal areas near Durban, a major urban and industrial hub, have shown elevated levels of pharmaceuticals in water and sediment samples. Two NSAIDs naproxen and ibuprofen were detected in estuarine and seawater in the Durban area. Their concentrations were 0.192 and 0.198 µg L^{-1} in Umgeni Estuary, 0.147 and 0.166 µg L^{-1} in Blue Lagoon Beach, and 0.157 µg L^{-1} and <LOD in Glen Ashley Beach, for naproxen and ibuprofen, respectively (Ngubane *et al.*, 2019). Effluents from WWTPs and river inputs from the Umgeni River have been shown to be significant sources. This was demonstrated when acetaminophen and atenolol were detected in concentrations exceeding 10 µg L^{-1} in the Umgeni River Estuary mouth (Agunbiade and Moodley, 2014). Another study reported the detection of efavirenz, emtricitabine and diclofenac in the Umgeni Estuary (Sigonya *et al.*, 2022). The sources of these compounds are linked to Umgeni River as a number of studies have reported the detection of these compounds in samples from the River (Matongo *et al.*, 2015; Gumbi *et al.*, 2017; Mlunguza *et al.*, 2019). The frequent detection of these drugs has raised concerns about their impact on local marine biodiversity (Rimayi *et al.*, 2018).

2.5.2 Pharmaceutical pollution along the coast of Western Cape

Research in Cape Town's coastal waters has highlighted the presence of pharmaceutical residues in areas with dense urban populations and high wastewater discharge. One such area with a dense urban population is Camps Bay, which is located 11 km Southwest of Cape Town. The detection of diclofenac, carbamazepine and acetaminophen has been reported in sea water samples from Camps Bay. These pharmaceutical compounds, as well as the antiretroviral drug lamivudine were also detected in beach sand and sediments from the same area. The study also evaluated the presence of these compounds in marine biota samples, focusing on two invertebrate and seaweed. Only diclofenac and lamivudine were detected in the marine biota samples (Ojemaye *et al.*, 2022). In False Bay, Cape Town, Diclofenac, was the most dominant compound identified. The concentrations reported range from 3.70–4.18 ng L^{-1} in seawater, 92.08–171.89 ng g^{-1} dry weight in sediment, 67.67–780.26 ng g^{-1} dry weight in marine invertebrates, and 101.50–309.11 ng g^{-1} dry weight in seaweed. These findings suggest that diclofenac persists and bioaccumulates across environmental compartments and trophic levels. The elevated levels in marine organisms raise concerns about potential ecological impacts and the transfer of contaminants through food webs (Ojemaye and Petrik, 2022b).

In seawater samples from Granger Bay, Cape Town, seven pharmaceutical compounds were detected in low concentrations up to 0.3 ng L^{-1}. These were acetaminophen, diclofenac, lamivudine, phenytoin, carbamazepine and sulfamethoxazole. Diclofenac showed the highest concentration of all pharmaceuticals that were investigated, highlighting its wide use in South Africa (Petrik *et al.*, 2017). A more recent and broader study investigated the presence of 58 pharmaceutical compounds in seawater samples from numerous sites spanning a wide area along the Cape coast, starting in Bloubergstrand, through Hout Bay beach and all the way to Kogel bay. Salicylic acid was the most prevalent, as it was detected at nearly all sites during both summer and winter. Frequently detected pharmaceuticals include acetaminophen, atenolol, codeine, and sulfamethoxazole, reflecting common usage in the region. Contamination was highest near river mouths and wastewater outlets, with False Bay showing greater pollution than the Atlantic Seaboard. Seasonal variations indicated higher pharmaceutical concentrations in summer, likely due to increased wastewater influence. Some of the compounds of interest were detected even in remote sites, highlighting their dispersion over long distances (Newman *et al.*, 2024).

2.5.3 Pharmaceutical pollution along the coast of Eastern Cape

In the Eastern Cape, coastal waters near Port Elizabeth and East London are affected by pharmaceutical runoff from rivers and agricultural activities. This was demonstrated in a recent study where the presence of five pharmaceuticals in Quenera and Gonubie River estuaries, as well as in seawater samples along the coast of East London was investigated. In the water samples from the estuaries, four of the five compounds under investigation were detected in concentrations up to 71 ng L^{-1}. These were ibuprofen, sulfamethoxazole, trimethoprim and efavirenz. Naproxen was not detected at all estuary sites. In the seawater samples, the concentration ranges in ng L^{-1} were 52–90, ND–57, 6–30, 1–15 and 15–76 for ibuprofen, naproxen, sulfamethoxazole, trimethoprim, and efavirenz, respectively (Netshithothole and Madikizela, 2024). In surface water and sediments from Buffalo and Sundays River estuaries, carbamazepine and trimethoprim were detected. These were present in concentrations that could potential pose a threat to aquatic life, based on the calculated risk quotients (Ohoro, Adeniji, et al., 2021). The authors also reported the detection of carbamazepine, trimethoprim and testosterone in sediments form Swartkops Estuary (Ohoro et al., 2021).

2.6 Traditional sampling techniques for pharmaceutical compounds in aquatic environment

Aquatic ecosystems are intricate environments with diverse physical and chemical properties that determine which species can thrive within them. These properties make pollution monitoring in aquatic systems a challenging task, as the conditions and characteristics of each ecosystem vary significantly (S. Giller *et al.*, 2004; Irfan and Alatawi, 2019). Three main types of aquatic ecosystems include marine, fresh and estuarine ecosystems.

Marine ecosystems are primarily composed of seas and oceans, characterized by salinities greater than 30 PSU. The high salinity and complex composition of marine waters support a distinct set of species and ecological interactions (Curtin and Prellezo, 2010). Freshwater environments, such as rivers, lakes, and dams, have significantly lower sodium content and salinities typically below 0.5 PSU. These ecosystems sustain a wide range of flora and fauna adapted to low-salinity conditions (Carpenter, Stanley and Vander Zanden, 2011). Estuaries represent a transitional zone between marine and freshwater systems, where salinity levels vary depending on the mixing ratio of saltwater and freshwater (Barbier *et al.*, 2011).

Aquatic ecosystems are highly dynamic and serve as critical habitats for numerous species, making their pollution monitoring particularly complex. To accurately assess pollution in

aquatic ecosystems, selecting an appropriate sampling technique is essential. The chosen method should provide a representative picture of water quality in the specific environment under investigation (Madrid and Zayas, 2007). This is important for understanding the impact of pollution on both the ecosystem and its inhabitants. This work will explore three key sampling techniques commonly used in pollution monitoring. Each technique has its strengths and limitations, making the selection of a suitable method dependent on the specific conditions and goals of the study. By examining these methods in detail, this discussion aims to highlight their roles in addressing the complexities of pollution monitoring, specifically pharmaceutical pollution monitoring in diverse aquatic ecosystems.

2.6.1 Grab sampling in aquatic systems

Grab sampling is a widely used method for collecting water samples at a specific location and time. It is the most commonly used sampling technique due to its straightforwardness. This technique involves using a container, such as a bottle, to collect a water sample, which is then transported to a laboratory for analysis. While the method is simple and useful for certain applications, it comes with both advantages and limitations (Martin, Smoot and White, 1992). The grab sampling process begins with sample collection, followed by laboratory preparation steps such as filtration to remove suspended particles and preconcentration to detect chemical contaminants. These preparatory steps can be labour intensive, particularly when dealing with large sample volumes, which adds complexity to the analysis process. The method is straightforward and requires minimal equipment, making it accessible and easy to implement in various settings. Grab sampling provides a momentary snapshot of water quality at a specific location and time. This makes it ideal for capturing episodic pollution events, such as spills or discharges, where immediate data is needed to assess the extent of contamination (Novic *et al.*, 2017).

Grab sampling provides data for a single point in time, making it insufficient for understanding variations in pollution levels over time or across different locations. If a sample is collected when a pollutant is absent, the method may fail to detect contamination, leading to a false negative. Water flow and weather conditions in natural systems are inconsistent, introducing variability into the data obtained through grab sampling. This variability can make it difficult to draw accurate conclusions about overall water quality. The accuracy of grab sampling is influenced by the location and timing of sample collection. Poor selection of sampling sites or times can lead to biased results that do not represent the broader water quality of the area. The

detection of trace-level contaminants may be limited due to the sensitivity of the instrumentation used for analysis. Without adequate preconcentration, pollutants present in low concentrations may go undetected (De Cesaris *et al.*, 2025).

Although the initial collection process is simple, the steps required to prepare water samples for laboratory analysis, particularly for large volumes, are time-consuming and labour intensive. While grab sampling is suitable for certain applications, such as detecting sudden pollution events or providing a quick assessment of water quality, its limitations must be carefully considered. For studies requiring long-term or broad-scale water quality monitoring, grab sampling may not provide the necessary data coverage. To overcome its limitations, grab sampling can be supplemented with other techniques, such as automatic sampling or passive sampling, to capture temporal and spatial variations more effectively (Wilson *et al.*, 2022). Ensuring that laboratory instruments are capable of detecting trace contaminants can improve the reliability of results (Tadić *et al.*, 2022). This can also be achieved by preconcentrating grab water samples prior to analysis (Lis, Stepnowski and Caban, 2021). By understanding its strengths and weaknesses, grab sampling can be effectively integrated into broader water quality monitoring programs, ensuring that data collected meets the specific needs of the study.

2.6.2 Automatic sampling

Automatic sampling is a method that utilizes specialized devices to collect water samples over a defined period without requiring continuous human intervention. These devices are equipped with pumps that regulate the volume of water collected, ensuring consistency and precision in sample acquisition (Martin, Thomas and Hartl, 2004). This technique has revolutionized water quality monitoring by offering a more streamlined and efficient approach, particularly in challenging and remote environments. Automatic samplers are convenient and efficient.

Once installed, automatic samplers operate independently, eliminating the need for onsite monitoring during sampling. This hands-off approach significantly reduces the workload for technicians, allowing them to focus on other tasks. Automatic samplers reduce the number of site visits. By minimizing the frequency of trips required to collect samples, this method reduces logistical challenges and travel expenses. For studies conducted in remote or hard-to-access areas, this advantage is particularly impactful, saving time, energy, and resources. Automatic sampling facilitates the collection of samples over an extended period, capturing temporal variations in pollution levels (Chapin, 2015). This provides a more comprehensive

representation of water quality compared to manual sampling, which might miss short-term fluctuations. Modern auto samplers come equipped with data logging and remote access capabilities.

These devices not only collect samples but can also perform preliminary analyses onsite, recording data that can be accessed remotely via the internet (Ore *et al.*, 2015). This feature enables real-time monitoring and decision-making, particularly beneficial for large-scale or long-term studies (Heinz *et al.*, 2013). While the initial investment in automatic samplers may be high, the long-term cost savings from reduced travel, labour, and logistical support can offset the upfront expenses. This is especially true for projects requiring extensive or continuous sampling. Despite all the attractive advantages, automatic sampling, as with any other sampling technique has its draw backs.

Automatic samplers are expensive to purchase, requiring a substantial upfront cost (Wilson, Miller and Lechner, 2024). This can be a barrier for small-scale studies or organizations with limited budgets. These devices are often deployed in outdoor environments, where they are exposed to harsh weather conditions, debris, and potential mechanical wear. Over time, these factors can lead to equipment malfunctions or failures, requiring repairs or replacements. Regular maintenance is essential to keep automatic samplers functioning effectively. Tasks such as battery replacements, cleaning, and calibration must be performed to ensure accurate and reliable operation. Neglecting maintenance can compromise data quality or result in equipment failure (Wahl, 1979). The risk of theft is a significant concern, particularly in unsecured or remote locations. Expensive equipment left unattended in the field may be targeted, leading to financial losses and interruptions in data collection (Fell, Pead and Winter, 2019). Automatic samplers, like all machinery, are not immune to errors. Failures in the pump mechanism, power supply, or data transmission systems can disrupt sampling efforts and compromise the integrity of the data collected.

2.7 Passive sampling

Passive sampling is an advanced analytical technique designed to accumulate contaminants from the environment over time (Vrana, Allan, *et al.*, 2005). It offers several advantages over traditional sampling methods such as grab sampling and automatic sampling, making it a valuable tool in pollution monitoring and environmental studies. Unlike grab sampling, which captures a single moment in time, passive sampling integrates contaminant levels over the

deployment period (Valenzuela, Menezes and Cardeal, 2020). This feature accounts for temporal variations, providing a more comprehensive representation of water quality.

Passive samplers are particularly effective at accumulating contaminants present in trace concentrations that might go undetected using grab sampling. By concentrating these chemicals in the sorbent phase of the sampler, they enhance the sensitivity of subsequent analysis (Shaw and Mueller, 2009). Passive samplers are simple to deploy and retrieve, requiring minimal technical expertise or operational complexity. They are ideal for long-term monitoring and do not require frequent site visits for sample collection, reducing logistical challenges and associated costs. Unlike automatic samplers that rely on a power supply for operation, passive samplers function without electricity. This makes them highly suitable for remote or inaccessible locations where power availability is limited.

Passive samplers can be customized to accumulate specific classes of compounds, such as hydrophobic organic pollutants or heavy metals (Roig *et al.*, 2011; Taylor *et al.*, 2021). This selectivity minimizes matrix interferences and eliminates the need for additional cleanup steps in the analytical process, saving time and effort. While passive sampling offers numerous advantages, it also comes with certain limitations that should be carefully considered before choosing this method for environmental monitoring.

Passive samplers provide time-integrated data, which means they cannot capture short-term or episodic pollution events effectively. Rapid changes in pollutant concentrations, such as those caused by spills or stormwater runoff, may be missed due to the averaging nature of the technique. Because passive samplers require deployment over an extended period to accumulate sufficient contaminants, they cannot provide real-time or immediate results. This can be a disadvantage in situations where prompt decision-making is required. In aquatic environments, biofouling can occur, potentially altering the sampling performance. Fouling can affect the rate of contaminant uptake and may require additional maintenance or cleaning during deployment (Schäfer, Paschke and Liess, 2008). Environmental factors such as water flow, temperature, and salinity can influence the diffusion or partitioning rates of contaminants into the sorbent phase (Kaserzon *et al.*, 2013). These variations can complicate data interpretation and introduce uncertainties in the calculation of pollutant concentrations.

Passive samplers require careful calibration to account for differences in uptake rates under various environmental conditions. Without proper calibration, the accuracy of the data may be compromised, particularly in complex or variable aquatic systems (Morin *et al.*, 2012; Castle *et al.*, 2018; Vrana *et al.*, 2021). While passive samplers can be tailored to target specific contaminants, their selectivity also limits their applicability. A single passive sampler may not be suitable for detecting a wide range of pollutants simultaneously, requiring the deployment of multiple devices for comprehensive monitoring (Kot-Wasik *et al.*, 2007). Deploying and retrieving samplers in dynamic or high-flow environments can be challenging. There is a risk of sampler loss or damage due to strong currents, vandalism, or other unforeseen factors. Although passive sampling is cost-effective over time, the initial cost of purchasing and calibrating the samplers may be high. Additionally, specialized training may be required to deploy, retrieve, and analyse data effectively. There is limited standardization in passive sampling methods compared to traditional techniques. Variability in sampler designs, deployment procedures, and data interpretation methods can lead to inconsistencies in results between studies (Godlewska, Stepnowski and Paszkiewicz, 2021).

2.7.1 Theory of passive samplers

The fundamental theory of passive sampling is based on chemical thermodynamics and kinetics, where contaminants move along a concentration gradient from areas of higher chemical potential to lower chemical potential. Contaminants naturally move from an area of higher concentration (the surrounding environment) to an area of lower concentration (the sampler's sorbent or receiving phase). This movement is governed by Fick's laws of diffusion, which describe the rate of contaminant transport across a boundary layer (Górecki and Namieśnik, 2002).

For certain passive samplers, such as those using polymer-based sorbents, contaminants partition between the environmental medium and the sampler's sorbent material. The equilibrium concentration in the sampler reflects the free or bioavailable fraction of the contaminant in the environment (Belháčová-Minaříková *et al.*, 2017). The accumulation of contaminants in a passive sampler occurs in three phases, which are the kinetic, intermediate and equilibrium phase (Figure 1). In the kinetic phase, rapid uptake of contaminants until the system approaches equilibrium. The intermediate phase of passive sampling refers to the kinetic uptake phase, which is a transitional period between the initial linear phase and the final equilibrium phase. In this phase, diffusion continues, but the rate of uptake begins to slow

down. Additionally, sampling is still useful, however the relationship between analyte concentration and time deviates from linearity. In the equilibrium phase, a steady state is reached, where the rate of contaminant uptake equals the rate of release. In aquatic systems, contaminants must diffuse through the water boundary layer (WBL) before entering the sorbent. The thickness of the WBL depends on factors such as water velocity, and it can influence the sampling rate (Vrana, Allan, *et al.*, 2005).

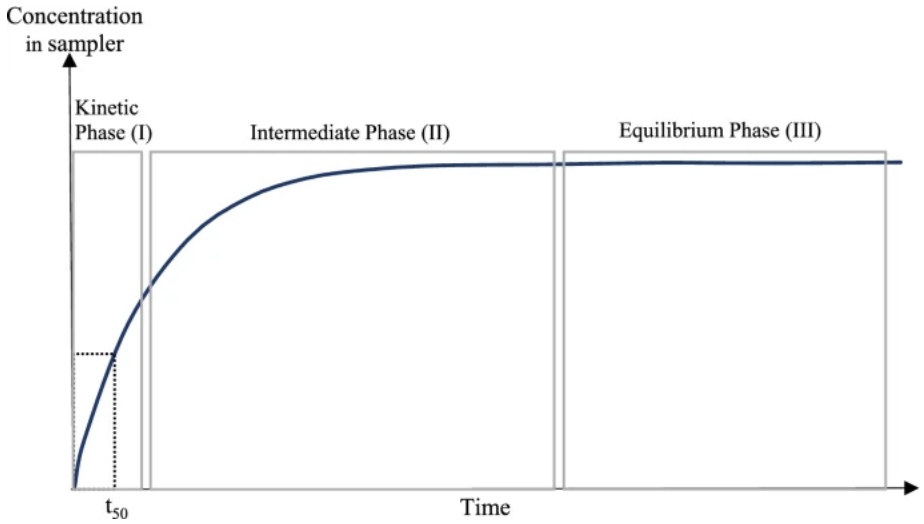

Figure 1 Kinetic and equilibrium uptake phases as a function of time

The mass of a contaminant accumulated by a passive sampler (m) over a deployment time (t) is described by the following equation:

$$m = R_s \times C \times t \qquad (1)$$

Where R_s is the sampling rate (volume of water sampled per unit time, usually in L day^{-1}), C is the concentration of the contaminant in the environmental medium and t is the deployment duration. For contaminants that do not reach equilibrium during the deployment period, adjustments must be made to account for non-equilibrium conditions using empirical or theoretical models.

With active sampling, the use of an autosampler is required to determine the TWA concentrations of the analytes (C_w). This is a costly and labour extensive operation. (C_w) in $\mu g L^{-1}$ can be calculated by equation (1) (Vrana, Allan, *et al.*, 2005).

$$C_w = \frac{C_s M_s}{R_s t} \qquad (2)$$

Where (C_s) is the sorbent concentration ($\mu g.g^{-1}$), (M_s) is the mass of the sorbent (g), (R_s) is the sampling rate in ($L.day^{-1}$), and (t) is the sampling duration (day). One of the issues encountered is determining the sampling rate as it depends on exposure conditions. As a result, the sampling rate determined under laboratory conditions and those obtained in *in situ* conditions differ. To overcome this, performance reference compounds (PRCs) are used. These are compounds that are not typically found in the environment and include compounds such as deisopropylatrazine-d5 (DIA-5 deuterium-labelled deisopropylatrazine). These compounds are also chemically similar but distinct to the target analytes. A PRC is added to the sorbent prior to deployment. The desorption of the PRC from the sorbent then gives data relating to the conditions of the matrix they are deployed in. The release of PRCs is supposed to follow the following first-order kinetic, equation (2) (Vrana, Allan, *et al.*, 2005):

$$ln\frac{C_t}{C_0} = K_e t \qquad (3)$$

Where (C_t) is the concentration of the PRC in the sorbent upon retrieval and (C_0) represent the concentration of the PRC in the sorbent before deployment. Both concentrations are recorded in ($\mu g.g^{-1}$). (t) represents the duration of deployment and (K_e) is the elimination rate constant. Equation (2) can be re-written as equation (3) (Vrana, Allan, *et al.*, 2005):

$$K_e = \frac{\ln \frac{C_0}{C_t}}{t} \qquad (4)$$

In order to determine the in-situ sampling rate (R_s), the ratio between the in-situ and calculated PCR elimination constants, ($K_{e\,(in-situ)}$) and ($K_{e\,(cal)}$) are used to adjust the calculated sampling rate ($R_{s\,(cal)}$) as shown in equation (4) (Vrana, Allan, *et al.*, 2005):

$$R_{s\,(in-situ)} = \frac{K_{e\,(in-situ)}}{K_{e\,(cal)}} R_{s\,(cal)} \qquad (5)$$

2.7.2 Types of passive samplers

Passive sampling began over a decade ago and was initially used for the evaluation of air pollution levels in trees. In the 1970's, concerns over volatile organic compounds lead to the refinement of passive sampling techniques for air. These new methods made use of single-use glass tubes packed with charcoal as the receiving phase. To extract the trapped contaminates, carbon disulphide was used, which was followed by analysis using GC-MS (Palmes and Gunnison, 1973). Since then, further developments have been made and in the present day, different types of passive samplers exist for the selective sampling of specific classes of compounds in air and water. The key differences in passive samplers arise from the type of receiving phase and rate-limiting membrane material used, in addition to the manner in which the sampler is assembled (Godlewska, Stepnowski and Paszkiewicz, 2021). This section will be focused on passive samplers used for monitoring contaminants in water.

2.7.2.1 Polar organic integrative sampler (POCIS)

The POCIS sampler is designed to monitor soluble polar organic compounds in water. The POCIS sampler consists of receiving phase, either in solid or powder form. The receiving phase is sandwiched between two-rate limiting diffusion polyether sulphone membranes. The two main configurations of POCIS commonly used are the pesticide (pest-POCIS) and the pharmaceutical (pharm-POCIS) (Alvarez *et al.*, 2004). As the name suggests, pest-POCIS is used for the monitoring of water-soluble pesticides. The receiving phase contains polystyrene divinylbenzene combined with active carbon. In the case of the pharm-POCIS, the receiving phase is an Oasis™ HLB sorbent. Both configurations have been calibrated for the uptake of numerous organic pollutants (Vermeirssen *et al.*, 2012; Kaserzon *et al.*, 2014; Martínez Bueno *et al.*, 2016; Berho *et al.*, 2020). The in situ calibration of the POCIS sampler was conducted for 76 pharmaceuticals and their metabolites. This was done in surface water with varying temperatures and flow rates. The sampling rates determined were found to range from 0.01 to 0.63 L d^{-1}, and it was observed that the varying water conditions had no effect on the sampling rates (Vrana *et al.*, 2021). In another study, the POCIS sampler was calibrated for the uptake of 49 pharmaceutical compounds. The sampling rates ranged from 0.004 L day^{-1} for enalaprilat and rasagiline to 0.536 L day^{-1} for rotigotine. The sampling rates of 33 of the 49 compounds that were investigated had not been previously reported. These include lopinavir, vildagliptin and desloratadine (Domínguez-García, Almirall and Gómez-Canela, 2025). Upon calibration, these samplers have been deployed in various water bodies for the monitoring of organic pollutants (Vrana *et al.*, 2021; Sultana and Metcalfe, 2022; Wirth *et al.*, 2024; Domínguez-

García, Almirall and Gómez-Canela, 2025). In France, POCIS samplers were calibrated for the uptake of numerous pollutants. The sampler was then deployed for 14 days in the Clain, the Gier and the Jalle Rivers for a duration of 14 days. passive sampling was found to be superior to grab sampling, with detection frequency being higher with passive sampling for the compounds under investigation (Mathon *et al.*, 2022).

2.7.2.2 Diffusive gradients in thin films (DGT)

The DGT sampler was developed in 1994 for the measurements and speciation of trace metals in marine water. The receiving phase of the sampler was made up of an ion exchange resin, enclosed in an ion-permeable gel membrane. Calculating the concentration of the analytes accumulated in the sampler is allowed by the mass transport being controlled by diffusion through the gel membrane as opposed to ion exchange resins enclosed in dialysis bags, which constrained mass transport (Davison and Zhang, 1994). In 2011, the sampler was adapted for the sampling of organic compounds, namely naphthalene in different soil types and this configuration of the sampler was named organic diffusive probe (ODP) (Bondarenko, Sani and Ruello, 2011). In 2012, the first use of the DGT sampler in monitoring organic compounds in water was reported. The organic DGT (o-DGT sampler made use of XAD18 as the binding agent. The study used the antibiotic sulfamethoxazole as the model pollutant in laboratory studies and highlighted the applicability of the novel sampler for the uptake of organic compounds (Chen, Zhang and Jones, 2012). Since then, several reports of the application of o–DGT for the monitoring of pharmaceuticals in aquatic matrices have emerged (Wacheski *et al.*, 2021; Bonnaud *et al.*, 2023; Martins de Barros *et al.*, 2023).

2.7.2.3 Semipermeable membrane devices (SPMDs)

The first use of the SPMDs sampler was reported in 1990 (Huckins *et al.*, 1990). The sampler consisted of a non-porous low-density polyethylene tube (70–90-µm wall thickness) as a membrane which housed an ultra-high purity lipid (triolein) that acted as the receiving phase. To keep the receiving phase in place, the membrane was heat sealed. This allowed for the membrane and receiving phase to be in close contact, which resulted in low interfacial tension between the two components. Since then, SPMDs variations where the LDPE tube is filled with isooctane, ionic liquids or triolein containing cellulose acetate have been reported (Leonard, Hyne and Pablo, 2002; Zhao *et al.*, 2006; Xu *et al.*, 2005). SPMDs are useful for the sampling of non-polar and semi-polar organic compounds such as PAHs, PCBs, OCPs, dioxins and other organics with octanol-water partitioning coefficients greater than three in aqueous

environments and air (Ockenden *et al.*, 1998; Lohmann *et al.*, 2001). These samplers have been shown to be effective for sampling a host of chemicals in different water types. These include organotin compounds in seawater, musk in river water, polychlorinated naphthalenes in sewage water, pesticides in estuaries and irrigation water (Følsvik, Brevik and Berge, 2000; Šetková *et al.*, 2005; Yusà, Pastor and de la Guardia, 2006; Scott *et al.*, 2002; Esteve-Turrillas, Pastor and de la Guardia, 2007). The ability of these to samplers mimic the adsorption of contaminants through cell membranes as well as the bioaccumulation of contaminants in the lipids of aquatic organisms make them important tools in ecotoxicology (Baussant *et al.*, 2001). Other advantages of SPMDs devices include their selectivity as only bioavailable contaminants are sampled, ease of use and low cost compared to active samplers. However SPMDs are limited by need for large volumes of organic solvents for extraction. Their selectivity toward lipophilic compounds limits the range of pharmaceutical compounds they can be used to monitor.

2.7.2.4 Peepers

Peepers were developed in 1976 for the sampling of inorganic contaminants in sediment porewater (Hesslein, 1976). Peepers are inert containers which consist of a volume of 1 to 100 mL of purified water (sorbent) which is capped with a semi-permeable membrane. To protect the receiving phase, the purified water is deoxygenated prior to deployment (Carignan, St-Pierre and Gachter, 1994). One advantage of this sampler over traditional sediment porewater sampling techniques is that it does not cause physical disturbances to the sampling site. Traditional pore water sampling involves the collection of large volumes of bulk sediment from which pore water to be analysed is extracted either mechanically or through centrifugation (Gruzalski *et al.*, 2016). Other methods involve mechanically suctioning pore water directly from the sediment (Peijnenburg *et al.*, 2014). These methods require tedious sample preparation steps, which are time consuming. These methods have also been shown to give analyte concentrations which deviate from actual concentrations in the sediments by either overestimating or underestimating the concentrations (Schroeder *et al.*, 2020). Peepers do not cause permanent physical disturbances to the sampling area and analyte concentrations determined from peepers are a good representation of the actual concentration in the sediments (Cleveland, Brumbaugh and MacDonald, 2017). For deployment, the peeper is inserted into the sediment. This allows for the diffusion of inorganic contaminants from the surrounding sediments, through the semi-permeable membrane and into the receiving phase. Over time, the concentration of the contaminants in the sediment and the concentration of the contaminants

in the sorbent reach equilibrium. After retrieval, the contents of the peepers are analysed to determine the concentration of the analytes accumulated.

2.7.2.5 Microporous polyethylene tubes (MPT)

Various passive samplers have been developed and calibrated for the monitoring of chemical contaminants in water. To ensure that the data obtained from these samplers is a true representation of the pollution levels, further optimization is still needed. One such example is effect of water velocity on sampling rates used to estimate TWA concentrations. Inconsistent water velocities have been reported to affect the thickness of the water boundary layer at the surface of sampling devices. At relatively low water velocities, the rate of transfer of chemicals into the sampler is influenced by diffusive transit of the WBL. This affects the sampling rates of the analytes of interest. At linear velocities greater than 2 cm s^{-1}, however, the sampling rates are not affected. To overcome this drawback, microporous polyethylene tube (MPT) sampler was developed to create a higher resistance to mass transfer of chemicals passing through the sampler itself (Fauvelle *et al.*, 2017). This decreases the importance of diffusion through the WBL and provides more stable sampling rates as well as reduces uncertainty in analyte concentration estimates. The MPT sampler was initially used for monitoring the herbicide glycophosphate and its metabolite aminomethyl phosphonic acid in water (Fauvelle *et al.*, 2017). Since then, the sampler has been used for monitoring an array of polar organic pollutants in water. In 2020, an MPT sampler with a WAX sorbent was calibrated for the uptake of 24 pesticides and pharmaceuticals. Sampling rates ranging from 0.00132 to 0.17 L d^{-1}. After which it was used in the monitoring of the pesticides and pharmaceuticals in the Chillan River, central Chile (Cárdenas-Soracá *et al.*, 2020). Another study calibrated the sampler for the uptake of illicit drugs, pharmaceuticals and personal care products, and applied the sampler in monitoring these compounds in wastewater influent. The sampler was found to show promise for providing necessary drug monitoring data in wastewater (McKay *et al.*, 2020).

2.7.2.6 Stabilized liquid membrane devices (SLMDs)

Stabilized liquid membrane devices are passive samplers used for monitoring labile ionic contaminants. They allow for the monitoring of bioavailable metal species such as cadmium, cobalt, copper, nickel, lead, and zinc, in the aquatic environment. The general design of the sampler consists of a 1:1 mixture of a hydrophobic metal complexing agent and a long chain organic acid as the sorbent material. The sorbent material is enclosed in a semi-permeable polyethylene tube. When deployed in water, the organic acid diffused thorough the semi-

permeable polyethylene tubing. A waxy layer is formed as the carboxylic portion forms complexes with sodium and magnesium ions in the water. As the waxy layer forms on the tubing, the complexing agent slowly mobilizes onto it. The complexing agent then forms metal complexes with the metal species present in the sampling medium. the most common complexing agent and organic acid combination is an alkylated 8-hydroxyquinoline and oleic acid. Upon retrieval, these passive sampling devices are washed with 20% nitric acid to extract the metal species that have formed metal complexes with the complexing agent (Brumbaugh *et al.*, 2002).

2.7.3 Chemcatcher passive sampler

The first use of the Chemcatcher® passive sampler was in 2000 for the sampling of organic compounds. Two passive samplers were used for the sampling of polar- and non-polar organic compounds. Both systems employed the same receiving phase which consisted of 47 mm C18 Empore™ disk, but different membranes – polysulphone for the polar and polyethylene for the non-polar analytes (Kingston *et al.*, 2000). In 2001, the development of a modified version of the original chemcatcher sampler, where the C18 disk was replaced with a chelating agent as the receiving phase, and a cellulose acetate membrane to allow for the sampling of inorganic contaminants was reported. The membrane was treated with Nafion prior to assembly in order to prevent fouling and (to exclude anions from diffusing though the membrane, into the receiving phase) (Persson *et al.*, 2001). Patents for these passive samplers were published first in 2004 Kingston et al, the second in 2006 by L Björklund. This led to first mention of the name Chemcatcher® in 2007 when the trademark was deposited. Since then, different designs of the chemcatcher have been reported, as well as their applications in the uptake of various organic and inorganic contaminants in water (Table 1) (Vermeirssen *et al.*, 2012; Kaserzon *et al.*, 2014; Petrie *et al.*, 2016; Glanzmann *et al.*, 2023; Robinson *et al.*, 2024).

2.7.3.1 Design of the chemcatcher sampler

The Chemcatcher sampler consists of a receiving phase and a membrane housed in either a PolyTetraFluoroEthylene (PTFE) or polycarbonate (PC) support. Three main designs of this sampler are currently in use (Figure 2) (Charriau *et al.*, 2016). The first design consists of two PTFE components screwed together to seal the receiving phase and membrane. The cavity that exposes the membrane to the sampling medium is approximately 20 mm deep (Kingston *et al.*, 2000). However, this depth significantly reduces the sampling rate due to the thicker WBL. To address the limitations of the first design, this version reduces the sampler cavity depth to

approximately 7 mm, which helps thin the WBL and thereby increases sampling rates. The sampler is composed of two PC parts clipped together around the membrane and receiving phase to create a seal. While the reduced cavity depth improves sampling efficiency, this design is more susceptible to variations in water velocity and turbulence. Additionally, the sampler is single use, making it less cost-effective (Lobpreis *et al.*, 2008). The third version reintroduces PTFE as the construction material for the cavity, which is made up of two parts screwed together to secure the disk and membrane. The cavity depth is reduced further to 2 mm, significantly improving sampling rates while maintaining structural stability (Charriau *et al.*, 2016). All three designs can be sealed with sampler caps for storage and transportation to and from deployment sites deployment sites.

Figure 2 Three main Chemcatcher sampler designs (Lissalde *et al*., 2016)

2.7.3.2 Calibration of the chemcatcher sampler

Calibrating Chemcatcher samplers is crucial for determining sampling rates, which are necessary for calculating TWA concentrations when deployed in real water systems. Several calibration methods have been explored. The first method makes use of a known volume of medium is spiked with a specific concentration of analytes of interest. The sampler is exposed to this medium for a fixed period, allowing for the determination of analyte uptake rates. This method is called the static calibration method (O'Brien, Bartkow and Mueller, 2011).

A second method is the static renewal experiment, where the spiked medium is periodically renewed to account for analyte depletion due to uptake by the receiving phase. On renewal days, aliquots of the medium are sampled and analysed to determine the uptake rate (Kaserzon *et al.*, 2014). The flowthrough or continuous flow method involves 20 to 50-liter tanks where a spiked medium with a known analyte concentration is pumped continuously (Vrana *et al.*, 2006). The goal is to maintain a constant analyte concentration, though this can be challenging due to analyte degradation, volatilization, or adsorption onto pump tubing (Vrana, Mills, *et al.*,

2005). In a 2021 study, the static renewal and flowthrough calibration methods were compared for the calibration of six pharmaceutical compounds. Four of the six compounds, namely, sulphamethaxole, carbamazepine, diclofenac and naproxen yielded higher sampling rates using the flow through method, whereas caffeine and atenolol yielded higher sampling rates using the static renewal method (Lis, Stepnowski and Caban, 2021). These findings highlight the dependence of the chemical properties of individual compounds on the appropriateness of each calibration method.

Chemcatcher samplers can be calibrated *in situ* where they are deployed directly in natural water bodies such as rivers, lakes, or dams. During the deployment, grab samples are frequently collected to compare against the analytes accumulated in the receiving phase of the sampler. In 2016, the *in situ* calibration of three types of passive samplers for monitoring of steroid hormones in municipal wastewater was compared. The three types of passive samplers instigated were POCIS, chemcatcher and an enhanced passive sampler. The passive sampling campaign was done concurrently with composite sampling in 24-hour intervals. POCIS yielded sampling rated ranging from 0.04 to 0.10 L d^{-1}. Slightly higher sampling rates were obtained with the Chemcatcher sampler, and these ranged from 0.07 to 0.18 L d^{-1}. The sampling rates obtained with the enhanced passive sampler were similar to those obtained with the Chemcatcher sampler, these ranged from 0.10 and 0.17 L d^{-1}. A direct comparison of sampling rates was not feasible due to differences in surface areas exposed to the sampling medium. The Chemcatcher and POCIS samplers had exposure from both sides, with surface areas of 34.6 cm^2 and 45.8 cm^2, respectively, whereas the enhanced sampler was exposed on one side with a surface area of 17.3 cm^2. The study highlighted certain limitations associated with using passive samplers for contaminant monitoring. One significant challenge was the poor uptake of steroid hormones, as evidenced by the low sampling rates. This highlights the necessity of tailoring sampler calibration experiments to align with the physicochemical properties of specific analytes for improved performance (Škodová *et al.*, 2016).

Artificial streams can be used to mimic natural water conditions. These artificial streams are constructed outdoors, and they allow for calibration under controlled yet realistic conditions. These setups enable the evaluation of external factors like biofouling, which can affect sampling rates. However, analyte loss due to adsorption onto sand particles remains a challenge. This method has shown particular success with municipal effluents, as analyte behaviour in artificial streams closely mirrors that in rivers (Schäfer, Paschke and Liess, 2008).

Each calibration method has its advantages and limitations, with the choice depending on the specific analytes and environmental conditions.

2.7.3.3 Applications of the chemcatcher passive sampler

Since the inception of the Chemcatcher sampler, the original design as well as modified versions of the original design have been applied in the monitoring of a mirid of contaminants in aquatic ecosystems. The application of the chemcatcher sampler to different classes of chemical contaminants is allowed by the ability to vary the receiving phase and the membrane to target specific classes of contaminants. Chemcatcher passive samplers have been applied in the monitoring of inorganic compounds such as metals and organic compounds such as pharmaceuticals, pesticides, PAH's and PCBs.

A chemcatcher sampler comprising of a 3M Empore™ Chelating Disks (47 mm in diameter) and a cellulose acetate membrane was evaluated for the uptake of rare earth elements in marine environments. To calibrate the sampler, a novel mobile continuous flow system was used. Using the laboratory derived sampling rates, TWA concentrations of rare earth elements up to 38.6 ng L^{-1} were observed near the shore of Bight, Germany. In the grab water samples, the rare earth metals could not be quantified as they are present in trace quantities. This study highlighted the applicability of passive sampling in matrices where the analytes under investigation are present in ultra trace level (Petersen *et al.*, 2016). A Chemcatcher based sampler consisting of a styrene-divinylbenzene reverse phase sulfonate disk and a PES membrane was calibrated for the uptake of 44 organic pollutants under varying hydrodynamic conditions. The sampling rates obtained ranged from 0.015 to 0.115 L day^{-1}. The sampling rates were observed to increase with an increasing flow rate. that is, at flow rates of 5 and 40 cm s^{-1}, the sampling rates for carbamazepine were 0.088 and 0.109 L day^{-1} (Glanzmann *et al.*, 2023). This study highlighted the dependence of sampling rates on hydrodynamic conditions of the sampling medium and showed one of the challenges associated with passive sampling.

Very recently, a Chemcatcher sampler for the monitoring of phosphorous in the aquatic environment. The sorbent consisted of A zirconium sulphate–surfactant micelle mesostructured embedded in a polysulphone matrix. The passive sampler was calibrated for the uptake of orthophosphate in the laboratory under varying conditions (temperature, and pH). Sampling rates ranging from 0.053 L d^{-1} to 0.166 L d^{-1} were obtained. These were then used to estimate

TWA concentrations of orthophosphate in a municipal WWTP and lakes in Sapporo, Japan (Hafuka *et al.*, 2023).

The Chemcatcher sampler's ability to be tailored to target specific pollutants by selecting appropriate receiving phases, as well its ability to provide time-weighted average concentrations of pollutants makes it a highly versatile and powerful tool for diverse environmental monitoring applications. Table 1 is a summary of studies in which the chemcatcher sampler has been applied in. it shows the matrix, deployment period, types of compounds sampled, their sampling rates range and the sorbent material used.

Table 1 Summary of Chemcatcher studies, along with water type, deployment duration, compounds sampled, sampling rates and sorbent material used

Water type	Deployment duration (days)	Compounds	Sampling rates (L day $^{-1}$)	Sorbent	Ref
River	14 & 28	PAHs & OCPs	Up to 0.5	C_{18} & SDB-XC	(Vrana *et al.*, 2010)
River	28	EDCs	NR	C_{18}	(Allinson *et al.*, 2015)
Marine		EDCs	NR	Empore™ SDB-RPS	(Tan *et al.*, 2007)
Marine	14	PAHs, PCBs & pesticides	NR	C_{18}	(El-Shenawy *et al.*, 2009)
Marine	10	Pesticides	0.14 – 0.19	C_{18}	(Stephens *et al.*, 2009)
River	21	Pesticides	0.025 – 0.068	HLB	(Grodtke *et al.*, 2021)
River	14	Mercury	0.029 – 0.009	Iminodiacetic chelate	(Aguilar-Martínez *et al.*, 2009)
Marine & river	14	Mercury & organotin	0.003 – 0.204	Iminodiacetic chelate & C_{18}	(Aguilar-Martínez, Gómez-Gómez and Palacios-Corvillo, 2011)
River	14 & 28	Trace metals		C_{18}	(Allan *et al.*, 2008)

Wastewater	3 & 7	Phosphorous	0.0527 – 0.166	PSfZS	(Hafuka *et al.*, 2023)
Marine	19	Short-chain chlorinated paraffins	Up to 0.53	C_8 & C_{18}	(Godere *et al.*, 2021)
Wastewater	14	Steroid hormones	0.1 – 0.17	SDB-RPS	(Škodová *et al.*, 2016)
River & sewage effluent	8	Pharmaceuticals	0.09 – 0.25	SDB-RPS	(Vermeirssen *et al.*, 2008)
Wastewater	9	PCPs & illicit drugs	0.01–0.10	HLB	(Petrie *et al.*, 2016)
River	14	Pesticides, pharmaceuticals, PCPs	0.0002 – 0.4	SDB-RPS	(Moschet *et al.*, 2015)
River	14	Steroid estrogens	0.077–0.304	C18	(Kuster *et al.*, 2010)
River	14	Pharmaceuticals	0.048 – 0.158	SDB-RPS	(Lindholm-Lehto *et al.*, 2016)
River	21	Pesticides, transformation products & pharmaceuticals	0.007 – 0.796	SDB-XC SDB-RPS HLB	(Römerscheid *et al.*, 2023)
River	21	Pesticides & pharmaceuticals	0.015 –0.115	SDB-RPS	(Glanzmann *et al.*, 2023)

C_8 – Octylsilane, C_{18} – Octadecylsilane, EDC – Endocrine disruptive compound, HLB – hydrophilic-lipophilic balance, OCP – Organochlorine pesticide, PAH –Polycyclic aromatic hydrocarbon, PCB – Polychlorinated biphenyl, PSfZS – zirconium sulfate–surfactant micelle mesostructure and polysulfone matrix, SDB-XC – poly(styrenedivinylbenzene) copolymer, SDB-RPS – styrenedivinylbenzene reverse phase sulfonate, NR – not reported

2.8 Analytical techniques for pharmaceutical compounds in aquatic ecosystems

The analysis of pharmaceuticals in the environment presents considerable challenges due to their trace concentrations and the complex nature of environmental matrices. These challenges necessitate the use of highly sensitive and accurate analytical techniques. Over the years, significant advancements in instrumentation have enabled the development of robust methods for detecting and quantifying pharmaceuticals in environmental samples. To date, several analytical methods for the determination of pharmaceuticals in the environment have been developed and reported.

2.8.1 Chromatographic techniques for analysis of pharmaceuticals in the environment

Chromatography has become an indispensable tool for analysing pharmaceuticals in the environment, with its evolution spanning decades. It has evolved from a simple technique for separating plant pigments to a sophisticated analytical tool widely used in various scientific fields. Its application to analysis of pharmaceuticals in the environment has evolved alongside advancements in analytical techniques and growing awareness of environmental pollution. Initially, gas chromatography (GC), introduced in the 1950s, was primarily used for analysing volatile organic compounds. Although pharmaceuticals were not the focus at the time, GC laid the groundwork for environmental analysis (James and Martin, 1952). In the 1970's, The concept of persistent organic pollutants emerged, leading to the inclusion of synthetic compounds such as pharmaceuticals in environmental studies (Tabak and Bunch, 1970). By the 1980s, the growing concern over pharmaceuticals in water systems coincided with the widespread adoption of high-performance liquid chromatography (HPLC), which enabled the separation of polar and non-volatile compounds, leading to early detections of APIs in municipal wastewater (Majors, 1980).

In the 1990s, the coupling of HPLC with ultraviolet (UV) and fluorescence detectors facilitated quantification of pharmaceuticals such as antibiotics and hormones in water systems. This period marked an increasing focus on the presence of pharmaceuticals in wastewater and surface water (Hartig, Storm and Jekel, 1999). By the 2000s, significant advancements in chromatography allowed for the development of methods capable of detecting trace-level pharmaceuticals. Techniques such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) became the gold standard for detecting pharmaceuticals and their metabolites at low concentrations (ng L^{-1} to µg L^{-1}) (Marchese *et al.*, 2003; Snyder *et al.*, 2004). Additionally, gas chromatography-mass spectrometry (GC-MS) was optimized for volatile and semi-volatile

pharmaceuticals, while high-resolution mass spectrometry (HRMS) was introduced, allowing for the identification of unknown transformation products and metabolites (Reddersen and Heberer, 2003; Kosjek and Heath, 2008).

The mid 2000s witnessed the introduction of ultra-high-performance liquid chromatography (UHPLC), which significantly improved the speed and resolution of separations. These advancements enabled the inclusion of transformation products in environmental monitoring programs, complementing the analysis of parent compounds (Petrovic, Gros and Barcelo, 2006). Presently, chromatographic techniques for pharmaceutical analysis are increasingly focusing on sustainability, emphasizing "green chromatography" through reduced solvent use. Moreover, multi-residue analysis methods have been developed to allow for the simultaneous detection of hundreds of pharmaceutical compounds in a single analytical run, enhancing efficiency and comprehensiveness (Kannaiah *et al.*, 2021).

2.8.2 Detection techniques for pharmaceuticals in the environment

The detection of pharmaceuticals in environmental samples often requires highly sensitive and selective techniques due to the trace and ultra-trace concentrations of these compounds and the complexity of the matrices. Mass spectrometry has emerged as a leading detection method, offering both qualitative and quantitative capabilities. Instruments such as quadrupole time-of-flight MS (QTOF-MS) and tandem MS/MS are widely used for their high sensitivity and selectivity (Miao and Metcalfe, 2003; Martínez-Piernas, Plaza-Bolaños and Agüera, 2021). Fluorescence detection is another effective technique, particularly for pharmaceuticals with natural fluorescence or those that can be derivatized to fluoresce. UV and Diode Array Detectors (DAD) are commonly integrated into HPLC systems for detecting pharmaceuticals with UV-absorbing groups, providing reliable results in targeted analyses (Turiel, Bordin and Rodríguez, 2003; Babić *et al.*, 2006).

2.8.3 Hyphenated techniques for analysis of pharmaceuticals in the environment

Hyphenated techniques combine two or more analytical methods to exploit their complementary strengths. In environmental analysis of pharmaceuticals, these techniques are essential for achieving high sensitivity, selectivity, and efficiency in complex matrices.

One such technique is GC-MS. It combines the separation power of gas chromatography with the identification and quantification capabilities of mass spectrometry. Although GC-MS is not

widely used for the analysis of pharmaceutical compounds, its use for the analysis of some volatile and semi-volatile pharmaceuticals such as steroids, antibiotics, and certain NSAIDs has been reported (Hao, Zhao and Yang, 2007). However, its applicability is limited to thermally labile compounds, and compounds which are polar have to be derivatized in order to be analysed with GC-MS. The sensitivity of GC-MS can be enhanced by incorporating tandem mass spectrometry (GC-MS/MS) for better quantification and identification. The additional mass filtering steps in GC-MS/MS significantly reduce background noise compared to conventional GC-MS, resulting in improved sensitivity and selectivity for trace-level analyte detection. GC-MS/MS also reduces interferences from complex matrices, which affect analysis with GC-MS (Luo *et al.*, 2021).

The most common hyphenated systems for pharmaceutical analyses are based on LC-MS. LC-MS is a versatile technique, which combines liquid chromatography's ability to separate polar and non-volatile compounds with the sensitivity of mass spectrometry. It is widely used for analysing a broad range of pharmaceuticals, including antibiotics, antidepressants, and beta-blockers. It has been shown to be effective for quantifying pharmaceuticals in complex matrices like wastewater and biota. Unlike GC-MS, LC-MS does not require derivatization of polar compounds. This makes it ideal for the analysis of polar, thermally labile, and non-volatile compounds. As with GC-MS, the specificity and sensitivity of LC-MS can be enhanced by adding a second analyser to yield LC-MS/MS. With LC-MS/MS, multi-residue analysis for hundreds of pharmaceuticals in a single run can be achieved. The enhanced sensitivity and selectivity also allows for accurate quantification, even in complex matrices (Simpson, Simpson and Gerber, 2024).

Separation in LC-MS can also be enhanced by using two different chromatographic columns before mass spectrometric analysis. This technique is called two-dimensional liquid chromatography-mass spectrometry (2D-LC-MS). It has been used for the analysis of pharmaceuticals in highly complex environmental samples, such as sludge and sediments. Although it offers better separation of analytes and matrix components, it requires long run times (Pugajeva *et al.*, 2021). Another way compound separation has been improved with LC-MS systems is by using high pressure in ultra-high-performance liquid chromatography-high-resolution mass Spectrometry (UHPLC-HRMS) (Fabregat-Safont *et al.*, 2023). By using ultra-high pressures for improved separation efficiency and high-resolution mass spectrometry, more precise measurements are achieved. This system is ideal for identifying unknown

pharmaceuticals and transformation products and has been used for environmental monitoring and risk assessment studies. The high resolution, which allows for accurate mass determination makes it a great tool for non-target screening. 48 pharmaceutical compounds and PCPs, including carbamazepine, trimethoprim and diclofenac, were detected in drinking water in Guangzhou. This was accomplished through suspect and non-target screening using in-house spectral libraries as well as HRMS spectral libraries (Wang *et al.*, 2022). Although this technique is a great tool for the analysis of pharmaceutical compounds in environmental samples, it is limited by its high cost and complexity.

High-performance liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD-MS) Combines HPLC for separation, a diode array detector (DAD) for UV/visible absorbance, and MS for compound identification. It is useful in the screening of pharmaceuticals with chromophores, as well as the analysis of transformation products and metabolites (Ricciutelli *et al.*, 2020). Despite combining spectral and mass data for compound identification, it is less sensitive than tandem MS techniques.

Another LC system which has been shown to be a powerful analytical technique is LC-QTOF-MS (Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry). It combines liquid chromatography (LC) for separating compounds and QTOF-MS for detecting and identifying analytes with high resolution and accuracy. This technique is able to resolve compounds with similar m/z values, aiding in the identification of co-eluting analytes (Martínez-Piernas, Plaza-Bolaños and Agüera, 2021). Other advantages with this technique include the simultaneous detection of pharmaceuticals at trace and higher concentrations, as well as its applicability to both polar and non-polar pharmaceuticals. Sixty parent pharmaceutical and pesticide compounds and their respective transformation products were identified in three Chinese WWTPs through non-target analysis and suspect screening using LC-QTOF_MS (Wang *et al.*, 2020). As with most advanced LC-MS systems, LC-QTOF-MS is expensive and complex.

2.9 Quality assurance and quality control for analysis of pharmaceuticals in the environment

Quality assurance (QA) and quality control (QC) measures are essential to ensure the reliability and accuracy of analytical results when using chromatographic techniques coupled to mass

spectrometry for detecting pharmaceuticals in environmental matrices. Some key QA/QC practices include the use of calibration standards, matrix matched standards and isotope labelled standards. Other QA and QC practices involve the use of method and instrument blanks, compound stability studies, replicate analysis, recovery tests, method validation and the inclusion of QC samples (Lescord and Lepage, 2025).

Calibration standards help establish the relationship between instrument response (signal intensity) and analyte concentration. These are essential for the accurate quantification of pharmaceuticals. Matrix matched standards compensate for matrix effects, which can alter analyte ionization efficiency due to the complexity of environmental samples. This helps reduce errors caused by ion suppression or enhancement, which then reflects the true response of the analyte in the presence of matrix components (Lescord and Lepage, 2025). Isotope labelled standards are used to correct for matrix effects, signal variability, and recovery losses during sample preparation. Isotope-labelled internal standards (ILIS) are structurally identical to the target analytes but contain stable isotopes (Caballero-Casero *et al.*, 2021). They help provide high precision and accuracy by accounting for instrument drift and matrix effects. This then enables more reliable quantification, especially in complex environmental samples.

Method blanks are processed without the addition of analytes to check for contamination during sample preparation, whereas instrument blanks are analysed to ensure the absence of carryover between samples. Often, analyte stability studies are conducted to determine the storage duration of prepared standards. The stability of QC standards was investigated for the ARV drug favipiravir. This was done for freeze/thaw cycles, at room temperature over 25 hours, at –70 ˚C for 72 days and in the autosampler at 15 ˚C for 25 hours. Insignificant loss (> 1% loss) of the tested drug occurred during repeated thawing and freezing conditions and long term storage. At room temperature and in the autosampler, significant loss of the tested drug was noticed (< 10% loss) (Morsy *et al.*, 2021). Replicate analyses is used to assess data reproducibility and helps identify inconsistencies caused by sample heterogeneity or processing errors. The inclusion of QC samples help to verify accuracy throughout the analytical run. The validation of an instrument method for parameters such as linearity, sensitivity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) ensures the robustness of the method and accuracy of results (Oberacher *et al.*, 2020).

In pharmaceutical analysis of environmental samples, implementing robust QA and QC practices and applying advanced analytical techniques is crucial for generating reliable and accurate data. By incorporating these practices, reliable data on the presence of pharmaceuticals and their transformation products in aquatic ecosystems can be generated. This will aid in the development of mitigation strategies to address pharmaceutical pollution.

REFERENCES

Abafe, O.A. *et al.* (2018) 'LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa', *Chemosphere*, 200, 660–670. doi.org/10.1016/j.chemosphere.2018.02.105.

Abdel Hady, K.K. *et al.* (2021) 'Simultaneous HPLC determination of vildagliptin, ampicillin, sulbactam and metronidazole in pharmaceutical dosage forms and human urine', *J. Iran. Chem. Soc.*, 18(3), 729–738. doi.org/10.1007/s13738-020-02065-z.

Aguilar-Martínez, R. *et al.* (2009) 'Application of Chemcatcher passive sampler for monitoring levels of mercury in contaminated river water', *Talanta*, 77(4), 1483–1489. doi.org/10.1016/j.talanta.2008.09.037.

Aguilar-Martínez, R. *et al.* (2011) 'Mercury and organotin compounds monitoring in fresh and marine waters across Europe by Chemcatcher passive sampler', *J. Environ. Anal. Chem.*, 91(11), 1100–1116. doi.org/10.1080/03067310903199534.

Agunbiade, F.O. and Moodley, B. (2014) 'Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa', *Environ. Monit. Assess.,* 186(11), 7273–7291. doi.org/10.1007/s10661-014-3926-z.

Allan, I.J. *et al.* (2008) 'Chemcatcher® and DGT passive sampling devices for regulatory monitoring of trace metals in surface water', *Environ. Monit. Assess.,* 10(7), 821. doi.org/10.1039/b802581a.

Allegaert, K. *et al.* (2015) 'Paracetamol pharmacokinetics and metabolism in young women', *BMC Anesthesiol.*, 15(1), 163. doi.org/10.1186/s12871-015-0144-3.

Allinson, G. *et al.* (2015) 'Combining Passive Sampling with Recombinant Receptor–Reporter Gene Bioassays to Assess the Receptor Activity of Victorian Rivers' *Bull Environ Contam Toxicol*., 95(6), 758–763. doi.org/10.1007/s00128-015-1577-6.

Alvarez, D.A. *et al.* (2004) 'Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments', *Environ Toxicol Chem.*, 23(7), 1640. doi.org/10.1897/03-603.

Álvarez-Muñoz, D. *et al.* (2015) 'Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe', *Environ. Res.,* 143, 56–64. doi.org/10.1016/j.envres.2015.09.018.

Ameller, T. *et al.* (2004) 'Drug delivery systems for oestrogenic hormones and antagonists: the need for selective targeting in estradiol-dependent cancers', *J Steroid Biochem Mol Biol*, 92(1–2), 1–18. doi.org/10.1016/j.jsbmb.2004.05.006.

Amos, S.P. *et al.* (2019) 'Naproxen, ibuprofen, and diclofenac residues in river water, sediments and *Eichhornia crassipes* of Mbokodweni river in South Africa: An initial screening', *Environ. Forensics*, 20(2), 129–138. doi.org/10.1080/15275922.2019.1597780.

Ang, K.L. *et al.* (2021) 'Sustainability framework for pharmaceutical manufacturing (PM): A review of research landscape and implementation barriers for circular economy transition', *J. Clean. Prod.*, 280, 124264. doi.org/10.1016/j.jclepro.2020.124264.

Ateba, C.N. *et al.* (2020) 'Occurrence of Antibiotic-Resistant Bacteria and Genes in Two Drinking Water Treatment and Distribution Systems in the North-West Province of South Africa', *J. Antibiot.*, 9(11), p. 745. doi.org/10.3390/antibiotics9110745.

Babić, S. *et al.* (2006) 'Solid phase extraction and HPLC determination of veterinary pharmaceuticals in wastewater', *Talanta*, 70(4), 732–738. doi.org/10.1016/j.talanta.2006.07.003.

Barbier, E.B. *et al.* (2011) 'The value of estuarine and coastal ecosystem services', *Ecol. Monogr.*, 81(2), 169–193. doi.org/10.1890/10-1510.1.

Baussant, T. *et al.* (2001) 'Bioaccumulation of polycyclic aromatic compounds: 1. Bioconcentration in two marine species and in semipermeable membrane devices during chronic exposure to dispersed crude oil', *Environ Toxicol Chem*, 20(6), 1175–1184. doi.org/10.1002/etc.5620200606.

Belháčová-Minaříková, M. *et al.* (2017) 'Investigation of cosolvent application to enhance POPs' mass transfer in partitioning passive sampling in sediment', *Environ. Sci. Pollut. Res.*, 24(35), 27334–27344. doi.org/10.1007/s11356-017-0223-8.

Bergeron, H.C. and Tripp, R.A. (2020) 'Emerging small and large molecule therapeutics for respiratory syncytial virus', *Expert Opin. Investig. Drugs.*, 29(3), 285–294. doi.org/10.1080/13543784.2020.1735349.

Berho, C. *et al.* (2020) 'Estimating 42 pesticide sampling rates by POCIS and POCIS-MIP samplers for groundwater monitoring: a pilot-scale calibration', *Environ. Sci. Pollut. Res*, 27(15), 18565–18576. doi.org/10.1007/s11356-020-08385-0.

Bisognin, R.P. *et al.* (2021) 'Occurrence and fate of pharmaceuticals in effluent and sludge from a wastewater treatment plant in Brazil', *Environ. Technol.*, 42(15), 2292–2303. doi.org/10.1080/09593330.2019.1701561.

Bondarenko, A. *et al.* (2011) 'Design and Calibration of an Organic Diffusive Probe to Extend the Diffusion Gradient Technique to Organic Pollutants', *Int. J. Environ. Res. Public Health.*, 8(8), 3318–3332. doi.org/10.3390/ijerph8083318.

Bonnaud, B. *et al.* (2023) 'Calibration comparison between two passive samplers -o-DGT and POCIS- for 109 hydrophilic emerging and priority organic compounds', *Sci. Total Environ.*, 869, 161720. doi.org/10.1016/j.scitotenv.2023.161720.

Boxall, A.B.A. *et al.* (2012) 'Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions?', *Environ. Health Perspect.*, 120(9), 1221–1229. doi.org/10.1289/ehp.1104477.

Brumbaugh, W.G. *et al.* (2002) 'Stabilized Liquid Membrane Device (SLMD) for the Passive, Integrative Sampling of Labile Metals in Water', *Water Air Soil Pollut.*, 133(1/4), 109–119. doi.org/10.1023/A:1012923529742.

Caballero-Casero, N. *et al.* (2021) 'Towards harmonised criteria in quality assurance and quality control of suspect and non-target LC-HRMS analytical workflows for screening of emerging contaminants in human biomonitoring', *TrAC, Trends Anal. Chem.*, 136, 116201. doi.org/10.1016/j.trac.2021.116201.

Cárdenas-Soracá, D.M. *et al.* (2020) 'In-situ calibration of a microporous polyethylene passive sampling device with polar organic micropollutants in the Chillan River, central Chile', *Environ. Res.*, 188, 109738. doi.org/10.1016/j.envres.2020.109738.

Carignan, R. *et al.* (1994) 'Use of diffusion samplers in oligotrophic lake sediments: Effects of free oxygen in sampler material', *Limnol. Oceanogr.*, 39(2), 468–474. doi.org/10.4319/lo.1994.39.2.0468.

Carpenter, S.R. *et al.* (2011) 'State of the World's Freshwater Ecosystems: Physical, Chemical, and Biological Changes', *Annu. Rev. Environ. Resour.*, 36(1), 75–99. doi.org/10.1146/annurev-environ-021810-094524.

Castle, G.D. *et al.* (2018) 'Calibration and field evaluation of the Chemcatcher® passive sampler for monitoring metaldehyde in surface water', *Talanta*, 179, 57–63. doi.org/10.1016/j.talanta.2017.10.053.

De Cesaris, M.G. *et al.* (2025) 'Extraction of organic contaminants from grab and composite water samples', in *Sample Handling and Trace Analysis of Pollutants*. Elsevier, 35–63. doi.org/10.1016/B978-0-323-85601-0.00031-X.

Chakrabarti, R. *et al.* (1989) 'Transfer of monoclonal antibodies into mammalian cells by electroporation', *J. Biol. Chem.*, 264(26), 15494–15500. doi.org/10.1016/S0021-9258(19)84857-3.

Chapin, T.P. (2015) 'High-frequency, long-duration water sampling in acid mine drainage studies: A short review of current methods and recent advances in automated water samplers', *J. Appl. Geochem.*, 59, 118–124. doi.org/10.1016/j.apgeochem.2015.04.004.

Charriau, A. *et al.* (2016) 'Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments Part A: Principles, calibration, preparation and analysis of the sampler', *Talanta*, 148, 556–571. doi.org/10.1016/j.talanta.2015.06.064.

Chen, C.-E. *et al.* (2012) 'A novel passive water sampler for in situ sampling of antibiotics', *J. Environ. Monit. Assess*, 14(6), 1523. doi.org/10.1039/c2em30091e.

Chmiel, T. *et al.* (2019) 'The impact of lipophilicity on environmental processes, drug delivery and bioavailability of food components', *Microchem. J.*, 146, 393–406. doi.org/10.1016/j.microc.2019.01.030.

Cleveland, D. *et al.* (2017) 'A comparison of four porewater sampling methods for metal mixtures and dissolved organic carbon and the implications for sediment toxicity evaluations', *Environ. Toxicol. Chem.*, 36(11), 2906–2915. doi.org/10.1002/etc.3884.

Curtin, R. and Prellezo, R. (2010) 'Understanding marine ecosystem based management: A literature review', *Mar. Policy.*, 34(5), 821–830. doi.org/10.1016/j.marpol.2010.01.003.

Cyrus, D.P. (1991) 'Fish conservation in South African estuaries: Pressures, problems and prospects', *South. Afr. J. Aquat. Sci.*, 17(1–2), pp. 19–27. https://doi.org/10.1080/10183469.1991.9631310.

Davison, W. and Zhang, H. (1994) 'In situ speciation measurements of trace components in natural waters using thin-film gels', *Nature*, 367(6463), 546–548. doi.org/10.1038/367546a0.

Desbiolles, F. *et al.* (2018) 'Occurrence and ecotoxicological assessment of pharmaceuticals: Is there a risk for the Mediterranean aquatic environment?', *Sci. Total Environ.*, 639, 1334–1348. doi.org/10.1016/j.scitotenv.2018.04.351.

Ding, C. and He, J. (2010) 'Effect of antibiotics in the environment on microbial populations', *Appl Microbiol Biotechnol.*, 87(3), 925–941. doi.org/10.1007/s00253-010-2649-5.

Domínguez-García, P. *et al.* (2025) 'A POCIS-based approach for the monitoring of pharmaceuticals in wastewater treatment plants: Calibration and deployment challenges', *Environ. Pollut.*, 367, 125641. doi.org/10.1016/j.envpol.2025.125641.

El-Shenawy, N.S. *et al.* (2009) 'Comparing the Passive and Active Sampling Devices with Biomonitoring of Pollutants in Langstone and Portsmouth Harbour, UK', *J. Environ. Sci. Technol.,* 3(1), 1–17. doi.org/10.3923/jest.2010.1.17.

Esteve-Turrillas, F.A. *et al.* (2007) 'Behaviour of semipermeable membrane devices in neutral pesticide uptake from waters', *Anal. Bioanal. Chem.*, 387(6), 2153–2162. doi.org/10.1007/s00216-006-1087-9.

Fabregat-Safont, D. *et al.* (2023) 'Searching for pharmaceutically active products and metabolites in environmental waters of Peru by HRMS-based screening: Proposal for future monitoring

and environmental risk assessment', *Chemosphere*, 337, 139375. doi.org/10.1016/j.chemosphere.2023.139375.

Farounbi, A.I. and Ngqwala, N.P. (2020) 'Occurrence of selected endocrine disrupting compounds in the eastern cape province of South Africa', *Environ. Sci. Pollut. Res.*, 27(14), 17268–17279. doi.org/10.1007/s11356-020-08082-y.

Fauvelle, V. *et al.* (2017) 'Glyphosate and AMPA passive sampling in freshwater using a microporous polyethylene diffusion sampler', *Chemosphere*, 188, 241–248. doi.org/10.1016/j.chemosphere.2017.08.013.

Feher, M. and Schmidt, J.M. (2003) 'Property Distributions: Differences between Drugs, Natural Products, and Molecules from Combinatorial Chemistry', *J. Chem. Inf. Comput.*, 43(1), 218–227. doi.org/10.1021/ci0200467.

Fell, J., Pead, J. and Winter, K. (2019) 'Low-Cost Flow Sensors: Making Smart Water Monitoring Technology Affordable', *IEEE Consum. Electron. Mag.*, 8(1), 72–77. doi.org/10.1109/MCE.2018.2867984.

Følsvik, N. *et al*. (2000) 'Monitoring of organotin compounds in seawater using semipermeable membrane devices (SPMDs)—tentative results', *J. Environ. Monit.*, 2(4), 281–284. doi.org/10.1039/b002768p.

Glanzmann, V. *et al.* (2023) 'An improved Chemcatcher-based method for the integrative passive sampling of 44 hydrophilic micropollutants in surface water – Part A: Calibration under four controlled hydrodynamic conditions', *Sci. Total Environ.*, 871, 162037. doi.org/10.1016/j.scitotenv.2023.162037.

Godere, M. *et al.* (2021) 'First study of passive sampling to monitor short-chain chlorinated paraffins in water: Comparing capabilities of Chemcatcher® and silicone rubber samplers', *Talanta*, 224, 121920. doi.org/10.1016/j.talanta.2020.121920.

Godlewska, K. *et al.* (2021) 'Pollutant analysis using passive samplers: principles, sorbents, calibration and applications. A review', *Environ. Chem. Lett.*, 19(1), 465–520. doi.org/10.1007/s10311-020-01079-6.

Górecki, T. and Namieśnik, J. (2002) 'Passive sampling', *TrAC, Trends Anal. Chem.*, 21(4), 276–291. doi.org/10.1016/S0165-9936(02)00407-7.

Grodtke, M. *et al.* (2021) 'Calibration and field application of the Atlantic HLB Disk containing Chemcatcher® passive sampler – Quantitative monitoring of herbicides, other pesticides, and transformation products in German streams', *J. Hazard. Mater.*, 410, 124538. doi.org/10.1016/j.jhazmat.2020.124538.

Gruzalski, J.G. *et al.* (2016) 'Pore water collection, analysis and avolution: The need for standardization', *Rev Environ Contam Toxicol.,* 37–51. doi.org/10.1007/978-3-319-23573-8_2.

Guler, Y. and Ford, A.T. (2010) 'Anti-depressants make amphipods see the light', *Aquat. Toxicol.*, 99(3), 397–404. doi.org/10.1016/j.aquatox.2010.05.019.

Gumbi, B.P. *et al.* (2017) 'Detection and quantification of acidic drug residues in South African surface water using gas chromatography-mass spectrometry', *Chemosphere*, 168, 1042–1050. doi.org/10.1016/j.chemosphere.2016.10.105.

Hafuka, A. *et al.* (2023) 'Innovative receiving phase for Chemcatcher® passive sampler for phosphorus in the water environment: Calibration of sampling rate by water temperature and pH', *Water Res.*, 243, 120412. doi.org/10.1016/j.watres.2023.120412

Hao, C. *et al.* (2007) 'GC-MS and HPLC-MS analysis of bioactive pharmaceuticals and personal-care products in environmental matrices', *TrAC, Trends Anal. Chem.*, 26(6), 569–580. doi.org/10.1016/j.trac.2007.02.011

Hartig, C. *et al.* (1999) 'Detection and identification of sulphonamide drugs in municipal waste water by liquid chromatography coupled with electrospray ionisation tandem mass spectrometry', *J. Chromatogr. A.*, 854(1–2), 163–173. doi.org/10.1016/S0021-9673(99)00378-7.

Heinz, E. *et al.* (2013) 'Set Up of an Automatic Water Quality Sampling System in Irrigation Agriculture', *J. Sens.*, 14(1), 212–228. doi.org/10.3390/s140100212.

Hesslein, R.H. (1976) 'An in situ sampler for close interval pore water studies1', *Limnol. Oceanogr.*, 21(6), 912–914. doi.org/10.4319/lo.1976.21.6.0912.

Hoang, A.T.P. *et al.* (2024) 'Environmental risk assessment of selected pharmaceuticals in hospital wastewater in northern Vietnam', *Chemosphere*, 356, 141973. doi.org/10.1016/j.chemosphere.2024.141973.

Howe, A.J., *et al*. (2009) 'Surface evolution and carbon sequestration in disturbed and undisturbed wetland soils of the Hunter estuary, southeast Australia', *Estuar. Coast. Shelf Sci.*, 84(1), 75–83. doi.org/10.1016/j.ecss.2009.06.006.

Huckins, J.N. *et al.* (1990) 'Polymeric Film Dialysis in Organic Solvent Media for Cleanup of Organic Contaminants', *J. AOAC Int.*, 73(2), 290–293. doi.org/10.1093/jaoac/73.2.290.

Iloms, E. *et al.* (2020) 'Investigating Industrial Effluent Impact on Municipal Wastewater Treatment Plant in Vaal, South Africa', *Int. J. Environ. Res. Public Health*, 17(3), 1096. doi.org/10.3390/ijerph17031096.

James, A.T. and Martin, A.J.P. (1952) 'Gas-liquid partition chromatography: the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid', *Biochem. J.*, 50(5), 679–690. doi.org/10.1042/bj0500679.

Kannaiah, K.P. *et al.* (2021) 'Environmental impact of greenness assessment tools in liquid chromatography – A review', *Microchem. J.*, 170, 106685. doi.org/10.1016/j.microc.2021.106685.

Kasanah, N. and Hamann, M.T. (2004) 'Development of antibiotics and the future of marine microorganisms to stem the tide of antibiotic resistance.', *Curr Opin Investig Drugs*, 5(8), 827–37.

Kaserzon, S.L. *et al.* (2013) 'Passive sampling of perfluorinated chemicals in water: Flow rate effects on chemical uptake', *Environ. Pollut.*, 177, 58–63. doi.org/10.1016/j.envpol.2013.02.002.

Kaserzon, S.L. *et al.* (2014) 'Characterisation and comparison of the uptake of ionizable and polar pesticides, pharmaceuticals and personal care products by POCIS and Chemcatchers', *Environ. Sci.: Processes Impacts*, 16(11), 2517–2526. doi.org/10.1039/C4EM00392F.

Kingston, J.K. *et al.* (2000) 'Development of a novel passive sampling system for the time-averaged measurement of a range of organic pollutants in aquatic environments', *J. Environ. Monit. Assess*, 2(5), 487–495. doi.org/10.1039/b003532g.

Kolář, P. *et al.* (2002) 'Solvent selection for pharmaceuticals', *Fluid Ph. Equilib.*, 194–197, 771–782. doi.org/10.1016/S0378-3812(01)00716-6.

Kosjek, T. and Heath, E. (2008) 'Applications of mass spectrometry to identifying pharmaceutical transformation products in water treatment', *TrAC, Trends Anal. Chem.*, 27(10), 807–820. doi.org/10.1016/j.trac.2008.08.014.

Kot-Wasik, A. *et al.* (2007) 'Advances in passive sampling in environmental studies', *Anal. Chim. Acta*, 602(2), 141–163 doi.org/10.1016/j.aca.2007.09.013.

Kroløkke, C.H.,*et al.* (2024) 'Fatal feminisation: Problematising endocrine-disrupting chemicals in Denmark', *Eur. J. Cult. Stud.* [Preprint]. doi.org/10.1177/13675494241229094.

Kuster, M. *et al.* (2010) 'Evaluation of two aquatic passive sampling configurations for their suitability in the analysis of estrogens in water', *Talanta*, 83(2), 493–499. doi.org/10.1016/j.talanta.2010.09.033.

Larsson, D.G.J. *et al.* (2007) 'Effluent from drug manufactures contains extremely high levels of pharmaceuticals', *J. Hazard. Mater.*, 148(3), 751–755. doi.org/10.1016/j.jhazmat.2007.07.008.

Leonard, A.W. *et al.* (2002) 'Trimethylpentane-containing passive samplers for predicting time-integrated concentrations of pesticides in water: Laboratory and field studies', *Environ. Toxicol. Chem.*, 21(12), 2591–2599. doi.org/10.1002/etc.5620211210.

Lescord, G.L. and Lepage, A.T. (2025) 'Making waves: report your quality assurance and control (QA/QC) information when publishing analytical data', *Water Res.*, 123167. doi.org/10.1016/j.watres.2025.123167.

Liang, Z. *et al.* (2022) 'A sludge bulking wastewater treatment plant with an oxidation ditch-denitrification filter in a cold region: bacterial community composition and antibiotic resistance genes', *Environ. Sci. Pollut. Res.*, 30(12), 33767–33779. doi.org/10.1007/s11356-022-24591-4.

Lindholm-Lehto, P.C. *et al.* (2016) 'Suitability of passive sampling for the monitoring of pharmaceuticals in Finnish surface waters', *Environ. Sci. Pollut. Res.*, 23(18), 18043–18054. doi.org/10.1007/s11356-016-6778-y.

Lis, H. *et al*. (2021) 'New sampling methods for detecting pharmaceutical residues in seawater and sediments', in *Pharmaceuticals in Marine and Coastal Environments*. Elsevier, 253–274. doi.org/10.1016/B978-0-08-102971-8.00014-7.

Lissalde, S. *et al*. (2014) 'Polar organic chemical integrative samplers for pesticides monitoring: Impacts of field exposure conditions', *Sci. Total Environ*, 488–489, 188–196. doi.org/10.1016/j.scitotenv.2014.04.069.

Lobpreis, T. *et al.* (2008) 'Effect of housing geometry on the performance of Chemcatcher™ passive sampler for the monitoring of hydrophobic organic pollutants in water', *Environ. Pollut.*, 153(3), 706–710. doi.org/10.1016/j.envpol.2007.09.011.

Lohmann, R. *et al.* (2001) 'Further Developments in the Use of Semipermeable Membrane Devices (SPMDs) as Passive Air Samplers for Persistent Organic Pollutants: Field Application in a Spatial Survey of PCDD/Fs and PAHs', *Environ. Sci. Tech.*, 35(12), 2576–2582. doi.org/10.1021/es0001862.

Luo, L. *et al.* (2021) 'Detection and risk assessments of multi-pesticides in 1771 cultivated herbal medicines by LC/MS-MS and GC/MS-MS', *Chemosphere*, 262, 127477. doi.org/10.1016/j.chemosphere.2020.127477.

Madikizela, L.M. *et al.* (2022) 'Target and Suspect Screening of Pharmaceuticals and their Transformation Products in the Klip River, South Africa, using Ultra-High–Performance Liquid Chromatography–Mass Spectrometry', *Environ. Toxicol. Chem.*, 41(2), 437–447. doi.org/10.1002/etc.5265.

Madrid, Y. and Zayas, Z.P. (2007) 'Water sampling: Traditional methods and new approaches in water sampling strategy', *TrAC, Trends Anal. Chem.*, 26(4), 293–299. doi.org/10.1016/j.trac.2007.01.002.

Majors, R.E. (1980) 'High Performance Liquid Chromatography Columns and Column Technology: A State-of-the-Art Review (Parts I and II)', *J. Chromatogr. Sci.*, 18(9), 393–393. doi.org/10.1093/chromsci/18.9.393.

Marchese, S. *et al.* (2003) 'Determination of Non-Steroidal Anti-Inflammatory Drugs in Surface Water and Wastewater by Liquid Chromatography-Tandem Mass Spectrometry', *Chromatographia*, 58(5–6), pp. 263–269. doi.org/10.1365/s10337-003-0052-4.

Martell, B. *et al.* (2017) 'Pharmacokinetics of Lidocaine and Its Metabolites Following Vaginal Administration of Lidocaine Gel to Healthy Female Subjects', *Clin Pharmacol Drug Dev.*, 6(1), 27–35. doi.org/10.1002/cpdd.286.

Martin, G.R., Smoot, J.L. and White, K.D. (1992) 'A comparison of surface-grab and cross sectionally integrated stream-water-quality sampling methods', *Water Environ. Res.*, 64(7), 866–876. doi.org/10.2175/WER.64.7.4.

Martin, J.B. *et al.* (2004) 'An inexpensive, automatic, submersible water sampler', *Limnol. Oceanogr.: Methods*, 2(12), 398–405. doi.org/10.4319/lom.2004.2.398.

Martínez Bueno, M.J. *et al.* (2016) 'POCIS passive samplers as a monitoring tool for pharmaceutical residues and their transformation products in marine environment', *Environ. Sci. Pollut. Res.*, 23(6), 5019–5029. doi.org/10.1007/s11356-014-3796-5.

Martínez-Piernas, A.B. *et al.* (2021) 'Assessment of the presence of transformation products of pharmaceuticals in agricultural environments irrigated with reclaimed water by wide-scope LC-QTOF-MS suspect screening', *J. Hazard. Mater.,* 412, 125080. doi.org/10.1016/j.jhazmat.2021.125080.

Martins de Barros, R. *et al.* (2023) 'Adaptation of the o-DGT for the sampling of 12 hormones: calibration, performance evaluation, and recommendation', *Environ. Sci. Pollut. Res.*, 30(26), 68177–68190. doi.org/10.1007/s11356-023-26975-6.

Masters, P.A. *et al.* (2003) 'Trimethoprim-Sulfamethoxazole Revisited', *Archives of Internal Medicine*, 163(4), p. 402. Available at: https://doi.org/10.1001/archinte.163.4.402.

Mathon, B. *et al.* (2022) 'Polar organic chemical integrative samplers as an effective tool for chemical monitoring of surface waters – Results from one-year monitoring in France', *Sci. Total Environ.*, 824, 153549. doi.org/10.1016/j.scitotenv.2022.153549.

Matongo, S. *et al.* (2015) 'Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa', *Environ. Sci. Pollut. Res.*, 22(13), 10298–10308. doi.org/10.1007/s11356-015-4217-0.

Mazzenga, G.C. and Berner, B. (1991) 'The transdermal delivery of zwitterionic drugs I: the solubility of zwitterion salts', *Journal of Controlled Release*, 16(1–2), 77–88. doi.org/10.1016/0168-3659(91)90032-9.

McKay, S. *et al.* (2020) 'Calibration and validation of a microporous polyethylene passive sampler for quantitative estimation of illicit drug and pharmaceutical and personal care product (PPCP) concentrations in wastewater influent', *Sci. Total Environ.*, 704, 135891. doi.org/10.1016/j.scitotenv.2019.135891.

Miao, X.S. and Metcalfe, C.D. (2003) 'Determination of Carbamazepine and Its Metabolites in Aqueous Samples Using Liquid Chromatography−Electrospray Tandem Mass Spectrometry', *Anal. Chem.*, 75(15), 3731–3738. doi.org/10.1021/ac030082k

Mlunguza, N.Y. *et al.* (2019) 'Adsorbents and removal strategies of non-steroidal anti-inflammatory drugs from contaminated water bodies', *J. Environ. Chem. Eng.*, 7(3), 103142. doi.org/10.1016/j.jece.2019.103142.

Moreno-González, R. *et al.* (2016) 'Do pharmaceuticals bioaccumulate in marine molluscs and fish from a coastal lagoon?', *Environ. Res. J.*, 146, 282–298. doi.org/10.1016/j.envres.2016.01.001.

Morin, N. *et al.* (2012) 'Chemical calibration, performance, validation and applications of the polar organic chemical integrative sampler (POCIS) in aquatic environments', *TrAC, Trends Anal. Chem.*, 36, 144–175. doi.org/10.1016/j.trac.2012.01.007.

Morsy, M.I. *et al.* (2021) 'A novel LC-MS/MS method for determination of the potential antiviral candidate favipiravir for the emergency treatment of SARS-CoV-2 virus in human plasma: Application to a bioequivalence study in Egyptian human volunteers', *J. Pharm. Anal.*, 199, 114057. doi.org/10.1016/j.jpba.2021.114057.

Moschet, C. *et al.* (2015) 'Evaluation of in-situ calibration of Chemcatcher passive samplers for 322 micropollutants in agricultural and urban affected rivers', *Water Res.*, 71, 306–317. doi.org/10.1016/j.watres.2014.12.043.

Ncube, S. *et al.* (2018) 'Environmental fate and ecotoxicological effects of antiretrovirals: A current global status and future perspectives', *Water Res.*, 145, 231–247. doi.org/10.1016/j.watres.2018.08.017.

Ncube, S. *et al.* (2021) 'Trace Detection and Quantitation of Antibiotics in a South African Stream Receiving Wastewater Effluents and Municipal Dumpsite Leachates', *Front. Environ. Sci.*, 9. doi.org/10.3389/fenvs.2021.733065.

Netshithothole, R. *et al.* (2024) 'Occurrence of selected pharmaceuticals in wastewater and sludge samples from wastewater treatment plants in Eastern Cape province of South Africa', *S. Afr. j. chem.*, 78, 7–14. doi.org/10.17159/0379-4350/2024/v78a02.

Netshithothole, R. and Madikizela, L.M. (2024) 'Occurrence of Selected Pharmaceuticals in the East London Coastline Encompassing Major Rivers, Estuaries, and Seawater in the Eastern Cape Province of South Africa', *ACS meas. sci. au.*, 4(3), 283–293. doi.org/10.1021/acsmeasuresciau.4c00004.

Newman, B.K. *et al.* (2024) 'Occurrence and potential hazard posed by pharmaceutically active compounds in coastal waters in Cape Town, South Africa', *Sci. Total Environ.*, 949, 174800. doi.org/10.1016/j.scitotenv.2024.174800.

Ngubane, N.P. *et al.* (2019) 'Determination of naproxen, diclofenac and ibuprofen in Umgeni estuary and seawater: A case of northern Durban in KwaZulu–Natal Province of South Africa', *Reg. Stud. Mar. Sci.*, 29, 100675. doi.org/10.1016/j.rsma.2019.100675.

Nguyen, P.Y. *et al.* (2021) 'A review of the biotransformations of priority pharmaceuticals in biological wastewater treatment processes', *Water Res.*, 188, 116446. doi.org/10.1016/j.watres.2020.116446.

Nieto-Juárez, J.I. *et al.* (2021) 'Pharmaceuticals and environmental risk assessment in municipal wastewater treatment plants and rivers from Peru', *Environ. Int.*, 155, 106674. doi.org/10.1016/j.envint.2021.106674.

Novic, A.J. *et al.* (2017) 'Monitoring Herbicide Concentrations and Loads during a Flood Event: A Comparison of Grab Sampling with Passive Sampling', *Environ. Sci. Tech.*, 51(7), 3880–3891. doi.org/10.1021/acs.est.6b02858.

Nyamba, I. *et al.* (2024) 'Pharmaceutical approaches for enhancing solubility and oral bioavailability of poorly soluble drugs', *Eur. J. Pharm. Biopharm.*, 204, 114513. doi.org/10.1016/j.ejpb.2024.114513.

Oberacher, H. *et al.* (2020) 'A European proposal for quality control and quality assurance of tandem mass spectral libraries', *Environ. Sci. Eur.*, 32(1), 43. doi.org/10.1186/s12302-020-00314-9.

O'Brien, D. *et al.* (2011) 'Determination of deployment specific chemical uptake rates for SDB-RPD Empore disk using a passive flow monitor (PFM)', *Chemosphere*, 83(9), 1290–1295. doi.org/10.1016/j.chemosphere.2011.02.089.

Ockenden, W.A. *et al.* (1998) 'Passive Air Sampling of PCBs: Field Calculation of Atmospheric Sampling Rates by Triolein-Containing Semipermeable Membrane Devices', *Environ. Sci. Tech.*, 32(10), 1538–1543. doi.org/10.1021/es971020c.

Ogunbanwo, O.M. *et al.* (2022) 'High Concentrations of Pharmaceuticals in a Nigerian River Catchment', *Environ. Toxicol. Chem.,* 41(3), 551–558. doi.org/10.1002/etc.4879.

Ohoro, C. R., Adeniji, A. O., Okoh, A. I., & Okoh, O. O. (2021). Spatial and seasonal variations of endocrine disrupting compounds in water and sediment samples of Markman Canal and Swartkops River Estuary, South Africa and their ecological risk assessment. Marine Pollution Bulletin, 173, 113012. https://doi.org/10.1016/j.marpolbul.2021.113012

Ohoro, C. R., Adeniji, A. O., Semerjian, L., Okoh, O. O., & Okoh, A. I. (2021). Occurrence and distribution of pharmaceuticals in surface water and sediment of Buffalo and Sundays River estuaries, South Africa and their ecological risk assessment. Emerging Contaminants, 7, 187–195. https://doi.org/10.1016/j.emcon.2021.09.002

Ojemaye, C.Y. *et al.* (2022) 'The burden of emerging contaminants upon an Atlantic Ocean marine protected reserve adjacent to Camps Bay, Cape Town, South Africa', *Heliyon*, 8(12), e12625. doi.org/10.1016/j.heliyon.2022.e12625.

Ojemaye, C.Y. and Petrik, L. (2022) 'Pharmaceuticals and Personal Care Products in the Marine Environment Around False Bay, Cape Town, South Africa: Occurrence and Risk-Assessment Study', *Environ. Toxicol. Chem.,*, 41(3), 614–634. doi.org/10.1002/etc.5053.

Ore, J. *et al.* (2015) 'Autonomous Aerial Water Sampling', *J. Field Robot.*, 32(8), 1095–1113. doi.org/10.1002/rob.21591.

Palmes, E.D. and Gunnison, A.F. (1973) 'Personal Monitoring Device for Gaseous Contaminants', *Am. Ind. Hyg. Assoc. J.*, 34(2), 78–81. doi.org/10.1080/0002889738506810.

Peijnenburg, W.J. *et al.* (2014) 'Passive sampling methods for contaminated sediments: State of the science for metals', *Integr. Environ. Assess. Manag.*, 10(2), 179–196. doi.org/10.1002/ieam.1502.

Persson, L.B. *et al.* (2001) 'Diffusional behaviour of metals in a passive sampling system for monitoring aquatic pollution', *J. Environ. Monit. Assess*, 3(6), 639–645. doi.org/10.1039/b107959j.

Petersen, J. *et al.* (2016) 'Development and field test of a mobile continuous flow system utilizing Chemcatcher for monitoring of rare earth elements in marine environments', *Environmental Science: Water Research & Technology*, 2(1), 146–153. doi.org/10.1039/C5EW00126A.

Petrie, B. *et al.* (2016) 'In Situ Calibration of a New Chemcatcher Configuration for the Determination of Polar Organic Micropollutants in Wastewater Effluent', *Environ. Sci. Tech.*, 50(17), 9469–9478. doi.org/10.1021/acs.est.6b02216.

Petrik, L. *et al.* (2017) 'Corrigendum: Desalination and seawater quality at Green Point, Cape Town: A study on the effects of marine sewage outfalls', *S. Afr. J. Sci.*, 114(1/2), 1. doi.org/10.17159/sajs.2018/a0244C.

Petrovic, M. *et al.* (2006) 'Multi-residue analysis of pharmaceuticals in wastewater by ultra-performance liquid chromatography–quadrupole–time-of-flight mass spectrometry', *J. Chromatogr. A.*, 1124(1–2), 68–81. doi.org/10.1016/j.chroma.2006.05.024.

Pugajeva, I. *et al.* (2021) 'Two-dimensional liquid chromatography - mass spectrometry as an effective tool for assessing a wide range of pharmaceuticals and biomarkers in wastewater-based epidemiology studies', *J. Pharm. Biomed. Anal.*, 205, 114295. doi.org/10.1016/j.jpba.2021.114295.

Ramm, A.E.L. (1990) 'Application of the community degradation index to South African estuaries', *Water Res.*, 24(3), 383–389. doi.org/10.1016/0043-1354(90)90018-2.

Rao, P.P.N. *et al.* (2010) 'Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): Progress in Small Molecule Drug Development', *J. Pharm.*, 3(5), 1530–1549. doi.org/10.3390/ph3051530.

Reddering, J. and Rust, I. (1990) 'Historical changes and sedimentary characteristics of southern African estuaries', *S. Afr. J. Sci.*, 86(7), 425.

Reddersen, K. and Heberer, T. (2003) 'Multi-compound methods for the detection of pharmaceutical residues in various waters applying solid phase extraction (SPE) and gas chromatography with mass spectrometric (GC-MS) detection', *J. Sep. Sci.*, 26(15–16), 1443–1450. doi.org/10.1002/jssc.200301533.

Reinholds, I. *et al.* (2017) 'Decomposition of multi-class pharmaceutical residues in wastewater by exposure to ionising radiation', *Int. J. Environ. Sci. Technol..*, 14(9), 1969–1980. doi.org/10.1007/s13762-017-1290-6.

Ricciutelli, M. *et al.* (2020) 'Study of the effect of marination treatment on garlic bioactive compounds through an innovative HPLC-DAD-MS method for alliin and curcuminoids analysis', *LWT*, 131, 109788. doi.org/10.1016/j.lwt.2020.109788.

Rimayi, C. *et al.* (2018) 'Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa', *Sci. Total Environ.*, 627, 1008–1017. doi.org/10.1016/j.scitotenv.2018.01.263.

Robinson, R.F.A. *et al.* (2024) 'Quantification and risk assessment of polar organic contaminants in two chalk streams in Hampshire, UK using the Chemcatcher passive sampler', *Sci. Total Environ.*, 939, 173316. doi.org/10.1016/j.scitotenv.2024.173316.

Róg, T. *et al.* (2021) 'Mechanistic Understanding from Molecular Dynamics in Pharmaceutical Research 2: Lipid Membrane in Drug Design', *J. Pharm.*, 14(10), 1062. doi.org/10.3390/ph14101062.

Roig, N. *et al.* (2011) 'Novel approach for assessing heavy metal pollution and ecotoxicological status of rivers by means of passive sampling methods', *Environ. Int.*, 37(4), 671–677. doi.org/10.1016/j.envint.2011.01.007.

Römerscheid, M. *et al.* (2023) 'Calibration of the Chemcatcher® passive sampler and derivation of generic sampling rates for a broad application in monitoring of surface waters', *Sci. Total Environ.*, 871, 161936. doi.org/10.1016/j.scitotenv.2023.161936.

S. Giller, P. *et al.* (2004) 'Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments', *Oikos*, 104(3), 423–436. doi.org/10.1111/j.0030-1299.2004.13253.x.

Schäfer, R.B. *et al.* (2008) 'Aquatic passive sampling of a short-term thiacloprid pulse with the Chemcatcher: Impact of biofouling and use of a diffusion-limiting membrane on the sampling rate', *J. Chromatogr. A.*, 1203(1), 1–6. doi.org/10.1016/j.chroma.2008.05.098.

Schoeman, F.R. (1979) 'DIATOMS AS INDICATORS OF WATER QUALITY IN THE UPPER HENNOPS RIVER (Transvaal, South Africa)', . *Limnol. Soc. South. Afr.,* 5(2), 73–78. doi.org/10.1080/03779688.1979.9633188.

Schroeder, H. *et al.* (2020) 'Sediment water (interface) mobility of metal(loid)s and nutrients under undisturbed conditions and during resuspension', *J. Hazard. Mater.*, 394, 122543. doi.org/10.1016/j.jhazmat.2020.122543.

Scott, G.I. *et al.* (2002) 'Toxicological Studies in Tropical Ecosystems: an Ecotoxicological Risk Assessment of Pesticide Runoff in South Florida Estuarine Ecosystems', *J. Agric. Food Chem.*, 50(15), 4400–4408. doi.org/10.1021/jf011356c.

Šetková, L. *et al.* (2005) 'Fast isolation of hydrophobic organic environmental contaminants from exposed semipermeable membrane devices (SPMDs) prior to GC analysis', *J. Chromatogr. A.*, 1092(2), 170–181. doi.org/10.1016/j.chroma.2005.07.059.

Shaw, M. and Mueller, J.F. (2009) 'Time Integrative Passive Sampling: How Well Do Chemcatchers Integrate Fluctuating Pollutant Concentrations?', *Environ. Sci. Tech.*, 43(5), 1443–1448. doi.org/10.1021/es8021446.

Sigonya, S. *et al.* (2022) 'Method optimisation and application based on SPE of non-steroidal anti-inflammatory drugs, antiretroviral drugs, and a lipid regulator from coastal areas of Durban, South Africa', *SN Appl. Sci.*, 4(8), 231. doi.org/10.1007/s42452-022-05120-x.

Simpson, J., Simpson, B.S. and Gerber, C. (2024) 'A LC-MS/MS method for the simultaneous quantification of 17 opioids in biosolids', *Talanta*, 272, 125775. doi.org/10.1016/j.talanta.2024.125775.

Škodová, A. *et al.* (2016) 'In situ calibration of three passive samplers for the monitoring of steroid hormones in wastewater', *Talanta*, 161, 405–412. doi.org/10.1016/j.talanta.2016.08.068.

Snyder, S.A. *et al.* (2004) 'Biological and Physical Attenuation of Endocrine Disruptors and Pharmaceuticals: Implications for Water Reuse', *Ground Water Monit Remediat.*, 24(2), 108–118 doi.org/10.1111/j.1745-6592.2004.tb00719.x.

Stephens, B.S. *et al.* (2009) 'Event monitoring of herbicides with naked and membrane-covered Empore disk integrative passive sampling devices', *Mar. Pollut. Bull.*, 58(8), 1116–1122. doi.org/10.1016/j.marpolbul.2009.04.012.

Sultana, T. and Metcalfe, C.D. (2022) 'Calibration and field validation of POCIS passive samplers for tracking artificial sweeteners as indicators of municipal wastewater contamination in surface waters', *Environ. Monit. Assess.*, 194(8),. 564. doi.org/10.1007/s10661-022-10150-4.

Tabak, H.H. and Bunch, R.L. (1970) 'Missouri River Basin Sterol Assay Project Report. Coprostanol, A Positive Marker of Domestic and Run-Off Pollution: Sterol Assay of Wastewater Plant Effluents and Surface Waters of the Lower Main Stem Missouri.', *(1970).*

Tadić, Đ. *et al.* (2022) 'Use of Passive and Grab Sampling and High-Resolution Mass Spectrometry for Non-Targeted Analysis of Emerging Contaminants and Their Semi-Quantification in Water', *Molecules*, 27(10), 3167. doi.org/10.3390/molecules27103167.

Tan, B.L.L. *et al.* (2007) 'Comprehensive study of endocrine disrupting compounds using grab and passive sampling at selected wastewater treatment plants in South East Queensland, Australia', *Environ. Int.*, 33(5), 654–669. doi.org/10.1016/j.envint.2007.01.008.

Taylor, A.C. *et al.* (2021) 'Applications for Passive Sampling of Hydrophobic Organic Contaminants in Water—A Review', *Crit. Rev. Anal. Chem.*, 51(1), 20–54. doi.org/10.1080/10408347.2019.1675043.

Teschner, S. and Burst, V. (2010) 'Leflunomide: A Drug with a Potential Beyond Rheumatology', *Int. J. Immunother.*, 2(5), 637–650. doi.org/10.2217/imt.10.52.

Truter, J. *et al*. (2015) '*In vitro* screening for endocrine disruptive activity in selected South African harbours and river mouths', *Afr. J. Mar. Sci.*, 37(4), 567–574. doi.org/10.2989/1814232X.2015.1105296.

Turiel, E., *et al.* (2003) 'Trace enrichment of (fluoro)quinolone antibiotics in surface waters by solid-phase extraction and their determination by liquid chromatography–ultraviolet detection', *J. Chromatogr. A*, 1008(2), 145–155. doi.org/10.1016/S0021-9673(03)00975-0.

Valenzuela, E.F. *et al.* (2020) 'Passive and grab sampling methods to assess pesticide residues in water. A review', *Environ. Chem. Lett.*, 18(4), 1019–1048. doi.org/10.1007/s10311-020-00998-8.

van Deventer, H., Apleni, P., Adams, J. B., Riddin, T., Whitfield, E., Machite, A., van Niekerk, L., & Madasa, A. (2025). Assessing the feasibility of mapping changes of ecosystem functional groups in South African estuaries using Landsat and Sentinel images of 1990, 2014, 2018 and 2020. Wetlands Ecology and Management, 33(1), 12. https://doi.org/10.1007/s11273-024-10027-y

Vaudreuil, M.A. *et al.* (2022) 'Pharmaceutical pollution of hospital effluents and municipal wastewaters of Eastern Canada', *Sci. Total Environ.*, 846, 157353. doi.org/10.1016/j.scitotenv.2022.157353.

Vermeirssen, E.L.M. *et al.* (2008) 'The role of hydrodynamics, matrix and sampling duration in passive sampling of polar compounds with Empore^{TM} SDB-RPS disks', *J. Environ. Monit.*, 10(1), 119–128. doi.org/10.1039/B710790K.

Vermeirssen, E.L.M. *et al.* (2012) 'Transfer Kinetics of Polar Organic Compounds over Polyethersulfone Membranes in the Passive Samplers Pocis and Chemcatcher', *Environ. Sci. Technol.*, 46(12), 6759–6766. doi.org/10.1021/es3007854.

Vrana, B., Allan, I.J., *et al.* (2005) 'Passive sampling techniques for monitoring pollutants in water', *TrAC, Trends Anal. Chem.*, 24(10), 845–868. doi.org/10.1016/j.trac.2005.06.006.

Vrana, B., Mills, G., *et al.* (2005) 'Performance optimisation of a passive sampler for monitoring hydrophobic organic pollutants in water', *J. Environ. Monit.*, 7(6), 612. doi.org/10.1039/b419070j.

Vrana, B. *et al.* (2006) 'Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water', *Environ. Pollut.*, 142(2), 333–343. doi.org/10.1016/j.envpol.2005.10.033.

Vrana, B. *et al.* (2010) 'Field performance of the Chemcatcher passive sampler for monitoring hydrophobic organic pollutants in surface water', *J. Environ. Monit.*, 12(4), p. 863. https://doi.org/10.1039/b923073d.

Vrana, B. *et al.* (2021) 'In situ calibration of polar organic chemical integrative sampler (POCIS) for monitoring of pharmaceuticals in surface waters', *Environ. Pollut.*, 269, 116121. doi.org/10.1016/j.envpol.2020.116121.

Vumazonke, S. *et al.* (2020) 'Detection of Pharmaceutical Residues in Surface Waters of the Eastern Cape Province', *Int. J. Environ. Res. Public Health*, 17(11), 4067. doi.org/10.3390/ijerph17114067.

Wacheski, T. *et al.* (2021) ‘o-DGT Devices for the Determination of Emerging Contaminants in Aqueous Matrices’, *J. Braz. Chem. Soc.* [Preprint]. doi.org/10.21577/0103-5053.20200157.

Wahl, B.J. (1979) ‘Automatic Sampling Systems at Alcan Smelters and Chemicals’, *JOM*, 31(8), 7–10. doi.org/10.1007/BF03354473.

Waleng, N.J. and Nomngongo, P.N. (2022) ‘Occurrence of pharmaceuticals in the environmental waters: African and Asian perspectives’, *J. Environ. Chem. Ecotoxicol.*, 4, 50–66. doi.org/10.1016/j.enceco.2021.11.002.

Wang, K. *et al.* (2021) ‘Antibiotic residues in wastewaters from sewage treatment plants and pharmaceutical industries: Occurrence, removal and environmental impacts’, *Sci. Total Environ.*, 788, 147811. doi.org/10.1016/j.scitotenv.2021.147811.

Wang, X. *et al.* (2020) ‘Suspect and non-target screening of pesticides and pharmaceuticals transformation products in wastewater using QTOF-MS’, *Environ. Int.*, 137, 105599. doi.org/10.1016/j.envint.2020.105599.

Wang, Y.Q. *et al.* (2022) ‘Suspect, non-target and target screening of pharmaceuticals and personal care products (PPCPs) in a drinking water system’, *Sci. Total Environ.*, 808, 151866. doi.org/10.1016/j.scitotenv.2021.151866.

Wepener, V. *et al.* (2011) ‘An assessment of the influence of multiple stressors on the Vaal River, South Africa’, *Phys. Chem. Earth*, 36(14–15), 949–962. doi.org/10.1016/j.pce.2011.07.075.

Wilson, M. *et al.* (2022) ‘Comparison of Auto Sampling and Passive Sampling Methods for SARS-CoV-2 Detection in Wastewater’, *J. Pathog.*, 11(3), 359. doi.org/10.3390/pathogens11030359.

Wilson, T.P., Miller, C. V. and Lechner, E.A. (2024) *Guidelines for the use of automatic samplers in collecting surface-water quality and sediment data*. doi.org/10.3133/tm1D12.

Wirth, E. *et al.* (2024) ‘Comparison of chemical contaminant measurements using CLAM, POCIS, and silicone band samplers in estuarine mesocosms’, *Integr. Environ. Assess. Manag.*, 20(5), 1384–1395. doi.org/10.1002/ieam.4953.

Xu, Y. *et al.* (2005) ‘Accumulation of Organochlorine Pesticides from Water Using Triolein Embedded Cellulose Acetate Membranes’, *Environ. Sci. Technol.*, 39(4), 1152–1157. doi.org/10.1021/es040454m.

Xu, Y. *et al.* (2021) ‘Sorption of pharmaceuticals and personal care products on soil and soil components: Influencing factors and mechanisms’, *Sci. Total Environ.*, 753, 141891. doi.org/10.1016/j.scitotenv.2020.141891.

Yamamoto, H. *et al.* (2009) ‘Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments’, *Water Res.*, 43(2),351–362. doi.org/10.1016/j.watres.2008.10.039.

Yusà, V. *et al*. (2006) 'Microwave-assisted extraction of polybrominated diphenyl ethers and polychlorinated naphthalenes concentrated on semipermeable membrane devices', *Anal. Chim. Acta.*, 565(1), 103–111. doi.org/10.1016/j.aca.2006.02.009.

Zhao, W. *et al.* (2006) 'Ionic liquid-containing semipermeable membrane devices for monitoring the polycyclic aromatic hydrocarbons in water', *Chemosphere*, 62(10), 1623–1629. doi.org/10.1016/j.chemosphere.2005.06.041.

Zubillaga, M.P. and Maerker, G. (1990) 'Determination of Safrole and Isosafrole in Ham by HPLC with UV Detection', *J. Food Sci.*, 55(4), 1194–1195. doi.org/10.1111/j.1365-2621.1990.tb01639.x.

CHAPTER 3 RESEARCH AIM AND OBJECTIVES

3.1 Aim of study

The primary aim of this study is to assess pharmaceutical pollution in two major South African rivers and one estuary through suspect screening and targeted analysis by employing a combination of grab sampling and Chemcatcher passive sampling techniques. This research focuses specifically on the Hennops River, Crocodile River, Hartbeespoort Dam, and the Durban Harbour estuary, which are critical aquatic ecosystems facing significant pollution challenges from various sources.

3.2 Specific Objectives

The aim will be met by achieving the following objectives:

- To develop and validate an HPLC-MS analysis method for the quantification of nine pharmaceutical compounds from various therapeutic classes. These compounds include: an anticonvulsant drug – carbamazepine, a corticosteroid – dexamethasone, an adrenergic agonist – etilefrine, antiretroviral agents – lopinavir and nevirapine, antidiabetic agent – metformin, muscle relaxant – methocarbamol, antibiotic – trimethoprim, and an antidepressant – venlafaxine.

- To calibrate the Chemcatcher passive samplers for the uptake and accumulation of six target pharmaceutical compounds, namely: carbamazepine, dexamethasone, etilefrine, methocarbamol, nevirapine, and venlafaxine.

- To apply the developed and validated HPLC-MS method to detect and quantify the nine target pharmaceutical compounds in grab water samples and samples from Chemcatcher samplers collected from the Hennops River, Crocodile River, Hartbeespoort Dam, and the Durban Harbour estuary.

- To compare the effectiveness of grab sampling and passive sampling techniques in detecting pharmaceuticals in the study sites by evaluating the detection frequencies and concentration ranges obtained from each method, highlighting their respective strengths and limitations for monitoring pharmaceutical pollution.

3.3 Significance of the Study

This study addresses critical knowledge gaps in the monitoring of pharmaceutical pollution in South African rivers and estuaries. By integrating grab sampling and passive sampling (which is underutilized in South Africa) with an advanced analytical method (HPLC-MS/MS), it aims to provide a comprehensive understanding of contaminant levels. The findings contribute to the development of effective water quality management strategies, enhance environmental monitoring programs, and support the conservation of aquatic ecosystems in South Africa.

3.4 Novelty of the study

This study employs a novel approach to investigating pharmaceutical pollution in Durban Harbour Estuary, Hennops River, Crocodile River, and Hartbeespoort Dam, utilizing a combination of a Chemcatcher passive sampler and grab sampling to identify and quantify the bioavailable fractions of these contaminants. These aquatic systems are subjected to varying anthropogenic pressures, including untreated wastewater inputs in the Hennops and Crocodile Rivers, urban run-off, and nutrient-rich agricultural runoff in Hartbeespoort Dam, contributing to a chemically diverse contaminant profile. This study focuses on the adsorption and diffusive transport of pharmaceuticals, including antibiotics, anti-inflammatories, and endocrine-disrupting compounds, into the Chemcatcher polar phases, allowing for their identification and quantification. By examining the physicochemical interactions between pharmaceuticals and environmental matrices across these distinct ecosystems, the research provides critical insights into the sources, transport, and distribution of emerging contaminants, contributing to the development of pollution mitigation strategies

3.5 Structure of the thesis

This thesis is organized into six chapters. These chapters address the research objectives and explore the issue of pharmaceutical pollution across different environments, from freshwater to marine ecosystems. Chapter 1 introduces the research topic by providing background information on pharmaceutical pollution and its environmental implications. Chapter 2 offers an in-depth review of relevant literature, discussing pharmaceutical pollution in freshwater and marine ecosystems. It also covers various sampling techniques employed to monitor pharmaceutical pollution, with particular emphasis on the Chemcatcher sampler and its applications in aquatic pollution monitoring studies. Chapter 3 introduces the primary aim of the study and expands on how the aim will be achieved. It also highlights the significance and

novelty of the study. Chapter 4 chapter focuses on the calibration of a Chemcatcher-like sampler for the uptake of six pharmaceutical compounds. It details the deployment of the sampler in the Hennops River (upstream) and its catchment, Hartbeespoort Dam (downstream). Additionally, it compares the effectiveness of Chemcatcher sampling to traditional grab sampling for targeted analysis of six pharmaceutical compounds and untargeted analysis of pesticides and pharmaceuticals in the study areas. Chapter 5 examines the presence of nine pharmaceutical compounds in the Durban Marina area, an estuarine environment on South Africa's east coast. It highlights how pharmaceutical pollutants from land-based activities eventually enter marine ecosystems, emphasizing the interconnectedness of aquatic environments. Chapter 6 builds on Chapter 4, it investigates the transport and fate of nine pharmaceutical compounds in the Crocodile River, downstream of Hartbeespoort Dam. It explores the dynamics of pharmaceutical contaminants as they move through aquatic ecosystems. Chapter 7 concludes the study, highlighting key findings, limitations, and recommendations for future research.

CHAPTER 4 MANUSCRIPT 1

Profiling diverse sources of pollution of urban rivers using target and nontarget analysis: A case of a heavily polluted river in South Africa

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ABSTRACT

Urban rivers in developing countries are under enormous strain from pollution mostly due to poor functioning and inadequately maintained wastewater treatment plants, industrial discharges among other sources. In this study, Chemcatcher passive sampling and solid phase extraction (SPE) followed by ultra-high performance liquid chromatography (UHPLC) Quadrupole-Orbitrap mass spectrometry (MS) were used to assess the extent of pollution of a heavily polluted river. Laboratory calibration of the Chemcatcher samplers yielded sampling rates ranging from 0.360 L day^{-1} for dexamethasone to 0.447 L day^{-1} for carbamazepine. Using the sampling rates obtained, the time-weighted average (TWA) concentrations were determined to range from not detected (ND) to 17.87 μg L^{-1} for venlafaxine and dexamethasone, respectively. The obtained SPE recoveries ranged from 83.92 and 80.52 %, for river and ultrapure waters, respectively. The concentrations in SPE samples were found to be greater than those in Chemcatcher samples and ranged from ND to 84.71 μg L^{-1} for carbamazepine and dexamethasone, respectively. In the suspect screening, 25 pesticides and 61 pharmaceutical compounds were detected with the detection frequency higher for Chemcatcher compared to SPE samples. This study reveals the extent of pollution in Hennops river and its catchment by various organic pollutants. Additionally, it highlights that the passive sampling approach is an excellent tool for the detection of compounds at higher frequency in suspect screening than SPE approach.

Keywords: Emerging pollutants; Urban River pollution; Chemcatcher sampler; Q-Orbitrap MS

4.1 Introduction

In recent years, the presence of pharmaceutical compounds and other related compounds in the aquatic environment has been a subject of growing concern in environmental chemistry. This is due to their potential threat to non-target aquatic organisms and the aquatic ecosystem as a whole (Feijão et al., 2020; Kayode-Afolayan et al., 2022; Khan & Barros, 2023; Porretti et al., 2022; Świacka et al., 2022). Pharmaceuticals are medicinal products used in human and veterinary medicine for diagnostic, preventive, and therapeutic purposes (Enick & Moore, 2007). A wide variety of these compounds exist, and the function they are designed for is determined by the chemical and physical properties these compounds possess. The majority of pharmaceutical compounds are polar and lipophilic, while some dissolve moderately in water. They typically possess more than one ionizable group, and the degree to which they can be ionized is largely dependent on the physiochemical properties of the medium they are in Bunally et al. (2019).

The presence of pharmaceutical compounds in the aquatic environment can be attributed to sources such as pharmaceutical manufacturing plants, hospital waste, poorly treated effluent from wastewater treatment plants (WWTP), household waste, and their excretion through incomplete metabolism in humans and animals (Freitas & Radis-Baptista, 2021; Khasawneh & Palaniandy, 2021; Rogowska & Zimmermann, 2022; Vaudreuil et al., 2022a, 2022b). Pharmaceuticals are excreted in various forms, including as their native compounds, metabolites, or transformation products. Upon entering the environment, these substances can undergo further transformations, creating complex mixtures of parent compounds, metabolites, and additional transformation products (Narayanan et al., 2022; Wang et al., 2020). This complexity complicates the monitoring of active pharmaceutical ingredients (APIs) in aquatic environments and hinders the assessment of their ecological impact.

In continents such as Europe and North America, more data is available on water pollution levels (Desai et al., 2022). Gani et al. (2021) reviewed literature available between January 2000 – April 2020 on emerging contaminants in South African waters. A total of 41 publications were found. It was revealed that majority of these publications focused on wastewaters, and very little information was available on the presence of emerging contaminants in surface and drinking water (Odendaal et al., 2015; Schoeman et al., 2017; Wood et al., 2015).

In recent years, numerous studies have reported the detection and quantification of pharmaceuticals in South African surface waters (Mosharaf et al., 2024; Ojemaye & Petrik, 2022; Waleng & Nomngongo, 2022; Wilkinson et al., 2022). A common feature in these studies is the reliance on grab sampling techniques, which may not provide a comprehensive picture of contaminant levels over time. Further, almost all these studies focused on target analysis. Passive sampling, on the other hand, has been increasingly recognized as a valuable complementary tool, enabling the accumulation of pollutants over extended periods and the detection of trace contaminants (Cao et al., 2024; Cristóvão et al., 2021; Fialová et al., 2024; Robinson et al., 2024; Römerscheid et al., 2023; Yu et al., 2024). However, its application remains underutilized in the African continent, despite its advantages especially when combined with non-target or suspect screening analysis. This may be attributed to issues such as theft and cost (Jiang et al., 2022).

This study addresses a critical gap by employing both passive and active sampling techniques to monitor the extent of pollution by pharmaceutical compounds and other related contaminants in one of the most polluted urban rivers in South Africa. Part of the upstream of Hennops River (Gauteng province, South Africa) has little to no aquatic life because of high dissolved solids from poorly treated wastewater resulting in dissolved oxygen below the minimum standard allowed to support aquatic. This has caused massive eutrophication downstream, which has resulted in the deterioration of value of property around the

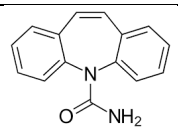
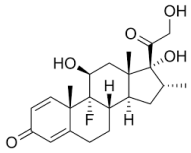
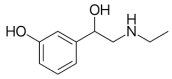
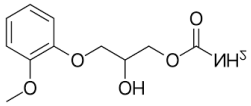
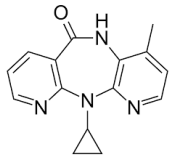
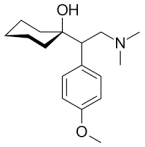
Hartbeespoort dam which is major catchment of the water. The use of Chemcatcher passive samplers and SPE allowed to provide the needed synergy for the detection of a wide range of pharmaceutical compounds, including those present at trace levels. By using both suspect screening and targeted approaches, this study provides a more detailed profile of the emerging pollutants in the urban river system.

4.2 Materials and methods

4.2.1 Reagents and Chemicals

Analytical grade nitric acid, HPLC grades acetonitrile, methanol, ammonium formate and formic acid were purchased from Merck (Johannesburg, South Africa). Analytical grades venlafaxine hydrochloride, methocarbamol, etilefrine hydrochloride, nevirapine, and carbamazepine, and dexamethasone were from Merck (Johannesburg, South Africa). For mass spectrometry, LC-MS grade water and methanol from Merck (Johannesburg, South Africa) were used along with formic acid and ammonium formate (Merck, Johannesburg, South Africa). AttractSPETM disks HLB (90 mm) and EXPRESS PLUS (PES) disk membranes (0.45 um, diameter 90 mm) were purchased from Anatech Pty Ltd (Johannesburg, South Africa). The SPE setup consisted of Supel-Select HLB cartridges with 500 mg sorbent material. The HLB cartridges were purchased from Sigma Aldrich (Johannesburg, South Africa). Ultrapure water was produced in-house using the Millipore DirectQ 3 UV water purification system from Millipore (Massachusetts, USA). All reagents and chemicals were used as received unless specified. A 1000 mg L^{-1} stock solution mixture of the six pharmaceutical standards was prepared by dissolving 10 mg each of the standards in 10 mL of methanol. This was kept refrigerated at 4 ºC and renewed every week. The nine calibration solutions were prepared in ultrapure water/methanol, 90:10, v/v with concentrations ranging from 0.1 to 500 µg L^{-1}.

Table 1
Properties of the target pharmaceutical compounds

Target compound	Chemical formula	Structure	Molecular weight g mol^{-1}	LogP	Water solubility mg L^{-1}
Carbamazepine	$C_{15}H_{12}N_2O$		236.27	2.77	35.40
Dexamethasone	$C_{22}H_{29}FO_5$		392.46	1.83	89.00
Etilefrine	$C_{10}H_{15}NO_2$		181.23	0.10	18.9
Methocarbamol	$C_{11}H_{15}NO_5$		241.24	0.61	29.9
Nevirapine	$C_{15}H_{14}N_4O$		266.21	2.50	100
Venlafaxine	$C_{17}H_{27}NO_2$		277.40	3.20	572 x 10^3

4.2.2 Instrumentation

pH, conductivity, and total dissolved solids (TDS) of the grab water samples were determined using a calibrated YSI multi-parameter meter (Model 556, YSI Inc., Yellow Springs, OH, USA) on the day of sampling. The bacteriological assessments for this study were carried out at the Agricultural Research Council (ARC), Centurion, South Africa. The grab

water samples were assessed for total aerobic count, coliform count and *E.coli* detection . The SPE vacuum manifold was from Pall Corporation (Fribourg, Switzerland). High resolution LC-MS analysis was performed using a Thermo Scientific Vanquish UHPLC^{+} Q Exactive Focus Orbitrap (Anatech, Johannesburg, South Africa).

4.2.3 Study area

The study was conducted between the provinces of Gauteng and the Northwest in South Africa. The study focused on the Hennops River (upstream) and the Crocodile River (downstream) that run through Hartbeespoort Dam. The Hennops River starts around OR Tambo international airport in Kempton Park, Johannesburg. It flows north and passes through Tembisa township before joining the Sesmylspruit River, which drains the Reitvlei Dam (located in Pretoria). The river's course then takes a north westerly direction towards the centre of Centurion Mall area passing through the foothills of Magalies valley, before joining the Crocodile River and then into the Hartbeespoort Dam. Major pollution occurs in Tembisa township where municipal wastewater infrastructure is poor coupled with improper municipal solid waste management dumping of solid waste directly into the river is common in some areas. Three municipal wastewater treatment plants discharge their water that end up in the river after the river leaves the township and before it joins the Jukskei River to form the Crocodile River. Water samples were collected from five sites, S1, S2, S3, S4 and S5 (Fig. 1). S1 (25° 43' 8,376" S 27° 50' 36,954" E) was located downstream along the Crocodile River, at the exit of Hartbeespoort dam. S2 (25° 45' 46,476" S 27° 48' 23,868" E) was situated in the Hartbeespoort Dam west channel. This site receives input mainly from agricultural activities. S3 (25° 47' 30,768" S 27° 53' 56,7" E) was located at the Hartbeespoort Dam entrance of the Crocodile River. S4 (25° 49' 46,392" S 28° 8' 9,906" E) was located along the Hennops riverbank near the Royal Elephant Hotel in Centurion, which was midway of the river from

upstream. S5 (25° 55' 24,006" S 28° 13' 36,702" E) was located upstream just as the river leaves the informal settlement of Tembisa.

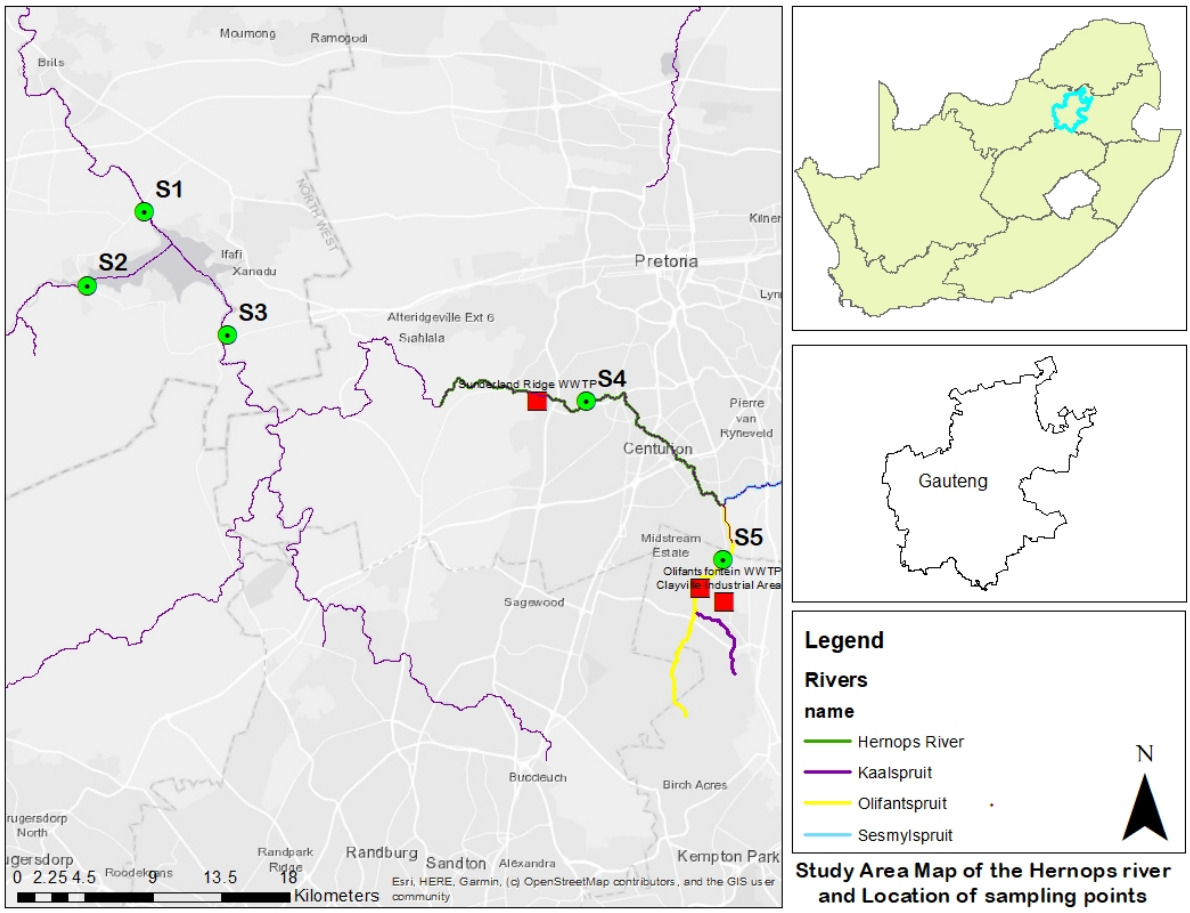


Fig. 1.
Study area map of Hennops River and Hartbeespoort Dam in Gauteng Province, South Africa

4.2.4. Assembly, laboratory calibration and extraction of Chemcatcher

Three component polytetrafluoroethylene (PTFE) Chemcatcher bodies (external diameter 6.6 cm, internal depth 0.8 cm and internal diameter 5.4 cm) were manufactured by the University of the Witwatersrand Physics workshop (Johannesburg, South Africa). This was similar to a design reported by Castle et al. (2018). The sampler holders were soaked in 10% nitric acid solution and rinsed with ultrapure water, which was followed by acetone before use.

AttractSPETM disks HLB (90 mm) and EXPRESS PLUS (PES) disk membranes (0.45 um, diameter 90 mm) were cut to the internal diameter of the sample holder and assembled. These were assembled by exposing the rough side of the polyether sulfone disk membranes on the sample holder's support plate. This was followed by placing the HLB disks, after which the sample holder lid was placed in such a manner that the HLB disks was sandwiched between the polyether sulfone disk membranes and sample holder lid. The assembled passive sampling devices were soaked in methanol overnight to activate the HLB disks, after which they were soaked in ultrapure water until use to prevent drying.

Chemcatcher passive sampler calibration experiments were conducted in ultrapure water using the static calibration method as described by Römerscheid et al. (2023). Ten Chemcatcher samplers were suspended vertically in a 5L glass tank spiked to 0.4 μg L^{-1} each of the target compounds. The experiment was conducted at a stirring rate of 100 rpm, at room temperature over 14 days. Two Chemcatcher samplers were retrieved after two, five, eight, eleven and sixteen days. Upon retrieval, the passive samplers were wrapped in aluminium foil and stored at – 4 °C until ready for extraction and analysis.

Extraction of the Chemcatcher samplers was done using a procedure modified from that described by Robinson et al. (2024). Briefly, the passive samplers were rinsed with ultrapure water to remove debris, after which they were disassembled, and the Oasis HLB disks were transferred into pre-washed glass vials. The Oasis HLB disks were allowed to air dry for five hours. Methanol (40 mL) was added to the glass vials for extraction. These were shaken for one hour. The sorbent material was removed, and the solvent was filtered through a syringe filter (0.22 μm) to remove the suspended disk particles. The volume of the filtrate was reduced to 1 mL under a stream of nitrogen gas, and the extracts were transferred to glass vials and stored in the freezer (–20 °C) until analysis. The sampling rates were calculated with linear uptake kinetics according to Römerscheid et al., (2023).

4.2.5 Field deployment of Chemcatcher samplers

Field deployed Chemcatcher samplers were housed in polyvinyl chloride pipes (25 mm x 15 cm) with holes (2 cm diameter) drilled on the sides. Cable ties were used to secure the Chemcatcher samplers, and a ski rope was used to secure the cages at the deployment sites. The cages were fully immersed in water at a depth of ~30 cm for the course of the deployment period. Three Chemcatcher samplers were deployed at each site for a duration of 14 days in the spring season (October 2023). Two field blanks were brought on site on the day of deployment and retrieval to monitor contamination. On retrieval, the Chemcatcher samplers were detached from the cages and lightly rinsed with ultrapure water before sealing with the Chemcatcher lids. The samplers were stored in a cooler for transportation to the laboratory, where they were stored at – 20 °C until extraction.

4.2.6 Grab sampling and SPE procedures

Polytetrafluoroethylene bottles (500 mL) were rinsed with 10% nitric acid solution followed by ultrapure water. On the day of Chemcatcher sampler deployment, two 500 mL water samples were collected at each deployment site. This was done by first rinsing the polytetrafluoroethylene bottles with river or dam water at the sampling site and then filling the bottles up to the neck. These were carefully closed and placed in a cooler box for transportation to the laboratory to preserve the integrity of the samples. One of the 500 mL water samples was kept for physiochemical property analysis and SPE, while the other bottle was sent to the ARC Irene, Centurion, South Africa for bacteriological analysis.

Grab water samples from sites S1, S2, S3, S4 and S5 were filtered through 0.45 µm filter paper in a Büchner funnel, and the filtrate was collected in clean glass bottles. The SPE

procedure used was modified from Tolić Čop et al. (2024). The cartridges were mounted on an SPE manifold and the whole set up was connected to a vacuum pump. The cartridges were pre-conditioned by passing 2 x 6 mL methanol at a rate of 1 drop per second, followed by 6 mL ultrapure water. This was followed by passing 200 mL of the filtered sample through the HLB cartridges at a rate of 1 drop per second.

After percolating, the HLB cartridges were vacuum dried, and 6 mL methanol was used to elute the sample. Each extract was collected in a clean glass vial, after which the solvent volume was reduced to 1 mL under a gentle stream of nitrogen gas. The 1 mL samples were then transferred to glass vials and stored in the freezer (– 20 °C) until analysis. The same procedure was repeated for the analyte recovery study, where 200 mL of ultrapure water was spiked to 10 µg L^{-1} and 25 µg L^{-1} using the six pharmaceutical standard mixture. 6 mL methanol was used for extraction. Each extract was collected in a clean glass vial, after which the solvent volume was reduced to 1 mL using nitrogen gas. The 1 mL samples were then transferred to glass vials and stored in the freezer (– 20 °C) until analysis. Each extraction of the samples and those of recovery studies was repeated three times to check for reproducibility.

4.2.7 UHPLC-Q Orbitrap MS analysis for target and suspect screening

The UHPLC-Q Orbitrap MS chromatographic separation was achieved using a Hypersil Gold 100 mm x 2.1mm x 1.9 µm column (Anatech, Johannesburg, South Africa) at a column compartment temperature of 30 ˚C and autosampler at room temperature. The instrument method developed was guided by compound chemical characteristics and instrument capabilities (Zabaleta et al., 2020) with the final optimised conditions consisting of solvent A as water in 0.1% formic acid with 5 mM ammonium formate and solvent B as methanol in 0.1% formic acid with 5 mM ammonium formate. The gradient method was initiated at 30% solvent B at 0.3 mL min^{-1} (hold 1 min) before increasing to 100% B with a curve set at 2 until

15 min. The flowrate was increased to 0.35 mL min^{-1} for 4 min before returning to 30% solvent B and 0.3 mL min^{-1}. A 10 µL injection volume was used. Tandem mass spectrometry was coupled to a heated electron spray ionisation in positive mode with the sheath gas flow rate at 40 aU, aux gas flow rate at 10 aU, spray voltage at 3.20 kV, S-lens RF level at 55.0, 300 ˚C capillary temperature and 400 ºC aux gas heater temperature. The full scan acquisition range was 83.4 to 1250 m/z at a resolution 70 000 and data dependent acquisition (dd-MS^2) in discovery at a resolution of 35 000. Stepped collision energy was selected at 15 and 30 eV with the AGC target set at 2×10^4. The accurate mass analysis for the compounds were determined by equation (3).

$$Mass\ error\ (ppm) = \frac{Theoretical\ mass\ expected\ (m/z)}{Mass\ observed\ (m/z)} \times 10^6 \qquad \ldots (3)$$

For HRMS, a ΔMass < 5ppm indicates accurate mass detection. The accurate mass detection used for identification and quantitation of the target pharmaceuticals is shown in Table 2 below.

Table 2.
Pharmaceutical accurate mass detection

Target compound	Retention time (min)	Confirmatory ion	Monoisotopic Mass (amu)	$[M+H]^+$ Theoretical Mass expected	Observed Mass (amu)	Mass error (ppm)
Carbamazepine	5.21	194.1175	236.0950	237.1028	237.1021	2.87
Dexamethasone	5.55	237.1272	392.1999	393.2077	393.2074	0.80
Etilefrine	1.08	164.1069	181.1103	182.1181	182.1176	2.77
Methocarbamol	4.21	118.0500	241.0950	242.1028	242.1023	2.23
Nevirapine	4.56	198.0147	266.1168	267.1246	267.1241	1.82
Venlafaxine	4.74	121.0649	277.2042	278.2120	278.2116	1.50

For suspect screening, the instrument data acquisition and analysis were performed using Thermo Scientific Xcalibur and TraceFinder 5.0. TraceFinder 5.0 was used for suspect screening where an unknown analysis method was developed for a min peak width of 0.30, max peak width of 1.20, RT window set at 35 seconds with alignment and gap filling selected for exhaustive search. The mass tolerance for the screening was set at 15 ppm with the cannabidiol (CBD) RT window at 40.00 seconds and the fragment intensity threshold at 1000 on the MS2 scan. Tentative identification was performed using two Thermo Scientific High-Resolution Accurate-Mass MS/MS Spectral Libraries; namely the environmental and food safety library and the clinical research and forensic toxicology library. An overall match score of 80% with more than two fragment ion matches were taken as highly probable identification.

4.2.8 Quality assurance procedures

As part of quality assurance procedures, parameters such as the coefficient of determination (R^2) from external calibration curves was determined as the indicator of linearity. The methods limit of detection (LOD) and limit of quantification (LOQ), which were taken as the sensitivity indicators, were measured at signal-to-noise of 3 and 10, respectively. Further, replicate analysis of samples were done to make sure that results were reproducible.

4.3. Results and Discussion

4.3.1. Quality assurance results

Quality assurance parameters obtained during SPE method optimisation are shown in Table 3. Carbamazepine and dexamethasone yielded 76.19 ±1.34 to 80.52 ±2.02 % recoveries which are similar to those reported in literature (Bahlmann et al., 2014; Cortés-Lagunes et al., 2024; Ivankovic et al., 2023; Mostafa et al., 2023; Xu, 2019). Recoveries for venlafaxine and

nevirapine ranged from 72.24 ±1.24 to 80.24 ±0.65 %, which is in line with data reported in literature (Chen et al., 2023; Nannou et al., 2019; Ngumba et al., 2016; Ngwenya & Mahlambi, 2023; Petromelidou et al., 2024). SPE recoveries for etilefrine and methocarbamol in water could not be found in literature. The recovery of etilefrine ranged from 55.18 ±0.51 to 64.82 ±3.80 %, which is the lowest of all six compounds. The low etilefrine recovery is linked to its poor stability in solution. Overall, good recoveries (> 70%) were obtained for all compounds, except etilefrine in both types of water (Lee et al., 2023).

The influence of matrices on the extraction was calculated by comparing recoveries in deionised water to those obtained from spiking river water. Matrix effects (be it ion suppression or enhancement) were observed for all six compounds (Table 3). Ion suppression was observed for three of the six compounds. This was seen for carbamazepine, dexamethasone and nevirapine and the values ranged from 2.21 to 4.24%. Signal enhancement was observed for etilefrine, nevirapine and venlafaxine and the values ranged from –1.00 to –12.21. Ion suppression was seen to be greatest for carbamazepine (4.24%), whereas signal enhancement was greatest for methocarbamol (–12.63%). It is worth noting that matrix effects have high variability, which makes it difficult to control and predict (Zhao & Metcalfe, 2008). The matrix effects were then used to correct for the concentrations of target analytes in the sampling medium.

Method LODs and LOQs are listed in (Table 3). The method LODs ranged from 0.15 ng L^{-1} for venlafaxine to 2.03 ng L^{-1} for methocarbamol, whereas the method LOQs ranged from 0.49 ng L^{-1} for venlafaxine to 6.75 ng L^{-1} for methocarbamol.

Table 3.
UHPLC-Q Orbitrap MS calibration regression coefficients, LOD, LOQ and SPE % recoveries ± RSD

Compound	R^2	Method LOD ($ng\ L^{-1}$)	Method LOQ $ng\ L^{-1}$)	Water type	SPE recovery	
					% recovery $\pm RSD$	% matrix effect
Carbamazepine	0.9884	0.21	0.75	River	76.19 ±1.34	4.24
				Ultrapure	79.56 ±2.62	–
Dexamethasone	0.9988	1.43	4.75	River	77.58 ±1.01	2.70
				Ultrapure	80.52 ±2.04	–
Etilefrine	0.9921	1.83	6.10	River	55.73 ±0.96	– 1.00
				Ultrapure	55.18 ±0.51	–
Methocarbamol	0.9952	2.03	6.75	River	83.92 ±0.70	–12.63
				Ultrapure	74.91 ±2.08	–
Nevirapine	0.9704	0.38	1.28	River	78.46 ±2.30	2.21
				Ultrapure	80.24 ±0.65	–
Venlafaxine	0.9884	0.15	0.49	River	70.18 ±2.65	2.85
				Ultrapure	72.24 ±1.24	–

Fig. 2 below shows the uptake of the Chemcatcher over 16 days in laboratory calibration. Linear uptake was obtained for all the compounds except etilefrine, whose uptake was linear until day eight, thereafter, the accumulated analyte mass decreased. Linear calibration curves were obtained for venlafaxine (R^2 = 0.998) and carbamazepine (R^2 =0.995) when Petrie et al. (2016) calibrated a chemcatcher sampler for the uptake of polar organic micropollutants.

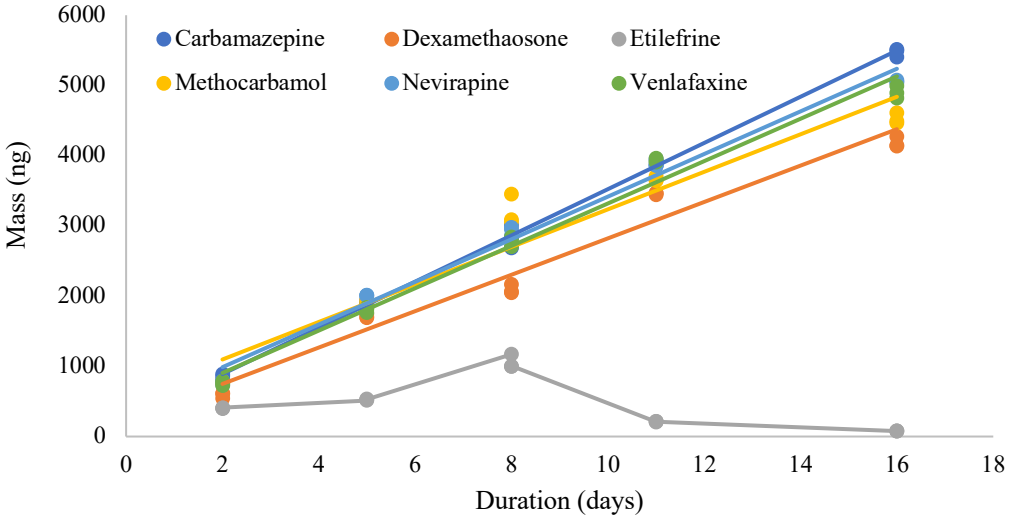


Fig. 2.
Chemcatcher laboratory calibration plot of the analyte mass accumulated in sampler over time

The calculated experimental sampling rates are listed in Table 4. These ranged from 0.360 L day^{-1} for dexamethasone to 0.447 L day^{-1} for carbamazepine. The sampling rates for carbamazepine and venlafaxine were found to be comparable to literature values (Bartelt-Hunt et al., 2009; Fedorova et al., 2014; Li et al., 2010; MacLeod et al., 2007). It is worth noting that literature sampling rates vary widely, and this is due to the varying experimental conditions that are employed in calibration experiments. The sampling rates for dexamethasone, methocarbamol and nevirapine and venlafaxine have not been reported in a Chemcatcher or POCIS samplers to our knowledge. One mention of sampling rates for methocarbamol, nevirapine and venlafaxine is by Khulu et al. (2022) where a membrane assisted solvent extraction-molecularly imprinted polymer based passive sampler was used for monitoring selected pharmaceuticals in surface waters. The sampling rates were determined to be 0.0011 ± 6.5, 0.0009 ± 7.9 and 0.0017 ± 5.6 L day^{-1} for etilefrine, methocarbamol and nevirapine, respectively. These low samplings rates can be attributed to the nature of the passive sampling

approach used as solid membrane used is known to have slow mass transfer of analytes across it.

Table 4.
Comparison of experimental and literature Chemcatcher sampling rates

Target compound	Correlation coefficients R^2	Experimental sampling rate (L day^{-1})	Literature mean sampling rate (L day^{-1})	References
Carbamazepine	0.983	0.447	0.288	(Bartelt-Hunt et al., 2009)
			0.348	(MacLeod et al., 2007)
			0.230, 0.397, 0.561	(Li et al., 2010)
Dexamethasone	0.990	0.360	_	–
Etilefrine	0.317	–	_	–
Methocarbamol	0.981	0.435	_	–
Nevirapine	0.985	0.439	_	–
Venlafaxine	0.992	0.431	0.23	(Fedorova et al., 2014)
			0.104, 0.167, 0.388,	(Li et al., 2010)
			0.521, 0.144	(Bayen et al., 2014)

4.3.2. Physicochemical parameters and bacteriological analysis

The values of the pH, electrical conductivity, total dissolved solids, total aerobic count, faecal coliform count and *E.coli* count are shown in Table 5. The pH values ranged from 7.120 –7.999. This ranged from neutral to basic pH. The pH values fell within the surface water standards according to the World Health Organisation (WHO) (6.5 – 8.5) and the South African Department of Water Affairs and Forestry (5 – 9.7) (Mike, 1996; Rickert et al., 2016). However, the basic pH supports input from wastewater as source of pollution. Electrical conductivity ranged from 183.7 – 376.0 μS cm^{-1}. This was considered normal for rivers and suggested that the river and dam did not contain unusually high dissolved salts. Total dissolved solids ranged

from 91.46 – 184.6 ppm, which fell within the recommended limits for surface water set by WHO and Department of Water Affairs and Forestry (Mike, 1996; Rickert et al., 2016).

The total aerobic count ranged from 0.9 x 10^5 to 3.2 x 10^7 CFU mL^{-1}. Downstream, site (S1), which was located along the crocodile river at the exit of Hartbeespoort dam showed the lowest total aerobic count. This observation suggested that the dam acts as a sink for bacterial matter that makes its way to the dam. The highest total aerobic count was recorded for S4 and S5 and elevated *E coli* levels were found to be the highest at S5 (2250 CFU mL^{-1}), which is followed by S3 (1450 CFU mL^{-1}). These values are 10- and 6-fold greater than the standard set by WHO (235 CFU mL^{-1}) for *E coli* levels in surface water (Rickert et al., 2016). This was in line with the locations of these sampling site. Site S5 was located upstream along the Hennops river, and shortly after the Olifantsfontein WWTP. Site S4 was located in Centurion, shortly before the Sunderland ridge WWTP. Potential sources of the high bacterial population detected at these sites are their close proximity to WWTP, sewage leaks and improper waste management happening upstream. Site S3 showed the highest coliform count, which indicates the presence of sewage contamination. S3 was located along the Crocodile River, after Hennops and Jukskei Rivers. The aggregation of sewage contamination from Hennops and Jukskei rivers as they join the Crocodile River may be the cause for the exacerbated coliform count at this sampling site. The bacteriological analysis values far exceeded the standard set by WHO and the Department of Water Affairs and Forestry, which implied that Hennops River and Hartbeespoort Dam are heavily polluted with faecal matter (Mike, 1996; Rickert et al., 2016).

Table 5.
Physicochemical parameters of water from sampling sites

Sample ID	pH	Conductivity ($\mu S.cm^{-1}$)	TDS (ppm)	Total aerobic count ($CFU\ mL^{-1}$)	Coliform count ($CFU\ mL^{-1}$)	*E.coli* count ($CFU\ mL^{-1}$)
S1	7.715	376.0	184.6	90000	2650	4
S2	7.872	275.8	187.8	1700000	495	30
S3	7.120	183.7	91.46	3150000	195000	1450
S4	7.999	190.9	95.40	400000000	180000	1150
S5	7.472	306.6	153.4	320000000	170000	2250
Mean	7.636	266.6	142.5	144988000	109629	976.8
Minimum	7.120	183.7	91.46	90000	495	4
Maximum	7.999	376.0	184.6	400000000	195000	2250
RSA drinking water standard	5 – 9.7	≤170	≤1200 (ppm)	ND	≤10	ND
RSA surface water standard	4 – 11	_	_	_	_	_
WHO drinking water standard	6.5 – 8.5	≤ 400	≤300 (ppm)	ND	<1	ND
WHO surface water standard:	6.5 – 9.5	_	≤600 (ppm)	_	_	235

ND – Not Detected, CFU – Colony Forming Units, ppt – parts per trillion

4.3.3. Targeted analysis of the samples using UHPLC-Q Orbitrap MS

The estimated TWA concentrations of the target compounds recovered from the Chemcatcher sampler are listed in Table 6. These were calculated using experimental sampling rates listed in Table 4. For etilefrine, a sampling rate of 0.115 L day^{-1} was used to estimate the TWA concentration. The concentrations in SPE samples from each sampling site are also listed in Table 6. With SPE, only five (carbamazepine, etilefrine, methocarbamol, nevirapine and

dexamethasone) of the six target compounds were detected. Carbamazepine was detected at S1, S2 and S3, whereas methocarbamol, etilefrine, nevirapine and dexamethasone were detected at all five sites. Venlafaxine, however, was not detected at any of the five sampling sites. Five of the six compounds were detected at all sampling sites in Chemcatcher samples. The exception was venlafaxine, which was detected at all sites but S2. This observation highlighted one of the advantages of passive sampling over grab sampling. Which is that passive sampling allows for the detection of compounds present in trace concentrations, that would otherwise go undetected with grab sampling.

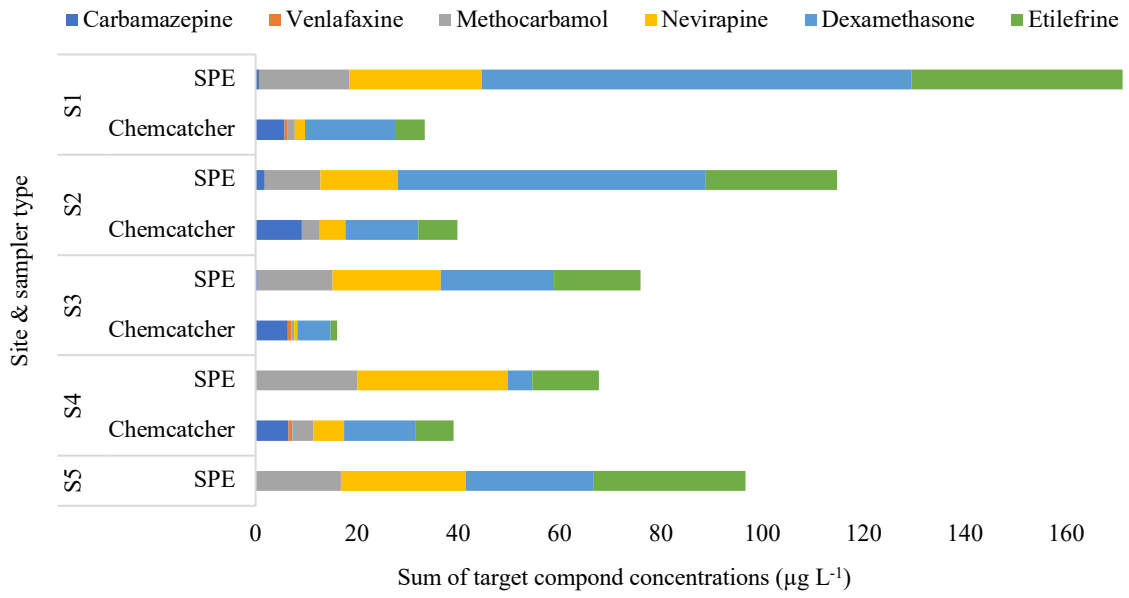


Fig. 3.
Comparison of pharmaceutical TWA concentration accumulated in Chemcatcher and SPE samples

Comparing Chemcatcher estimated TWA concentrations to SPE concentrations, it was evident that SPE was superior to passive sampling in determining the total concentration in the water (Fig. 3). This is despite passive sampling showing superiority to SPE when it comes to detection frequency. It is worth noting that TWA concentrations are estimates computed using sampling rates obtained under laboratory experimental conditions, which may not be an

accurate representation of environmental conditions. Further, passive samplers measure mostly accumulate the bioavailable fraction.

The highest concentration was observed for S1 site with SPE with a total concentration of 171 µg L^{-1}. This was followed by S2, S5, S3 then S4 with SPE total concentrations of 114.82, 96.66, 75.96 and 67.81 µg L^{-1}, respectively. Chemcatcher TWA concentrations for all sites except S4 were 4 times less than those from SPE. The order of Chemcatcher total concentration obtained was S2 > S4 > S1 > S3. In Chemcatcher samples, estimated TWA concentrations of dexamethasone were higher than those of all the other compounds at all sites, ranging from 4.88 ±1.31 to 84.71 ±30.57 µg L^{-1}. These are lower than those reported in Umgeni River (up to 2.11 ng L^{-1}) (Matongo et al., 2015).

The second highest TWA concentrations were those of carbamazepine at all sites except for S4. These ranged from 5.78 ±0.67 to 9.07 ±0.38 µg L^{-1}. These are similar to those reported by Matongo et al. (2015), where carbamazepine was detected in ranges of 0.77 ± 0.62 to 4.56 ± 0.81 µg L^{-1} in water from Umgeni River, KwaZulu-Natal, South Africa. In the case of S4, the TWA concentration of etilefrine (7.53 ±2.08 µg L^{-1}) exceeded that of carbamazepine (6.44 ±0.05 µg L^{-1}). The estimated concentrations of venlafaxine (ND to 0.83 ±0.13 µg L^{-1}) were the lowest at all sampling sites except for S3, where the estimated concentration of methocarbamol was the lowest (0.50 ±0.04 µg L^{-1}). Although the concentrations of venlafaxine were low, they are higher than those reported by Archer et al. (2017) (up to 35.4 ng L^{-1}).

Table 6.
Estimated TWA concentrations of the six target compounds recovered from Chemcatcher sampler and total concentrations from SPE

Site	Carbamazepine		Etilefrine		Venlafaxine		Methocarbamol		Nevirapine		Dexamethasone	
	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE
S1	5.78 ±0.67	0.63 ±0.26	5.74 ±0.67	41.67 ±0.87	0.50 ±0.27	<MLOD	1.48 ±0.59	17.84 ±2.29	2.03 ±0.87	26.20 ±3.59	17.87 ±3.70	84.71 ±30.57
S2	9.07 ±0.38	1.81 ±0.75	7.71 ±2.47	25.94 ±3.24	<MLOD	<MLOD	3.54 ±0.41	10.95 ±0.44	5.09 ±0.60	15.39 ±0.70	14.42 ±1.58	60.73 ±0.81
S3	6.32 ±0.19	0.45 ±0.27	1.32 ±1.53	17.02 ±2.20	0.83 ±0.13	<MLOD	0.50 ±0.04	14.74 ±1.11	0.58 ±0.07	21.33 ±1.74	6.56 ±0.50	22.42 ±3.05
S4	6.44 ±0.05	<MLOD	7.53 ±2.08	13.12 ±2.54	0.82 ±0.08	<MLOD	4.17 ±0.14	20.09 ±0.27	6.03 ±0.21	29.72 ±0.42	14.04 ±1.55	4.88 ±1.31
S5	–	<MLOD	–	30.00 ±1.15	–	<MLOD	–	16.87 ±0.78	–	24.67 ±1.22	–	25.12 ±6.23
Min	5.78 ±0.67	<MLOD	0.93 ±1.03	13.12 ±2.54	<MLOD	<MLOD	0.50 ±0.04	10.95 ±0.44	0.58 ±0.07	15.39 ±0.70	6.56 ±0.50	4.88 ±1.31
Max	9.07 ±0.38	11.81 ±0.75	5.19 ±1.66	41.67 ±0.87	0.83 ±0.13	<MLOD	4.17 ±0.14	`20.09 ±0.27	6.03 ±0.21	29.72 ±0.42	17.87 ±3.70	84.71 ±30.57
Mean	6.70 ±0.32	0.58 ±0.26	5.58 ±1.69	25.55 ±2.17	0.35 ±0.12	–	2.42 ±0.30	16.10 ±0.98	3.43 ±0.45	30.00 ±1.53	13.22 ±1.83	39.57 ±8.39

Concentration in (μg L^{-1}) ±RSD; Chem – Chemcatcher sampler, MLOD – Method limit of detection

4.3.4 Suspect screening of Chemcatcher and SPE water samples

In suspect screening, it is important to evaluate the qualitative protocol used in terms of a confidence level linked to probability of positive identification (Asghar et al., 2018; El-Deen and Shimizu, 2022; Manz et al., 2023). El-Deen and Shimizu. (2022) employed such a scheme where absolute identification was set at Level 1, linked to a reference standard. Levels 2 to 4 related to probable and tentative identification based on features, library matches and molecular formula generation (El-Deen and Shimizu. 2022). In this study, suspect screening aimed to achieve a Level 2 confidence level following the suspect screening protocol described in methodology was used.

4.3.4.1 Comparison of pesticides detected in Chemcatcher and SPE samples

The pesticides detected were categorized as fungicides, herbicides, insecticides and broad-spectrum pesticides (Table S1, Fig. 4). These made up 33, 33, 29 and 4% of the number of pesticides detected, respectively. Overall, a sum of 25 pesticides were detected in both Chemcatcher and SPE samples. Detection frequency of fungicides was highest in Chemcatcher samples for S1 and S2, which were located at the exit and west channel of the Dam, respectively (Fig. 5). In both samples, seven out of the nine fungicides were detected. This is in line with the heavy agricultural activities that take place in the area. A total of eight herbicides were detected at the sampling sites of interest. Detection frequency of this class of pesticides was observed to be low compared to fungicides. Sebuthylazine, an obsolete herbicide was detected at S1, S2, S3 and S4 in the Chemcatcher samples.

Eight of the 25 pesticides were identified as belonging to insecticides. Three of the most commonly detected insecticides are bufencarb, terbucarb and methacrifos. Methacrifos was detected in all four Chemcatcher samples. Terbucarb was detected in all five SPE sample and

bufencarb was detected in all four Chemcatcher samples and SPE samples from S2, S3 and S4. DEET and Sebuthylazine are two of the pesticides that were detected by Rimayi et al. (2019) in Hennops river when the group used the Chemcatcher passive sampler and time-of-flight mass spectrometry to screen for emerging pollutants in rivers in Gauteng Province of South Africa. Generally, greater detection frequency was observed in chemcatcher samples compared to SPE samples (Fig. 4).

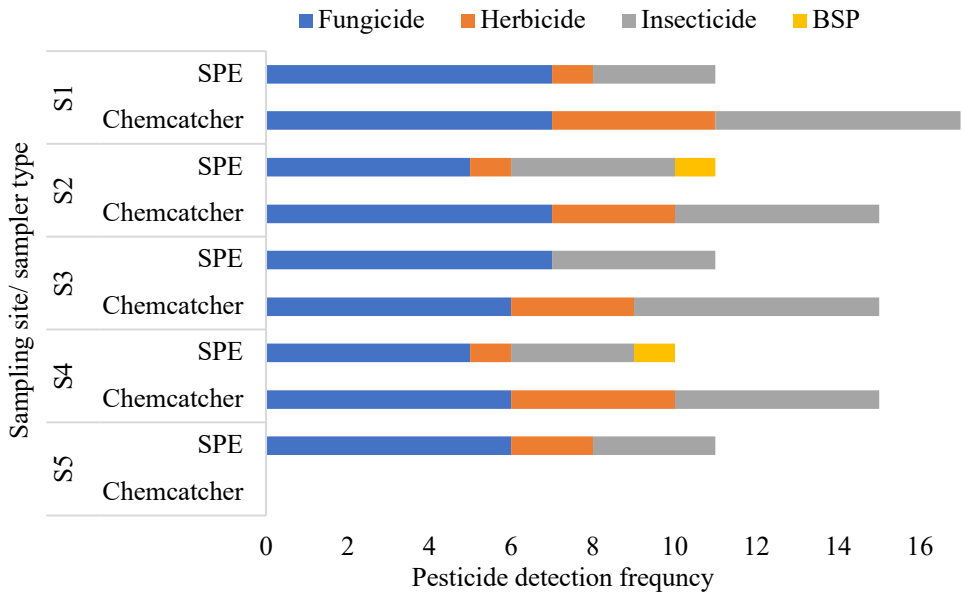


Fig. 4.
Comparison of pesticide detection frequency from chemcatcher and SPE samples

Fig. 5 shows a comparison of peak areas of pesticides detected in Chemcatcher and SPE samples. Despite differences in detection frequency being statistically insignificant from site to site, the differences in peak areas were notable (Fig. 5). These ranged from a sum of 394.31×10^6 for S2 to 3220.72×10^6 for S5. It is worth noting that quantification cannot be done with suspect screening, however, peak areas of the suspect analytes can be used to compare their abundance at each sampling site. The sum of peak areas for chemcatcher samples ranged from 1734.04×10^6 for S1 to 2865.01×10^6 for S4. The order of pesticide peak areas

for SPE samples was found to be S5> S4> S3> S1> S2 whereas those for Chemcatcher samples were found to be S3> S2> S4> S1. As with detection frequency, peak areas were generally higher in Chemcatcher samples compared to SPE samples. This observation can be explained by the fact the passive sampling allows for the accumulation of pollutants over time, whereas SPE only reveals information regarding samples that were collected at a particular point and time. The SPE sample from S5 was an exception, in that the sum of pesticide peak areas were higher than those from all other sampling sites. With no chemcatcher sample to compare this to, it cannot be concluded that SPE was superior to chemcatcher for this site. In fact, this site is located shortly after the Olifantsfontein WWTP, in an area surrounded by farms. It is possible that the sum of pesticide peak areas for chemcatcher could've been higher than those of SPE for this site due to heavy pesticide output from the surrounding farms.

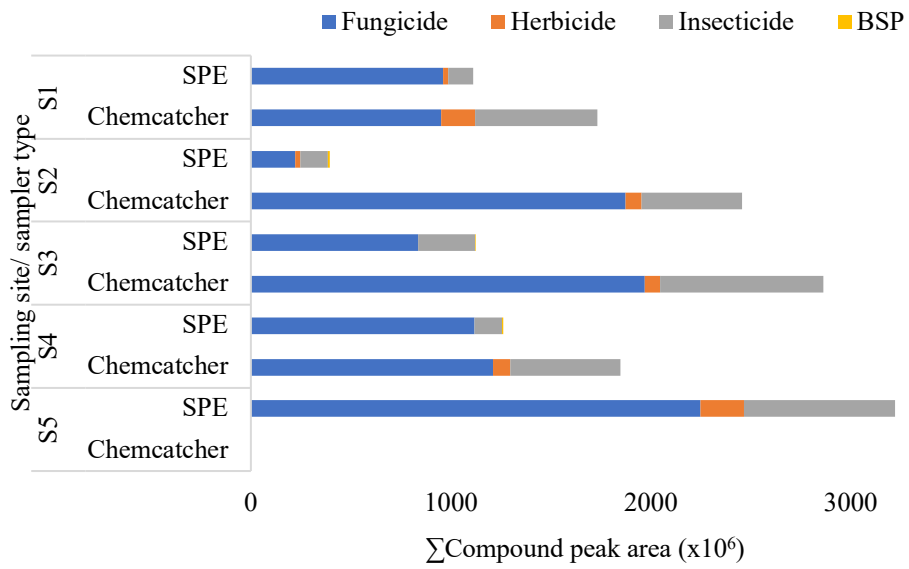


Fig. 5.
A comparison of pesticides compound peak areas in chemcatcher and SPE samples

4.3.4.2 Comparison of pharmaceuticals detected in Chemcatcher and SPE samples

The pharmaceutical compounds detected were sub-categorized based on their general uses (Table S2). This classification scheme is similar to that used by Rimayi et al. (2019). In

instances where the general use was unclear, their mode of action was used to classify them. With Chemcatcher, S4 had the highest detection frequency with 45 compounds (Fig. 6). This was followed by S1 with 44 compounds, then S3 with 43 followed and lastly S2 with 42 compounds detected. Statistically, the differences in detection frequency were insignificant.

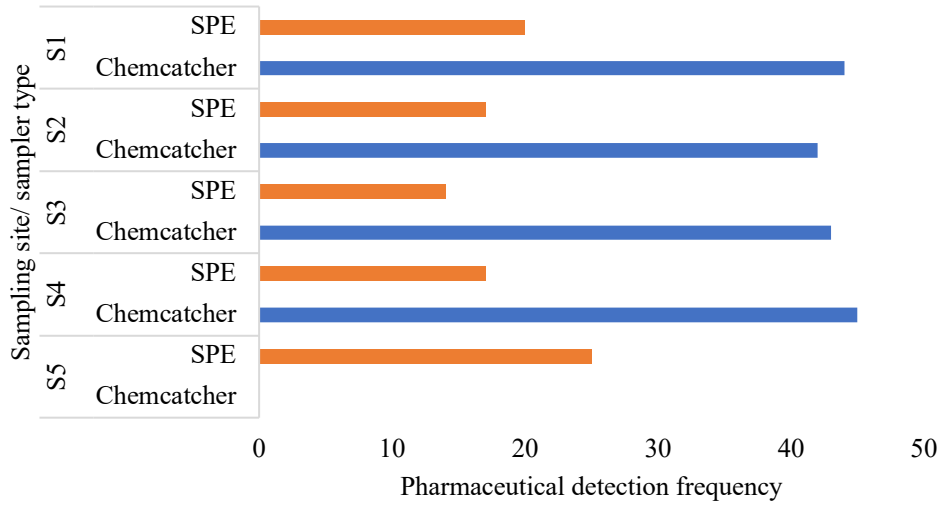


Fig. 6.
A comparison of pharmaceutical detection frequency in chemcatcher and SPE samples

With SPE, S5 had the highest detection frequency with a total of 25 compounds. This relatively high detection frequency can be explained by the site's close proximity to the Oliphantsfontein WWTP. S1 had the second highest detection frequency with a total of 20 compounds. This was followed by S2 and S4 with 17 compounds detected at each site. S3 had the least number of compounds detected. This observation was not anticipated as S3 was expected to show the highest compound detection frequency. This is because S3 is located shortly after Jukskei and Hennops river merge with the Crocodile River before flowing into Hartbeespoort Dam. It was thus expected that compounds present in Jukskei and Hennops river would be detected at this site. However, due to Jukskei river being less polluted than Hennops

river, it was possible that pollutants flowing from Hennops river are diluted by the flow from Jukskei river.

Overall, the highest number of pharmaceuticals were detected at S4. The greater pharmaceutical detection frequency with Chemcatcher is shown in (Fig. 6) and was attributed to the ability of the sampler to accumulate pollutants present in trace concentrations, which would otherwise go undetected with SPE.

Peak areas of the suspect pharmaceutical analytes were summed to compare their relative abundances at each sampling site (Fig. 7). S1 showed the highest abundance for both chemcatcher and SPE samples with sums of 436.52×10^7 and 212.43×10^7, respectively. This site is located along the Crocodile River at the exit of the Dam, where contaminants that accumulate in the dam exit the dam. The S3 chemcatcher sample gave the second highest peak areas (283.51×10^7). This was followed by S4 (217.10×10^7) then S2 (131.83×10^7). As with pesticide peak areas, pharmaceutical peak areas were greater for chemcatcher samples compared to samples.

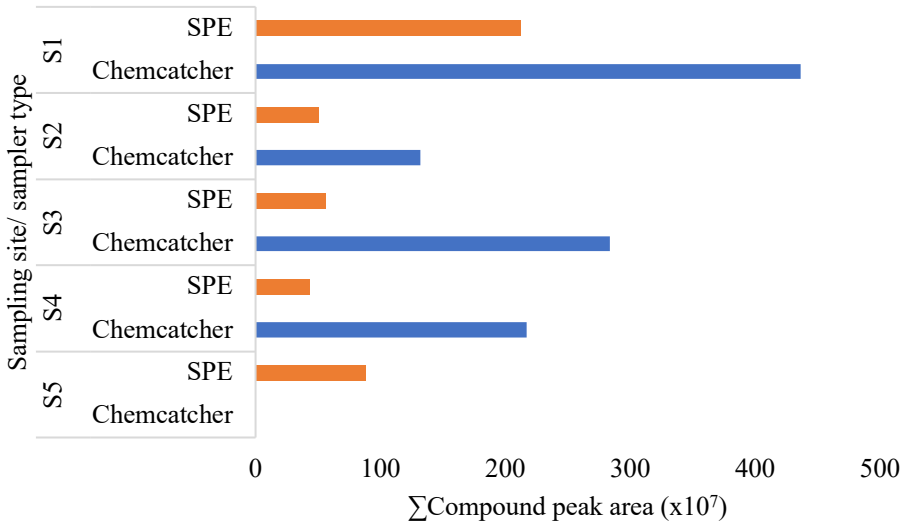


Fig. 7.
A comparison of pharmaceutical compound peak areas in chemcatcher and SPE samples

4.3.4.3 Peak area-based prioritisation from passive sampler screening results

A comparison of peak areas of the pesticides and pharmaceutical compounds detected was used to estimate their abundance (Fig. S1 & S2). The order of five most abundant pesticides in Chemcatcher samples is dodemorph> fenpropimorph> carboxin> methacrifos> diethyl toluamide. In SPE samples, the order is dodemorph> fenpropimorph> diofenolan> isoprocarb> sedaxane. It is worth noting that although the peak areas of these compounds are generally higher than those of the other compounds detected, it is not the case at every sampling site. An example of such a case is dodemorph in Chemcatcher samples. The peak areas of dodemorph at sites S2 (774×10^6) and S3 (749×10^6) were the most intense of all pesticides, however at sites S1 (358×10^5) and S4 (241×10^5), the peaks for dodemorph were some of the least intense of all pesticides detected. At sites S1 and S4, the peak intensities of fenpropimorph were the highest of all pesticides detected. In SPE samples, the intensity of fenpropimorph was highest at S1 and S3, isoprocarb was highest at S2 and dodemorph was highest at S4 and S5. 17α estadiol, estradione and the antibiotic netilmicin were abundant of all pharmaceutical compounds in chemcatcher samples, with peak areas ranging from (725×10^5) to (466×10^6) Chemcatcher samples from all sampling sites.

The peak intensity of netilmicin was the highest in S2 and S4. 3,4-DMMC, which is a designer drug showed the highest peak intensity at site S3. This drug was also detected at S4, which is located upstream in centurion as well as S1 which is located at the exit of the dam but not detected in S2. In the SPE samples, the antibiotic sulphamethaxole had the highest peak intensities at sites S1 and S2. The intensity of trihexyphenidyl, which is an anti-spasmodic drug was highest at site S3. Aspirin had the highest peak intensity at S4, whereas caffeine had the highest peak intensity at site S5. Although the peak intensities may be used to compare the relative abundance of the compounds detected per sampling site, they do not reveal the concentrations these compounds are present in.

4.4. Conclusions

This study presents a comprehensive evaluation of emerging contaminants. By employing both Chemcatcher passive sampling and SPE followed by UHPLC-Q Orbitrap MS for targeted and suspect screening, we demonstrated the extensive presence of organic pollutants in this urban river system. Chemcatcher passive samplers proved effective in detecting a broader range of trace contaminants, with higher detection frequencies than SPE, although SPE yielded higher concentrations for some compounds.

The findings highlight the significant pollution burden from agricultural runoff, WWTP effluents, and urban waste, which contribute to the contamination of these water bodies. The study identified 86 pollutants, including 25 pesticides and 61 pharmaceutical compounds, revealing a substantial overlap with known environmental contaminants, while also detecting several previously unreported substances. These results highlight the urgent need for more rigorous monitoring and management strategies in urban water systems, particularly in developing regions, to mitigate the ecological and public health risks posed by these emerging contaminants.

This study not only advances the understanding of pollutant distribution in South African water bodies but also emphasizes the importance of using both passive and active sampling techniques for a more accurate assessment of environmental pollution.

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REFERENCES

Archer, E. *et al.* (2017). The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. *Chemosphere*, *174*, 437–446. doi.org/10.1016/j.chemosphere.2017.01.101

Asghar, M. A. *et al.* (2018). Suspect screening and target quantification of human pharmaceutical residues in the surface water of Wuhan, China, using UHPLC-Q-Orbitrap HRMS. *Sci. Total Environ.*, *635*, 828–837. doi.org/10.1016/j.scitotenv.2018.04.179

Bahlmann, A. *et al.* (2014). Carbamazepine and its metabolites in wastewater: Analytical pitfalls and occurrence in Germany and Portugal. *Water Res.*, *57*, 104–114. doi.org/10.1016/j.watres.2014.03.022

Bartelt-Hunt, S. L. *et al.* (2009). The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. *Environ. Pollut.*, *157*(3), 786–791. doi.org/10.1016/j.envpol.2008.11.025

Bayen, S. *et al.* (2014). Application of Polar Organic Chemical Integrative Sampler (POCIS) to monitor emerging contaminants in tropical waters. *Sci. Total Environ.*, *482–483*, 15–22. doi.org/10.1016/j.scitotenv.2014.02.082

Bunally, S. B. *et al.* (2019). Using Physicochemical Measurements to Influence Better Compound Design. *SLAS Discov.*, *24*(8), 791–801. doi.org/10.1177/2472555219859845

Cao, H. *et al.* (2024). Evaluation of the DGT passive samplers for integrating fluctuating concentrations of pharmaceuticals in surface water. *Sci. Total Environ.*, *926*, 172067. doi.org/10.1016/j.scitotenv.2024.172067

Castle, G. D. *et al.* (2018). Calibration and field evaluation of the Chemcatcher® passive sampler for monitoring metaldehyde in surface water. *Talanta*, *179*, 57–63. doi.org/10.1016/j.talanta.2017.10.053

Chen, L. *et al.* (2023). Advances in the on-line solid-phase extraction-liquid chromatography-mass spectrometry analysis of emerging organic contaminants. *TrAC, Trends Anal. Chem.*, *160*, 116976. doi.org/10.1016/j.trac.2023.116976

Cortés-Lagunes, R. S. *et al.* (2024). Optimizing solid phase extraction and HPLC-MS/MS parameters for reliable quantification of COVID-19 pharmaceuticals in Mexico City's wastewater: A design of experiments approach. *Microchem. J.*, *200*, 110493. doi.org/10.1016/j.microc.2024.110493

Cristóvão, M. B. *et al.* (2021). Detection of anticancer drugs in wastewater effluents: Grab versus passive sampling. *Sci. Total Environ.*, *786*, 147477. doi.org/10.1016/j.scitotenv.2021.147477

Desai, M., Njoku, A., & Nimo-Sefah, L. (2022). Comparing Environmental Policies to Reduce Pharmaceutical Pollution and Address Disparities. *Int. J. Environ. Res. Public Health*, *19*(14), 8292. doi.org/10.3390/ijerph19148292

El-Deen, A. K., & Shimizu, K. (2022). Suspect and non-target screening workflow for studying the occurrence, fate, and environmental risk of contaminants in wastewater using data-independent acquisition. *J. Chromatogr. A*, *1667*, 462905. doi.org/10.1016/j.chroma.2022.462905

Enick, O. V., & Moore, M. M. (2007). Assessing the assessments: Pharmaceuticals in the environment. *Environ. Impact Assess. Rev.*, *27*(8), 707–729. doi.org/10.1016/j.eiar.2007.01.001

Fedorova, G. *et al.* (2014). A passive sampling method for detecting analgesics, psycholeptics, antidepressants and illicit drugs in aquatic environments in the Czech Republic. *Sci. Total Environ.*, *487*, 681–687. doi.org/10.1016/j.scitotenv.2013.12.091

Feijão, E. *et al.* (2020). Fluoxetine Arrests Growth of the Model Diatom Phaeodactylum tricornutum by Increasing Oxidative Stress and Altering Energetic and Lipid Metabolism. *Front. microbiol.*, *11*. doi.org/10.3389/fmicb.2020.01803

Fialová, P. *et al.* (2024). Performance comparison of three passive samplers for monitoring of polar organic contaminants in treated municipal wastewater. *Sci. Total Environ.*, *908*, 168153. doi.org/10.1016/j.scitotenv.2023.168153

Freitas, L. de A. A., & Radis-Baptista, G. (2021). Pharmaceutical Pollution and Disposal of Expired, Unused, and Unwanted Medicines in the Brazilian Context. *J. Xenobiot.*, *11*(2), 61–76. doi.org/10.3390/jox11020005

Gani, K. M. *et al.* (2021). Emerging contaminants in South African water environment- a critical review of their occurrence, sources and ecotoxicological risks. *Chemosphere*, *269*, 128737. doi.org/10.1016/j.chemosphere.2020.128737

Ivankovic, K. *et al.* (2023). Multiclass determination of drug residues in water and fish for bioaccumulation potential assessment. *Talanta*, *264*, 124762. doi.org/10.1016/j.talanta.2023.124762

Jiang, A. Z., Nian, F., Chen, H., & McBean, E. A. (2022). Passive Samplers, an Important Tool for Continuous Monitoring of the COVID-19 Pandemic. Environmental Science and Pollution Research, 29(22), 32326–32334. https://doi.org/10.1007/s11356-022-19073-6

Kayode-Afolayan, S. D. *et al.* (2022). Impacts of pharmaceutical effluents on aquatic ecosystems. *Sci. Afr.*, *17*, e01288. doi.org/10.1016/j.sciaf.2022.e01288

Khan, A. H. A., & Barros, R. (2023). Pharmaceuticals in Water: Risks to Aquatic Life and Remediation Strategies. *Hydrobiol. J.*, *2*(2), 395–409. doi.org/10.3390/hydrobiology2020026

Khasawneh, O. F. S., & Palaniandy, P. (2021). Occurrence and removal of pharmaceuticals in wastewater treatment plants. *Process Saf. Environ. Prot.*, *150*, 532–556. doi.org/10.1016/j.psep.2021.04.045

Khulu, S. *et al.* (2022). Development and application of a membrane assisted solvent extraction-molecularly imprinted polymer based passive sampler for monitoring of selected

pharmaceuticals in surface water. *Water Res.*, *225*, 119145. doi.org/10.1016/j.watres.2022.119145

Lee, H., Cho, Y., Jung, G., Kim, H., & Jeong, W. (2023). Comparison of recovery efficiency and matrix effect reduction in pesticide residue analysis: QuEChERS with d-SPE, SPE, and FaPEx in apples and Korean cabbage. Analytical Methods, 15(30), 3709–3716. https://doi.org/10.1039/D3AY00612C

Li, H., Helm, P. A., & Metcalfe, C. D. (2010). Sampling in the Great Lakes for pharmaceuticals, personal care products, and endocrine-disrupting substances using the passive polar organic chemical integrative sampler. *Environ. Toxicol. Chem.*, *29*(4), 751–762. doi.org/10.1002/etc.104

MacLeod, S. L. *et al.* (2007). Laboratory calibration and field deployment of the Polar organic chemical integrative sampler for pharmaceuticals and personal care products in wastewater and surface water. *Environ. Toxicol. Chem.*, *26*(12), 2517–2529. doi.org/10.1897/07-238.1

Manz, K. E. *et al.* (2023). Non-targeted analysis (NTA) and suspect screening analysis (SSA): a review of examining the chemical exposome. *J Expo Sci Environ Epidemiol.*, *33*(4), 524–536. doi.org/10.1038/s41370-023-00574-6

Matongo, S. *et al.* (2015). Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. *Environ. Sci. Pollut. Res.*, *22*(13), 10298–10308. doi.org/10.1007/s11356-015-4217-0

Mike, S. (1996). Water quality guidelines aquatic ecosystems, *Department of Water Affairs and Forestry*.

Mosharaf, M. K. *et al.* (2024). Wastewater reuse and pharmaceutical pollution in agriculture: Uptake, transport, accumulation and metabolism of pharmaceutical pollutants within plants. *Chemosphere*, *364*, 143055. doi.org/10.1016/j.chemosphere.2024.143055

Mostafa, A. *et al.* (2023). Multi-class determination of pharmaceuticals as emerging contaminants in wastewater from Eastern Province, Saudi Arabia using eco-friendly SPE-UHPLC-MS/MS: Occurrence, removal and environmental risk assessment. *Microchem. J.*, *187*, 108453. doi.org/10.1016/j.microc.2023.108453

Nannou, C. *et al.* (2019). Analytical strategies for the determination of antiviral drugs in the aquatic environment. *Trends in Envirn. Anal. Chem.*, *24*, e00071. doi.org/10.1016/j.teac.2019.e00071

Narayanan, M. *et al.* (2022). Current status of microbes involved in the degradation of pharmaceutical and personal care products (PPCPs) pollutants in the aquatic ecosystem. *Environ.l Pollut.*, *300*, 118922. doi.org/10.1016/j.envpol.2022.118922

Ngumba, E. *et al.*. (2016). A multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using SPE-LC-MS/MS and matrix-matched standards. *Anal. Methods*, *8*(37), 6720–6729. doi.org/10.1039/C6AY01695B

Ngwenya, N., & Mahlambi, P. (2023). Methods optimization and application: Solid phase extraction, ultrasonic extraction and Soxhlet extraction for the determination of antiretroviral drugs in river water, wastewater, sludge, soil and sediment. *J. Pharm. Biomed. Anal.*, *230*, 115358. doi.org/10.1016/j.jpba.2023.115358

Odendaal, C. *et al.* (2015). An LC-MS/MS based survey of contaminants of emerging concern in drinking water in South Africa. *S. Afr. J. Sci.*, *111*(9/10), 6. doi.org/10.17159/sajs.2015/20140401

Ojemaye, C. Y., & Petrik, L. (2022). Pharmaceuticals and Personal Care Products in the Marine Environment Around False Bay, Cape Town, South Africa: Occurrence and Risk-Assessment Study. *Environ. Toxicol. Chem.*, *41*(3), 614–634. doi.org/10.1002/etc.5053

Petrie, B. *et al.* (2016). In Situ Calibration of a New Chemcatcher Configuration for the Determination of Polar Organic Micropollutants in Wastewater Effluent. *Environ. Sci. Tech.*, *50*(17), 9469–9478. doi.org/10.1021/acs.est.6b02216

Petromelidou, S. *et al.* (2024). Unravelling psychoactive substances and their metabolites and transformation products: High-Resolution Mass Spectrometry approaches for comprehensive target and suspect screening in wastewater. *Sci. Total Environ.*, *931*, 172867. doi.org/10.1016/j.scitotenv.2024.172867

Porretti, M. *et al.* (2022). Impact of pharmaceutical products on zebrafish: An effective tool to assess aquatic pollution. *Comp. Biochem. Physiol. C, Comp. Pharmacol. Toxicol.*, *261*, 109439. doi.org/10.1016/j.cbpc.2022.109439

Rickert, B. *et al.* (2016). Protecting surface water for health identifying, assessing and managing drinking-water quality risks in surface-water catchments. http://www.who.int

Rimayi, C. *et al.* (2019). Use of the Chemcatcher® passive sampler and time-of-flight mass spectrometry to screen for emerging pollutants in rivers in Gauteng Province of South Africa. *Environ. Monit. Assess.*, *191*(6), 388. doi.org/10.1007/s10661-019-7515-z

Robinson, R. F. A. *et al.* (2024). Quantification and risk assessment of polar organic contaminants in two chalk streams in Hampshire, UK using the Chemcatcher passive sampler. *Sci. Total Environ.*, *939*, 173316. doi.org/10.1016/j.scitotenv.2024.173316

Rogowska, J., & Zimmermann, A. (2022). Household Pharmaceutical Waste Disposal as a Global Problem—A Review. *Int. J. Environ. Res. Public Health*, *19*(23), 15798. doi.org/10.3390/ijerph192315798

Römerscheid, M. *et al.* (2023). Calibration of the Chemcatcher® passive sampler and derivation of generic sampling rates for a broad application in monitoring of surface waters. *Sci. Total Environ.*, *871*, 161936. doi.org/10.1016/j.scitotenv.2023.161936

Schoeman, C. *et al.* (2017). The impact of a Wastewater Treatment Works in Southern Gauteng, South Africa on efavirenz and nevirapine discharges into the aquatic environment. *Emerg. Contam.*, *3*(2), 95–106. doi.org/10.1016/j.emcon.2017.09.001

Świacka, K. *et al.* (2022). Presence of pharmaceuticals and their metabolites in wild-living aquatic organisms – Current state of knowledge. *J. Hazard. Mater.*, *424*, 127350. doi.org/10.1016/j.jhazmat.2021.127350

Tolić Čop, K. *et al.* (2024). Comparison and application of the extraction methods for the determination of pharmaceuticals in water and sediment samples. *Microchem. J.*, *205*, 111283. doi.org/10.1016/j.microc.2024.111283

Vaudreuil, M.A. *et al.* (2022). Pharmaceutical pollution of hospital effluents and municipal wastewaters of Eastern Canada. *Sci. Total Environ.*, *846*, 157353. doi.org/10.1016/j.scitotenv.2022.157353

Waleng, N. J., & Nomngongo, P. N. (2022). Occurrence of pharmaceuticals in the environmental waters: African and Asian perspectives. *J. Environ. Chem. Ecotoxicol.*, *4*, 50–66. doi.org/10.1016/j.enceco.2021.11.002

Wang, X. *et al.* (2020). Suspect and non-target screening of pesticides and pharmaceuticals transformation products in wastewater using QTOF-MS. *Environ. Int.*, *137*, 105599. doi.org/10.1016/j.envint.2020.105599

Wilkinson, J. L. *et al.* (2022). Pharmaceutical pollution of the world's rivers. *Proc. Natl. Acad. Sci. U. S. A*, *119*(8). doi.org/10.1073/pnas.2113947119

Wood, T. P. *et al.* (2015). The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. *Environ. Pollut.*, *199*, 235–243. doi.org/10.1016/j.envpol.2015.01.030

Xu, Q. (2019). Incorporating solid-phase extraction into compendial procedures for the determination of dexamethasone and impurities in low-dose drug products. *J. Pharm. Biomed. Anal.*, *175*, 112773. doi.org/10.1016/j.jpba.2019.07.021

Yu, X. *et al.* (2024). Application of passive sampling device for exploring the occurrence, distribution, and risk of pharmaceuticals and pesticides in surface water. *Sci. Total Environ.*, *908*, 168393. doi.org/10.1016/j.scitotenv.2023.168393

Zabaleta, I. *et al.* (2020). Occurrence of per- and polyfluorinated compounds in paper and board packaging materials and migration to food simulants and foodstuffs. *Food Chem.*, *321*, 126746. doi.org/10.1016/j.foodchem.2020.126746

Zhao, X., & Metcalfe, C. D. (2008). Characterizing and Compensating for Matrix Effects Using Atmospheric Pressure Chemical Ionization Liquid Chromatography−Tandem Mass Spectrometry: Analysis of Neutral Pharmaceuticals in Municipal Wastewater. *Anal. Chem.*, *80*(6), 2010–2017. doi.org/10.1021/ac701633m

Appendix 1

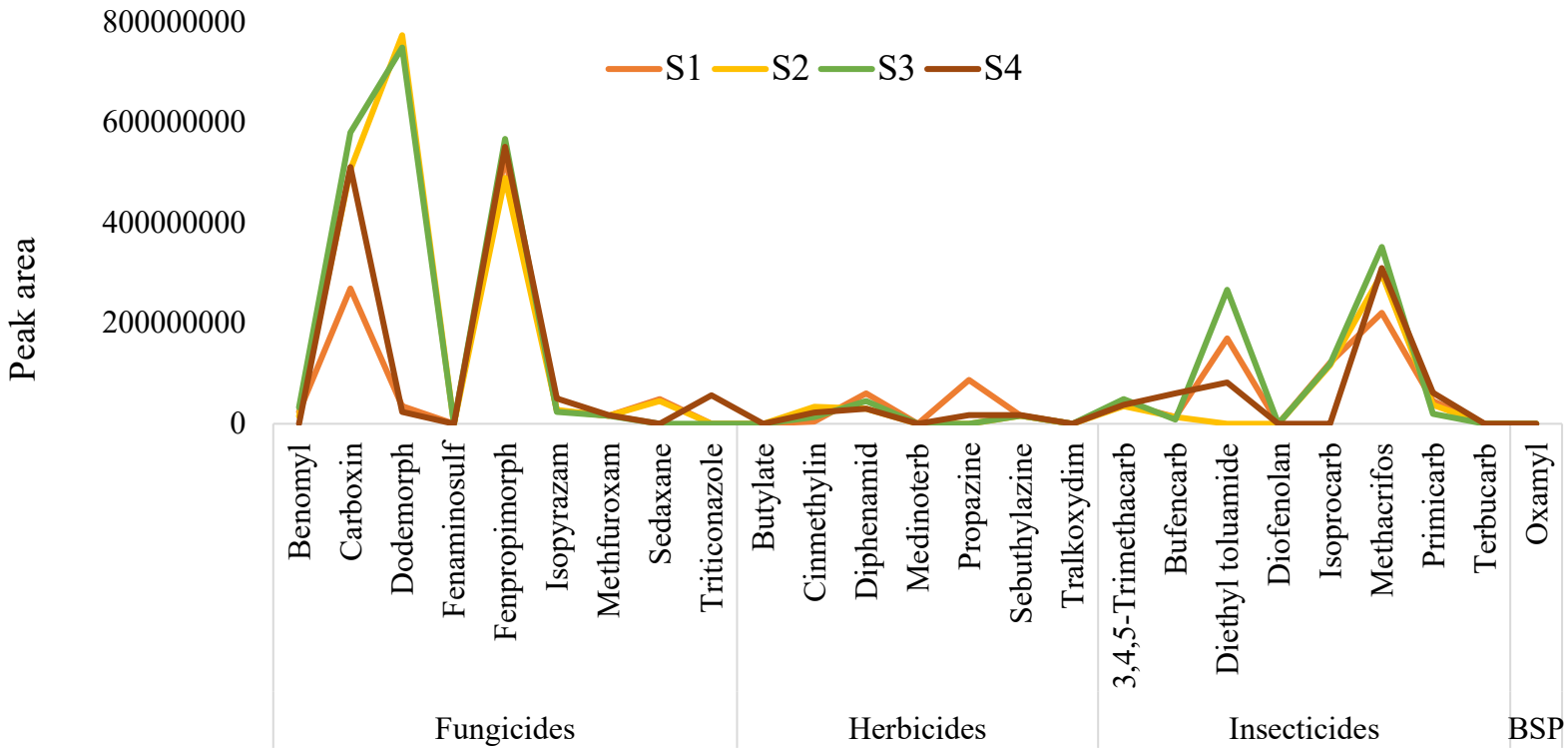

Fig. 1S. Compound areas of pesticides accumulated in Chemcatcher sampler

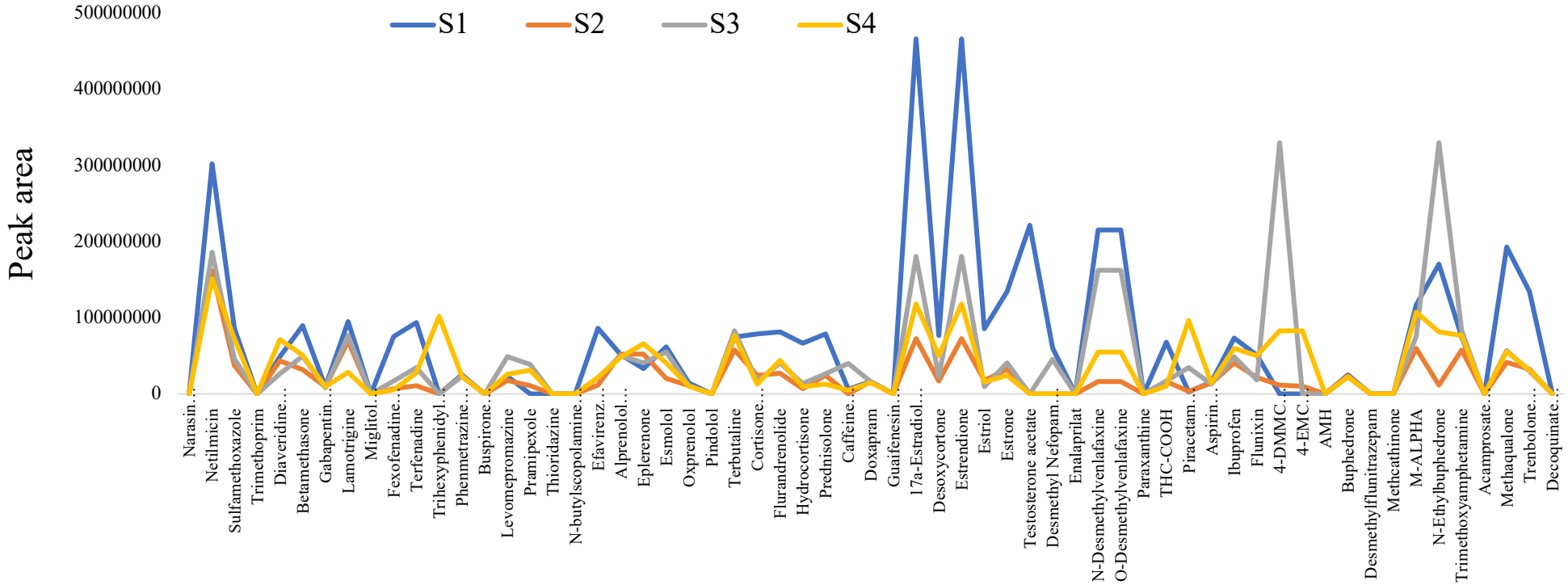

Fig. 2S. Compound areas of pharmaceutical compounds accumulated in Chemcatcher sampler

Table 1S.
Comparison of the pesticides detected in Hennops River and Hartbeespoort Dam

Type	Pollutant	S1		S2		S3		S4		S5
		Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	SPE
Fungicide	Benomyl	√	√	√	X	√	X	X	X	√
	Carboxin	√	X	√	X	√	X	√	X	X
	Dodemorph	√	√	√	√	√	√	√	√	√
	Fenaminosulf	√	√	X	√	X	√	X	√	√
	Fenpropimorph	X	√	√	X	√	√	√	√	√
	Isopyrazam	√	√	√	√	√	X	√	√	√
	Methfuroxam	√	X	√	X	√	X	√	X	X
	Sedaxane	√	√	√	√	X	√	X	√	X
	Triticonazole	X	√	X	√	X	√	√	X	√
Herbicide	Butylate	X	X	X	X	X	X	X	X	√
	Cinmethylin	√	X	√	X	√	X	√	X	X
	Diphenamid	√	X	√	X	√	X	√	X	X
	Medinoterb	X	√	X	√	X	X	X	X	X
	Propazine	√	X	X	X	X	X	√	X	X
	Sebuthylazine	√	X	√	X	√	X	√	X	X
	Tralkoxydim	X	X	X	X	X	X	X	√	√
Insecticides	3,4,5-Trimethacarb	√	X	√	X	√	X	√	X	X
	Bufencarb	√	X	√	√	√	√	√	X	X
	Diethyl toluamide	√	X	X	X	√	X	√	X	X
	Diofenolan	X	√	X	√	X	√	X	√	√
	Isoprocarb	√	√	√	√	√	√	X	√	√
	Methacrifos	√	X	√	X	√	X	√	X	X
	Primicarb	√	X	√	X	√	X	√	X	X
	Terbucarb	X	√	X	√	X	√	X	√	√
BSP	Oxamyl	X	X	X	√	X	X	X	√	X
Total number detected		17	11	15	11	15	8	15	10	11
		22		21		19		22		

BSP – Broad spectrum pesticide, Chem –Chemcatcher

Table S2.
Comparison of pharmaceuticals detected in Hennops River and Hartbeespoort Dam

Type	Pollutant	S1		S2		S3		S4		S5
		Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	SPE
ANTB	Narasin	X	√	X	X	X	X	X	X	X
	Netilmicin	√	X	√	X	√	X	√	X	X
	Sulfamethoxazole	√	√	√	√	√	X	√	√	√
	Trimethoprim	X	√	X	√	X	√	X	√	√
ANTIBA	Diaveridine	√	X	√	X	√	X	√	X	√
	Betamethasone	√	X	√	X	√	X	√	X	√
ANTCOV	Gabapentin	√	X	√	X	√	X	√	X	X
	Lamotrigine	√	X	√	X	√	X	√	X	X
ANTDIA	Miglitol	X	X	X	X	X	√	X	X	X
ANTHIS	Fexofenadine	√	X	√	X	√	X	√	X	X
	Terfenadine	√	X	√	X	√	X	√	X	X
ANTMU	Trihexyphenidyl	X	X	X	X	X	√	√	X	√
ANTOBE	Phenmetrazine	√	√	√	√	√	√	√	√	√
ANTPSY	Buspirone	X	V	X	X	X	X	X	√	X
	Levomepromazine	√	X	√	X	√	X	√	X	X
	Pramipexole	X	√	√	√	√	√	√	√	√
	Thioridazine	X	X	X	X	X	X	X	X	√
ANTSPAS	N-butylscopolamine bromide	X	X	X	X	X	X	X	X	√
ARV	Efavirenz	√	X	√	X	√	X	√	X	X
BETA-	Alprenolol	√	√	√	√	√	√	√	√	√
BLOCKER	Eplerenone	√	√	√	√	√	√	√	√	√
	Esmolol	√	√	√	X	√	X	√	X	X
	Oxprenolol	√	X	√	X	√	X	√	X	X
	Pindolol	X	√	X	√	X	√	X	√	√

	Terbutaline	√	√	√	√	√	√	√	√	√
Corticosteroi	Cortisone	√	X	√	X	√	X	√	X	X
d	Flurandrenolide	√	X	√	X	√	X	√	X	X
	Hydrocortisone	√	X	√	X	√	X	√	X	X
	Prednisolone	√	X	√	X	√	X	√	X	X
CNS	Caffeine	√	X	X	X	√	X	√	X	√
	Doxapram	√	X	√	X	√	X	√	X	X
FLU	Guaifenesin	X	√	X	√	X	X	X	√	√
HOR	17a-Estradiol	√	X	√	X	√	X	√	X	X
	Desoxycortone	√	X	√	X	√	X	√	X	X
	Estrendione	√	X	√	X	√	X	√	X	X
	Estriol	√	X	√	X	√	X	√	X	X
	Estrone	√	X	√	X	√	X	√	X	X
	Testosterone acetate	√	X	X	X	X	X	X	X	X
META	Desmethyl Nefopam	√	X	X	X	√	X	√	X	X
	Enalaprilat	X	X	X	X	X	X	X	X	√
	N-Desmethylvenlafaxine	√	X	√	X	√	X	√	X	X
	O-Desmethylvenlafaxine	√	X	√	X	√	X	√	X	X
	Paraxanthine	X	X	X	X	X	X	X	X	√
	THC-COOH	√	√	√	√	√	X	√	√	X
Nootropics	Piracetam	√	X	√	X	√	X	√	X	X
NSAID	Aspirin	√	√	√	√	√	√	√	√	√
	Ibuprofen	√	X	√	X	√	X	√	X	X
	Flunixin	√	√	√	√	√	√	√	√	√
PSYC	4-DMMC	X	X	√	X	√	X	√	X	X
Illicit	4-EMC	X	X	√	X	X	X	√	X	X
	AMH	X	√	X	X	X	X	X	X	√
	Buphedrone	√	√	√	√	√	√	√	√	√
	Desmethylflunitrazepam	√	√	X	√	X	X	X	X	X
	Methcathinone	X	√	X	√	X	√	X	√	√

	M-ALPHA	√	X	√	X	√	X	√	X	X
	N-Ethylbuphedrone	√	X	√	X	√	X	√	X	X
	Trimethoxyamphetamine	√	√	√	√	√	√	√	√	√
SEDA	Acamprosate	√	√	X	√	X	X	X	X	X
	Methaqualone	√	X	√	X	√	X	√	X	X
STEROID	Trenbolone	√	X	√	X	√	X	√	X	X
VET	Decoquinate	X	X	X	X	X	X	X	X	√
Total number detected		§	20	42	17	43	14	45	17	24

Chem – Chemcatcher, AMH - Anhydroecgonine Methyl Ester, ANTB – Antibiotics, ANTBA – antibacterial, ANTCOV – Anticonvulsants, ANTDIA – antibiabetic, ANTHIS – antihistamine, ANTMU – Antimuscarinic, ANTOBE – anitiobesity, ANTPSY– antipsychotic, ANTSPA – antispasmodic, ARV – Antiretroviral, FLU – Flu medicine, HOR – Hormones, M-ALPHA – 1-Methylamino-1-(3,4-methylenedioxyphenyl)propane, META – Metabolites, PSYC - Psychotropic drugs, SEDA – Sedative, THC-COOH – delta-9 carboxy tetrahydrocannabinol, VET – veterinary medicine

CHAPTER 5 MANUSCRIPT 2

Investigating the occurrence of pharmaceuticals in urban estuarine water using grab and passive sampling approaches with UHPLC Q-Orbitrap MS analysis – A case of Durban Marina, South Africa

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Abstract

Recent literature has highlighted the presence of pharmaceutical contaminants in marine ecosystems, but there are still gaps in similar studies from developing countries. This research focuses on the detection and distribution of nine pharmaceutical compounds of various therapeutic classes in the Durban Marina, an urban estuarine environment in South Africa. Both passive sampling using Chemcatcher devices and solid-phase extraction (SPE) methods were used to detect and quantify carbamazepine, dexamethasone, etilefrine, lopinavir, metformin, methocarbamol, trimethoprim, and venlafaxine. Analysis was conducted using a Thermo Scientific Vanquish UHPLC^{+} Q Exactive Focus Orbitrap. Method optimisation yielded limits of detection and quantification ranging from 0.15 to 2.18, and 0.50 to 7.27 ng L^{-1}, for venlafaxine and methocarbamol, respectively. SPE recoveries fell within acceptable ranges for all compounds in both seawater and ultrapure water (74.82 –103.57%). Correlation coefficients > 0.981 were obtained in Chemcatcher calibration experiments for all compounds considered, except etilefrine which showed linearity only until day eight. Etilefrine was not detected by either sampling method. Whereas metformin was detected across all sites with both sampling approaches. Passive sampling revealed a higher frequency of detection for carbamazepine, which was found at all sampling sites but was absent in all SPE samples. Additionally, SPE failed to detect lopinavir and trimethoprim in three and four samples, respectively, while these compounds were detected in all Chemcatcher samples. Chemcatcher extract concentration sums were higher and ranged from 824.81– 2574.52 ng L^{-1} for S3 and S1, compared to SPE extract concentration sums 36.07 ng L^{-1} – 221.66 ng L^{-1} for S1 and S2. Additionally, time-weighted average (TWA) concentrations were higher with Chemcatcher, reaching a maximum of 290.18 ±9.01 ng L^{-1} for dexamethasone at S5. This study highlights the complexities involved in detecting trace contaminants in estuarine waters due to dilution effects from the sea. The findings emphasise the need for integrating passive sampling techniques into water

quality assessments to better capture low concentration pollutants in dynamic marine environments.

Keywords: Durban Marina; Passive Sampling; Solid-phase extraction; UHPLC-Q Orbitrap MS

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5.1 Introduction

Over the past few decades, the occurrence of pharmaceutical compounds in various water bodies has been a subject of interest due to their daily discharge from various systems like wastewater treatment plant effluent. Their daily usage, and hence discharge has led to their persistence in the aquatic environment. Studies into the occurrence of these compounds have been focused mainly on wastewater treatment plant (WWTP) effluent, freshwater bodies such as rivers, drinking water, dams, and lakes (Blair et al., 2013; Gyesi et al., 2022; Ranjan et al., 2022). Whilst this is important, estuarine and marine environments are often overlooked.

Estuaries are biodiverse ecosystems which play an important role in the health of the marine environment. They serve as breeding grounds for aquatic organisms such as fish and invertebrate (Potter et al., 1993). They aid in carbon storage which helps with climate regulation and serve as a channel for species to move between fresh and saline water (Krauss et al., 2018). Despite forming a small portion of the marine environment, estuaries are among the most productive ecosystems in the world (Meire et al., 2005). South Africa is home to 290 estuaries, 250 of which are functional (Griffiths et al., 2010). Many of these have now been labelled as functionally degraded (Adams et al., 2020). This is due to the wide range of environmental and anthropogenic stressors they have been subjected to (Reddering & Rust, 1990). It is estimated that 840 million litres of wastewater is discharged into South African estuaries daily (Olisah et al., 2021). This in addition to industrial and other human activities upstream activities may be responsible for the current state of South African estuaries (Olisah et al., 2021).

Continued exposure to these stressors has been shown to have detrimental effects on the quality of estuaries. These include loss of habitats, which results in loss of species as many aquatic organisms exhibit specific habitat preferences (Fernandes & Adams, 2016). In more developed nations, comprehensive literature profiling estuarine and marine pollution by

various contaminants exits (Branchet et al., 2021; Cantwell et al., 2017; Pavlović et al., 2013). This has aided in putting regulatory measures in place to address issues associated with marine pollution (Hartnett et al., 2011; Oliveira et al., 2024). Developing countries on the other hand are lagging behind as limited literature on profiling the extent of estuary pollution can be found.

Estuarine and marine pollution in South Africa has been evaluated by looking at pollution from persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs), perchlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) (Olisah, Adeniji, et al., 2019; Vogt et al., 2018). Other estuarian environment studies have looked at pollution by microplastics, heavy metals and a few other organic pollutants (Naidoo et al., 2015; Nel et al., 2023; Yahaya et al., 2017). These studies mainly focus on sediments and the bioaccumulation of these contaminants in marine organisms (Buah-Kwofie & Humphries, 2017; Olisah, Okoh, et al., 2019). Unfortunately, literature on estuarine water pollution, especially by pharmaceutical compounds remains limited. The few that are present have reported the detection of naproxen and ibuprofen in northern Durban from samples acquired from the Umgeni River Estuary. These compounds were present in concentrations ranging from of 0.16 to 0.36 μg L^{-1} (Ngubane et al., 2019). In a fairly recent study, trimethoprim, and carbamazepine were detected in concentrations ranging from 0.39 ± 0.87 to 8.75 ± 1.45 μg kg^{-1} in sediments from Buffalo and Sundays River estuaries (Ohoro et al., 2021). A number of pharmaceutical compounds including carbamazepine and diclofenac were detected in seawater from False Bay. Concentrations ranging from ranging from 0.73 to 3.70 μg L^{-1} were reported (Ojemaye & Petrik, 2022). In a recent study, trimethoprim was reported in concentrations ranging from 3 – 4 ng L^{-1} at Quenera and Gonubie River estuaries in the Eastern Cape. Ibuprofen, naproxen, sulfamethoxazole and efavirenz were also detected in concentrations up to 55 ±0.687 ng L^{-1} (Netshithothole & Madikizela, 2024). A more comprehensive study investigated the occurrence and potential hazard posed by pharmaceutically active compounds

in coastal waters in Cape Town, South Africa over two seasons. Total concentration ranging from 41 and 109 ng L^{-1} were reported (Newman et al., 2024). All of these studies highlight the presence of pharmaceutical compounds in South African marine water. One common thread in these studies is that the sampling approach used is grab sampling.

Since the early 2000s, passive sampling has been demonstrated to be a powerful tool for concentrating trace contaminants to detectable levels (Kingston et al., 2000). This sampling approach is underutilized in developing countries including South Africa. Passive sampling is especially important when studying the extent of pollution in estuarine and marine waters because of the complexity of these matrices and high dilution factors by water from the sea (Jones et al., 2015. In this study, both passive and grab sampling approaches were used to assess the presence and quantity of nine pharmaceutical compounds of different therapeutic classes in the Durban marina harbour. One of the goals is to emphasize the importance of passive sampling in assessing pollution in environments where dilution may hinder the detection of low concentration pollutants.

5.2 Materials and Methods

5.2..1 Reagents and chemicals

Analytical grade nitric acid, HPLC Grade acetonitrile, methanol, formic acid, lopinavir, metformin, trimethoprim, venlafaxine hydrochloride, methocarbamol, etilefrine hydrochloride, nevirapine, carbamazepine, and dexamethasone were purchased from Merck (Johannesburg, South Africa). AttractSPE™ disks HLB (90 mm) and EXPRESS PLUS (PES) disk membranes (0.45 µm, 90 mm diameter) were purchased from Anatech Pty Ltd (Johannesburg, South Africa). The SPE setup consisted of Supel-Select Hydrophilic-lipophilic balance (HLB) cartridges with 500 mg sorbent material. The HLB cartridges were purchased from Sigma Aldrich (Johannesburg, South Africa). Ultrapure water was produced inhouse

using a Millipore DirectQ 3 UV water purification system (Massachusetts, USA). All reagents and chemicals were used as received unless specified. A 1000 mg L^{-1} stock solution of the nine pharmaceutical standards was prepared by dissolving 10 mg of each standard in 10 mL of methanol. This was kept refrigerated at 4 ºC and renewed every week. The nine calibration solutions were prepared in ultrapure water/methanol (90:10 v/v), with concentrations ranging from 0.1 to 500 µg L^{-1}. The physical-chemical properties of the analytes of interest are shown in **Table 1S**.

5.2.2 Instrumentation

pH, conductivity, total dissolved solids (TDS), and salinity were measured *in situ* using a calibrated Hanna HI98194/40 multi-parameter water quality meter (Hanna Instruments., Johannesburg, South Africa). The SPE vacuum manifold was from Pall Corporation (Fribourg, Switzerland). High resolution LC-MS analysis was performed using a Thermo Scientific Vanquish UHPLC$^+$ Q Exactive Focus Orbitrap (Anatech, Johannesburg, South Africa).

5.2.3 Study area

The Durban harbour is situated in the eThekwini metropolitan area on the northeast coast of South Africa in the province of Kwazulu Natal. The harbour is second busiest shipping harbour in sub-Saharan Africa, with the highest container capacity in Africa. A wide range of bulk products are imported and exported through the harbour. The harbour also services cruise liners and small fishing vessels. There are two yacht clubs in the harbour. The seven sampling sites were situated in the Point Yacht Club Basin (**Fig. 1**). The yacht basin was identified as the survey area since it has been plagued by the overflow of sewage from the wastewater reticulation system in the central part of Durban. The sampling sites were positioned at increasing distances from the point where sewage overflows into the basin, with the expectation that there were would be a concentration gradient for target analytes at the sites. S1 (29° 51’

51,996” S 31° 1’ 31,8” E) is furthest away from the sewer outlet and closest to the open sea. S2 (29° 51’ 48,888” S 31° 1’ 24,773” E) moves away from the open sea, but on the opposite end of the sewer outlet. S3 (29° 51’ 46,938” S 31° 1’ 20,078” E) and S4 (29° 51’ 45,276” S 31° 1’ 19,429” E) continue to move away from the open sea, but close to the sewer outlet on the opposite end. S5 (29° 51’ 46,212” S 31° 1’ 22,454” E) is close to the sea, but on the opposite end of S1. S6 (29° 51’ 47,886” S 31° 1’ 26,879” E) moves away from S7 (29° 51’ 50,598” S 31° 1’ 31,391” E) which is close to the sewer outlet.

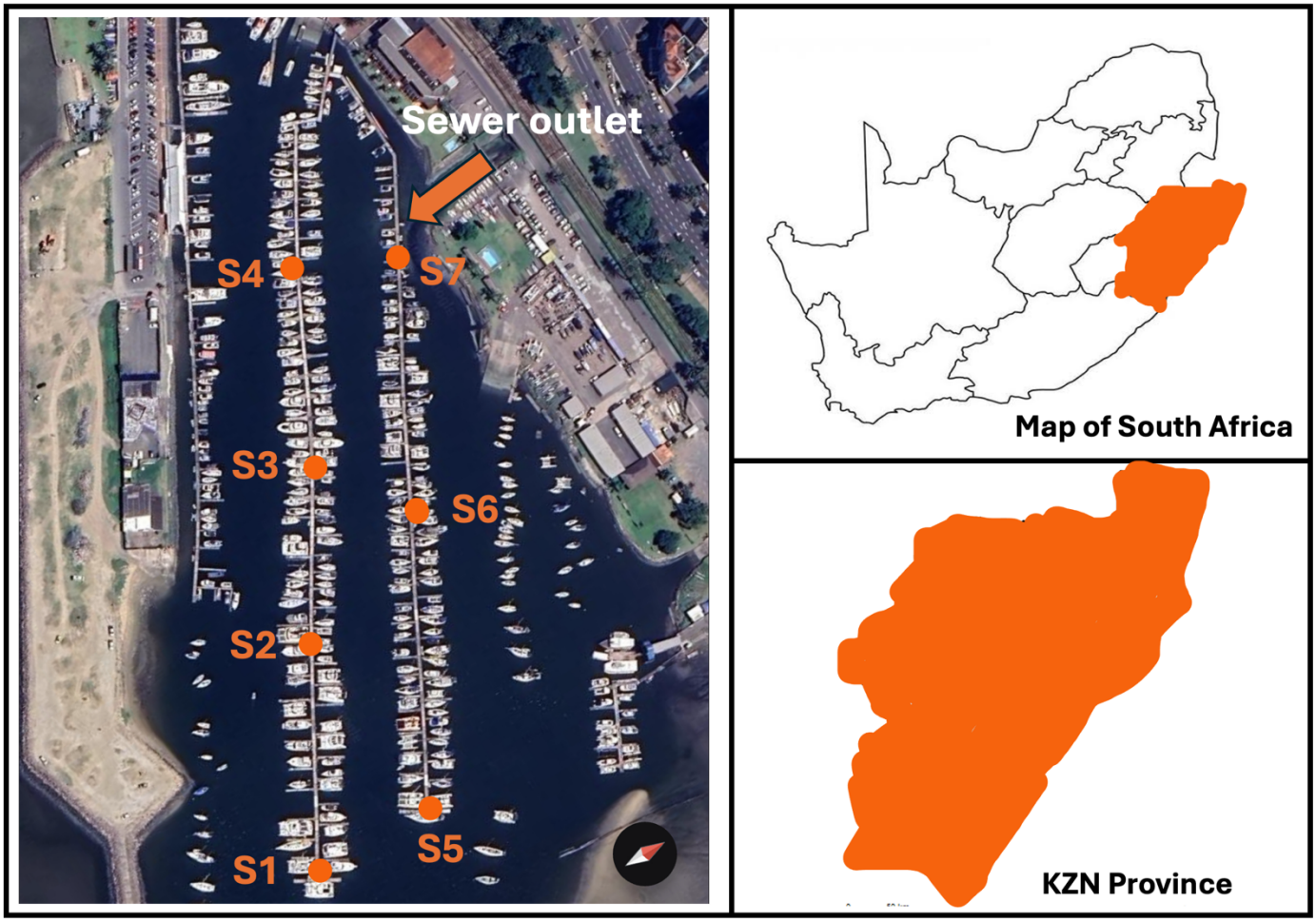

Fig. 1.
Aerial images (Google Earth) showing the location of Durban Marina harbour in South Africa, and the location of sites in the Point Yacht Club Basin.

5.2.4 Assembly, laboratory calibration, and extraction of Chemcatcher sampler

Three component polytetrafluoroethylene (PTFE) Chemcatcher bodies (external diameter 6.6 cm, internal depth 0.8 cm, internal diameter 5.4 cm) were manufactured by the University of the Witwatersrand Physics workshop (Johannesburg, South Africa). The sampler holders were soaked in 10% HNO_3 and rinsed with ultrapure water followed by acetone before use. Affinisep Hydrophilic-lipophilic balance (HLB) receiving phase disks (90 mm) and polyether sulfone (PES) membranes (0.22 µm) were cut to the internal diameter of the sample holder and assembled. The Chemcatcher samplers were assembled by first placing the PES membrane onto the sample holder's support ring, ensuring that the rough side faced outward. Next, the HLB disk was carefully positioned on top of the soft side of the PES membrane. The sample holder support base was then secured in such a way that the HLB disk was sandwiched between the PES membrane and the support ring, with the rough side of the membrane remaining exposed to the external environment. The assembled passive sampling devices were soaked in methanol overnight to activate the HLB disks, after which they were soaked in ultrapure water until use to prevent drying (Castle et al., 2018).

Chemcatcher passive sampler calibration experiments were conducted in seawater using the static calibration method described by Morin et al., (2012a). The pH, TDS, conductivity and salinity of the calibration medium was 8.55, 3516 ppt, 4957 $\mu S.cm^{-1}$ and 30.14 PSU, respectively. Ten Chemcatcher samplers were suspended vertically in a 5L glass tank spiked to 0.4 µg L^{-1} with etilefrine, dexamethasone, carbamazepine, methocarbamol, nevirapine and venlafaxine. Lopinavir, trimethoprim and metformin were not included in the calibration experiment. Seawater was used for this experiment since the water column in the Point Yacht Club Basin is highly saline. The experiment was conducted at a stirring rate of 200 rpm, at room temperature over 16 days. Two Chemcatcher samplers were retrieved after two, five,

eight, eleven and sixteen days. Upon retrieval, the passive samplers were wrapped in aluminum foil and stored at -4°C until ready for extraction and analysis.

The sampler extraction procedure was modified from that described by Kaserzon et al., (2014). To remove debris, the samplers were rinsed with ultrapure water. The samplers were then disassembled and the HLB sorbent transferred to clean 50 mL high density polyethylene (HDPE) centrifuge tubes where they were allowed to air dry for 5 h. Methanol (40 mL) was added to the centrifuge tubes, which were then vortexed for 5 min. The HLB sorbent was removed, and the solvent was filtered through a syringe filter (0.22 μm) into clean 50 mL centrifuge tubes. The filtrate volume was reduced to 1 mL under a gentle stream of nitrogen gas and then transferred to glass vials. These were stored at (-20°C) until analysis.

5.2.5 Field deployment of Chemcatcher samplers

Field deployed Chemcatchers were housed in PVC pipes (25 mm x 15 cm) with 2 cm holes drilled in the sides. Cable ties were used to secure the Chemcatcher samplers to the pipes, and a nylon rope was used to secure the pipes to walk on yacht moorings at each site. The pipes were immersed in water at a depth of ~1 m. Three Chemcatchers were deployed at each site for 14 days in summer (December 2023). On retrieval, the Chemcatcher samplers were detached from the cages and lightly rinsed with site water before sealing with Chemcatcher lids. The Chemcatcher samplers were kept in a cooler for transportation to the laboratory, where they were stored at -20°C until extraction.

5.2.6 Grab water sample processing using SPE

PTFE bottles were rinsed in a 10% nitric acid solution, followed by ultrapure water. On the day of Chemcatcher deployment, 500 mL water samples were collected at each site about 30 cm below the surface in duplicates. This was done by firstly rinsing the containers with site

water three times, then filling the bottles to the neck with site water. The bottles were held in a cooler for transportation to the laboratory, where they were refrigerated at 4°C. The samples were filtered (0.45 µm) in a Büchner funnel, and the filtrate collected in clean glass bottles. The SPE procedure used was modified from literature (Mutavdžić Pavlović et al., 2010). The cartridges were mounted on an SPE manifold connected to a vacuum pump. Pre-conditioning of the cartridges was carried out by passing 6 mL MeOH at a rate of 1 drop per second, followed by 6 mL ultrapure water. This was followed by passing 200 mL of the filtered sample through the HLB cartridges at a rate of 1 drop per second. After percolating, the HLB cartridges were vacuum dried and 2 x 6 mL HPLC grade MeOH was used to elute the sample. Each extract was collected in 15 mL HDPE centrifuge tubes, after which the solvent volume was reduced to precisely 1 mL under a gentle stream of nitrogen gas. The 1 mL samples were then transferred to glass vials and stored in the freezer (-20°C) until analysis. The same procedure was followed for the analyte recovery study, where triplicate 200 mL seawater samples were spiked with 10 µg L^{-1} and 25 µg L^{-1} using the nine pharmaceutical standard mixture. 2 x 6 mL HPLC-grade MeOH was used for elution. The eluent was collected in 15 mL HDPE centrifuge tubes, where it was reduced to 1 mL under a stream of nitrogen gas. The extract was stored in the freezer (-20°C) until analysis.

5.2.7 UHPLC-Q Orbitrap MS analysis for targeted analysis

Analysis was carried out using a Thermo Scientific Vanquish UHPLC$^+$ Q Exactive Focus Orbitrap (Anatech, Cape Town, South Africa) equipped with a Phenomex Luna Omega 1.6 µm C_{18} (100 A 100 x 2.1 mm) column (Anatech, Johannesburg, South Africa). Instrument data acquisition was performed using Thermo Scientific Chromeleon™ Chromatography Data System (CDS) Software. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), both acidified with 0.1% volume formic acid, kept at room temperature. A constant

flow rate of 0.400 mL.min^{-1} was maintained for the duration of the run (15 min). Initially, the instrument was allowed to equilibrate at 5% solvent B for 1 minute. The percentage of solvent B was increased to 55 % for 5 min with a curve of 3. This was held for 0.5 min, after which the percentage of solvent B was increased to 65% for 1.5 min with a curve of 3. The percentage of solvent B was further increased to 100% for 3 min with a curve of 3. The percentage of solvent B was then linearly reduced to 5% for 3 min, after which it was allowed to equilibrate at 5% for 2 min. The sample injection volume was 5 μL.

Tandem mass spectrometry was coupled to a heated electron spray ionisation in positive mode with the sheath gas flow rate at 40 aU, aux gas flow rate at 10 aU, spray voltage at 3.20 kV, S-lens RF level at 55.0, 300 ˚C capillary temperature and 400 ˚C aux gas heater temperature. The full scan acquisition range was 120 – 1000 m/z at a resolution 70 000 and data dependent acquisition (dd-MS^2) in positive mode at a resolution of 35 000, with the AGC target set at 1×10^6. The optimized MS parameters are described in **Table 1**.

5.2.8 Quality assurance

A field blank was prepared for each deployment. A field blank was a chemcatcher exposed to air during the deployment process, and this was done as the chemcatcher samplers were being assembled into cages at each field site. The field blank chemcatcher was opened at the beginning of deployment and remained opened until deployment was complete. After deployment, the field blank was covered with the Chemcatcher lid and transported to the laboratory in a cooler box. To track contamination during sample extraction, procedural blanks for both Chemcatcher sampler and HLB cartridges for SPE were processed alongside the Chemcatcher and SPE samples during sampler extraction and SPE process.

For Chemcatcher procedural blanks, a sampler was assembled then disassembled. After disassembly, the PES membrane and Oasis HLB disk were transferred to 50 mL HDPE

centrifuge tubes. 40 mL methanol was added and the centrifuge tube was vortexed for 5 min. The PES membrane and Oasis HLB sorbent were removed, and the solvent was filtered through a syringe filter (0.22 µm) into clean 50 mL centrifuge tubes. The filtrate volume was reduced to 1 mL under a gentle stream of nitrogen gas and then transferred to glass vials. These were stored at (-20°C) until analysis.

For the SPE procedural blanks, 200 mL ultrapure water was collected in clean PTFE bottles and filtered (0.45 µm) in a Büchner funnel, after which the filtrate was collected in clean 250 mL conical flasks. The filtrate was passed through HLB cartridges which were mounted on an SPE manifold connected to a vacuum pump at a rate of 1 drop per second. Prior to this, the HLB cartridges were pre-conditioning by passing 6 mL MeOH at a rate of 1 drop per second, followed by 6 mL ultrapure water. After percolating, the HLB cartridges were vacuum dried and 2 x 6 mL HPLC grade MeOH was used for elution. Each extract was collected in 15 mL HDPE centrifuge tubes, after which the solvent volume was reduced to precisely 1 mL under a gentle stream of nitrogen gas. The 1 mL samples were then transferred to glass vials and stored in the freezer (-20°C) until analysis.

SPE % recoveries were determined to assess the accuracy of the method and to quantify how efficiently the target analytes were extracted from the matrix. Potential interferences from the sample matrix were evaluated to ensure that the presence of co-extracted substances did not impact the analytical results. This was done by spiking ultrapure and seawater samples were spiked to concentrations (10 and 25 µg L^{-1}). The spiked ultrapure and seawater samplers were processed in the same manner as the SPE procedural blanks.

During analysis, for every five samples analyzed, a quality check (QC) was included. This was a standard sample consisting of the nine pharmaceutical standards prepared in MeOH at a concentration of 10 µg L^{-1}. This was done to account for instrument sensitivity deviation during the sample run period.

For each triplicate sample analyzed, a solvent blank was included. This consisted of pure MeOH. This was done to prevent analyte carryover between each sample run.

5.3 Results and Discussion

5.3.1 Quality assurance

In the field blank and procedural blank samples, none of the analytes of interest were detected. This implies that contamination between samples during sample processing did not occur. Representative chromatograms and calibration curves are shown in Appendix 4 (Figures 1–15).

Table 1 shows the SPE analyte mean recoveries and relative standard deviations, which reflect the analytical precision. Acceptable recoveries (>75%) were achieved for most compounds, except etilefrine (74.82 ±3.80%) in seawater and venlafaxine (72.24 ±1.24%) in deionized water. The recovery for etilefrine in seawater couldn't be compared to other studies due to a lack of available data for environmental matrices, as existing values are typically reported for biological matrices such as blood (Kolmonen et al., 2007). Recoveries for carbamazepine, dexamethasone, lopinavir, methocarbamol, nevirapine, and trimethoprim ranged between 75.40 ±3.33% and 100.52 ±3.61%, aligning with acceptable recovery ranges reported in literature (Borecka et al., 2013; Huysman et al., 2017; Kötke et al., 2019; Loos et al., 2013; Reig et al., 2006). Relative standard deviations varied between 0.51% and 8.24%, suggesting good precision.

Ion suppression was observed for carbamazepine, dexamethasone, and etilefrine, with etilefrine exhibiting the highest suppression (26%). Conversely, ion enhancement occurred for lopinavir, methocarbamol, nevirapine, trimethoprim, and venlafaxine, with venlafaxine showing the greatest enhancement (16.45%). It is worth noting that matrix effects have high

variability, which makes it difficult to control and predict. The matrix effects were then used to correct for the concentrations of target analytes in the sampling medium.

As part of quality assurance procedures, parameters such as the coefficient of determination (R^2) from external calibration curves was determined as the indicator of linearity. All calibration curves gave R^2 values > 0.99, suggestion acceptable linearity. The method detection limits (MLODs) and method quantification limits (MLOQs) were computed as the analyte concentration that produced a peak with a signal-to-noise ratio of 3 and 10, respectively. The MLODs and MLOQs for the analytes of interest are listed in (**Table 1**). The MLODs ranged from 0.15 to 2.18, and the MLOQs from 0.50 to 7.27 ng L^{-1} for venlafaxine and methocarbamol.

Table 1. Target compound's optimized LC-MS/MS parameters, method limits of detection and quantification, solid phase extraction recoveries and matrix effects

Compound	Mass spec parameters						Solid phase extraction recovery				
	Precursor ion	Confirming ion	CE (V)	RT (min)	MLOD ng L^{-1}	MLOQ ng L^{-1}	Matrix	10 µg $.L^{-1}$ % Recovery	ME	25 µg $.L^{-1}$ % Recovery	ME
Carbamazepine	237.10219	194.09657	23	5.184	0.22	0.72	Sea	76.86 ±2.86	3.40	95.73 ±1.54	4.76
							Deionised	79.56 ±2.62	–	100.52 ±3.61	–
Dexamethasone	393.20724	147.08052	29	5.274	1.41	4.70	Sea	78.76 ±5.14	2.19	92.38 ±6.51	3.10
							Deionised	80.52 ±2.04	–	95.34 ±3.32	–
Etilefrine	182.11760	164.10703	17	1.553	1.64	5.48	Sea	74.82 ±3.80	21.39	76.53 ±6.35	26
							Deionised	95.18 ±0.51	–	103.42 ±3.01	–
Lopinavir	629.37006	183.11285	25	8.129	0.48	1.60	Sea	85.57 ±3.93	– 8.84	90.52 ±5.87	-
							Deionised	78.62 ±2.46	–	81.78 ±2.44	10.68 –
Metformin	130.10870	85.05141	18	1.504	–	–	Sea	–	–	–	–
							Deionised				
Methocarbamol	242.10225	118.05008	14	4.163	2.18	7.27	Sea	77.30 ±1.48	–3.19	97.97 ±8.24	-1.92
							Deionised	74.91 ±2.08	–	96.12 ±4.66	–
Nevirapine	267.12411	80.05001	40	4.384	0.41	1.36	Sea	75.45 ±2.84	5.97	98.52 ± 6.82	-7.83

							Deionised	80.24 ±0.65	–	91.37 ±1.76	–
Trimethoprim	291.14508	123.06671	35	3.564	0.34	1.16	Sea	89.74 ±2.81	- 10.39	99.45 ±2.14	-16.45
							Deionised	81.29 ±1.57	_	85.40 ±3.33	–
Venlafaxine	278.21152	121.06503	45	4.076	0.15	0.50	Sea	77.94 ±3.37	– 7.89	93.55 ±3.88	-7.06
							Deionised	72.24 ±1.24	–	87.38 ±2.22	–

CE – Collision Energy, RT – Retention Time, MLOD – Method Limit of Detection, MLOQ – Method Limit of Quantification, ME – Matrix Effect, % Recovery ±RSD (n=3)

5.3.2 Chemcatcher calibration

Fig. 2 presents the calibration plot from Chemcatcher experiments in seawater, while **Table 2** provides laboratory sampling rates and correlation coefficients from these experiments. The calibration showed good linearity (>0.98) for five of the six pharmaceutical compounds. However, the uptake of etilefrine was linear only up to day eight, after which it decreased due to possible degradation. As a result, its sampling rate was excluded from the calculation of the TWA concentration.

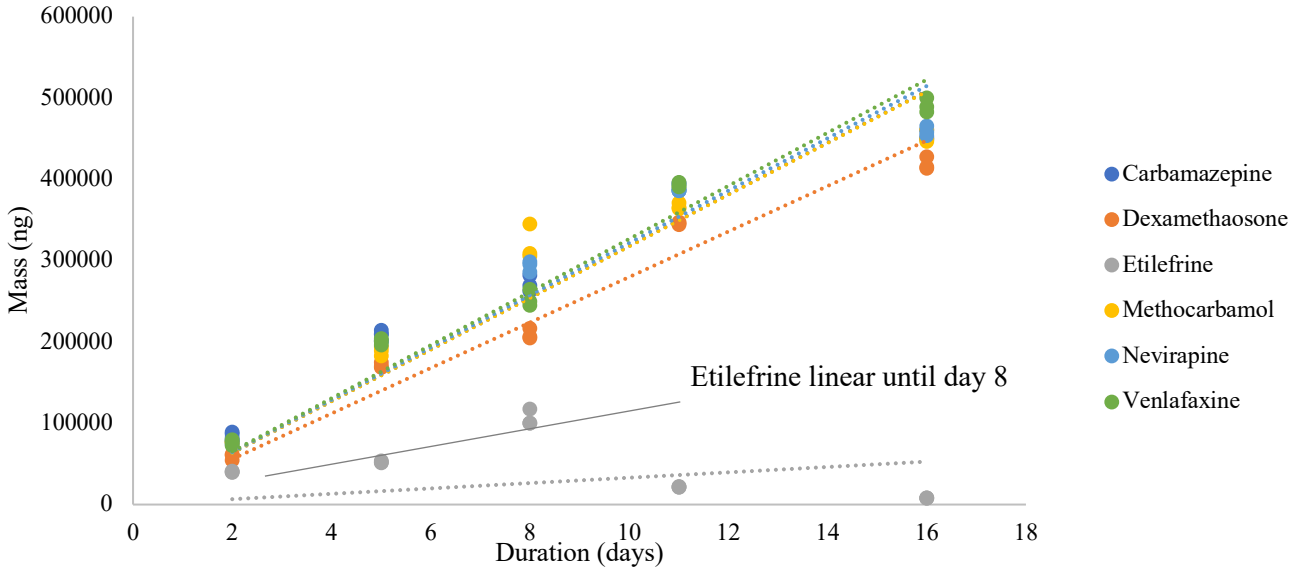

Fig. 2.
Calibration plot of pharmaceutical uptake in Chemcatcher passive samplers over time in seawater

The experimental sampling rates were compared to those reported in the literature. While there is extensive data on the laboratory calibration of Chemcatcher and POCIS for compounds like carbamazepine, trimethoprim, and venlafaxine in various water types, studies on dexamethasone, etilefrine, methocarbamol, and nevirapine are limited. In this study, the

sampling rates for carbamazepine and venlafaxine were both calculated at 0.42 L day^{-1}, aligning with previously reported values for saline water (Bayen et al., 2014; Li et al., 2011).

For dexamethasone, sampling rates in saline water could not be found, as such the experimental rate was compared to data from freshwater, which matched well with previous findings (Guibal et al., 2020). Due to the high water affinity of metformin, its sorption to HLB disks was weak, leading to low sampling rates (Bäuerlein et al., 2012) as a result, this compound could not be quantified. Similarly, there is a lack of literature on the uptake of lopinavir using Chemcatcher and POCIS in seawater. For this reason, the sampling rate (0.124 L day^{-1}) for lopinavir in freshwater was used to determine its TWA concentration (Domínguez-García et al., 2025). As with lopinavir, a literature sampling rate was used to calculated the TWA concentrations for Trimethoprim (Bayen et al., 2014).

Passive samplers, such as POCIS and Chemcatcher, have been calibrated for a wide range of compounds to determine their sampling rates (Morin et al., 2012b). However, these rates vary significantly across different laboratory studies (Li et al., 2010; MacLeod et al., 2007). Factors such as pH, stirring rate, and temperature significantly impact sampling rates (Li et al., 2010), making it difficult to control them in field conditions. Despite these challenges, laboratory-derived sampling rates remain crucial for estimating contaminant concentrations in natural water bodies.

Table 2.
Comparison of experimental and literature Chemcatcher sampling rates in seawater

Target compound	Correlation coefficients R^2	Experimental sampling rate (L day^{-1})	Literature sampling rate (L day^{-1})	References
Carbamazepine	0.983	0.42	0.342	(Martínez Bueno et al., 2016)
			0.497	(Bayen et al., 2014)
			0.372	(Li et al., 2010)

Dexamethasone	0.990	0.34	0.154 – 0.388	(Guibal et al., 2020)
Etilefrine	0.320	–	_	_
Lopinavir	_	_	0.124	(Domínguez-García et al., 2025)
Metformin	_	_	0.086 ± 0.018	(Kim & Homan, 2020)
Methocarbamol	0.981	0.39	_	–
Nevirapine	0.985	0.41	_	–
Trimethoprim	_	_	0.346	(Bayen et al., 2014)
			0.370	(Li et al., 2010)
			0.178	(Domínguez-García et al., 2025)
Venlafaxine	0.992	0.42	0.372	(Li et al., 2010)

R^2 – Correlation coefficient, L day^{-1} – Litre per day

Note: The sampling rate for etilefrine was not included due to deviation from linearity after day eight in the Chemcatcher calibration experiment. Lopinavir, metformin and trimethoprim experimental sampling rates are not included in the table because the compounds were not considered in the laboratory calibration experiment.

5.3.3 Field determined physicochemical parameters

Table 3 shows the determined physiochemical parameters from the sampling sites determined on the day of grab sampling and Chemcatcher deployment. The pH along the sampling sites ranged from 8.14 to 8.43, with a mean of 8.23 (n=7). These fall within the 7.5 to 8.5 pH range for seawater. There is no universally acceptable pH standard range for marine water as this parameter is heavily influenced by local conditions (Marion et al., 2011). Electrical conductivity ranged from 43200 to 44800 (µS.cm^{-1}), with a mean of 44200 (µS.cm^{-}

[1]). These values are below the marine electrical conductivity standard (~ 45000 – 60000 µS.cm^{-1}) range for water with a salinity of ~ 35 PSU (Tyler et al., 2017). This was expected as the sampling sites are situated in an estuary, with salinities ranging from 28.18 to 29.03 PSU. As with conductivity and salinity, the TDS were lower than those expected for seawater (10000 – 35000 ppm), but much greater than those for fresh water (< 1000 ppm). This can be explained by the heavy marine influence on this estuary. TDS factor was calculated as the ratio between the TDS and conductivity per sampling site. These were found to fall between 0.72 to 0.73, which is in line with the expected 0.7 factor for saline water (Al-Kubaish et al., 2024).

Table 3.
Physicochemical parameter analysis of water samples

Sample ID	pH	Salinity (PSU)	Conductivity (µS.cm^{-1})	TDS (ppm)	TDS factor
S1	8.43	28.12	43600	31790	0.73
S2	8.16	28.95	44730	32360	0.72
S3	8.14	28.43	43980	31970	0.73
S4	8.14	28.29	43570	31870	0.73
S5	8.17	28.69	44290	32160	0.73
S6	8.24	28.71	44400	32200	0.73
S7	8.30	29.03	44840	32430	0.72
Mean	8.23	28.60	44200.12	32111.43	0.73
Minimum	8.14	28.12	4320	31790	0.72
Maximum	8.43	29.03	4484	32430	0.73
Saline water standard	7.5 – 8.5	35	45000 - 60000	10000 – 35000	0.7

PSU – Practical Salinity Unit, µS cm^{-1} –micro siemens per centimetre, TDS – Total Dissolved Solid, ppm – parts per million

5.3.4 Pharmaceutical concentrations in the Durban Marina

5.3.4.1 Detection and total pharmaceutical concentrations in SPE and Chemcatcher extracts

Fig. 3. shows the detection frequency of pharmaceuticals using chemcatcher and SPE for all sites. Chemcatcher consistently shows a higher detection frequency (88.89%) across all sampling sites, whereas SPE detection frequencies vary more across sites (55.56 – 77.78%). The higher detection frequency of pharmaceuticals using Chemcatcher suggests that passive sampling is more effective at detecting these compounds over time compared to SPE, and that SPE may be missing some pharmaceuticals that are present at lower concentrations or intermittently in water, while Chemcatcher integrates contaminants over time, making it more sensitive.

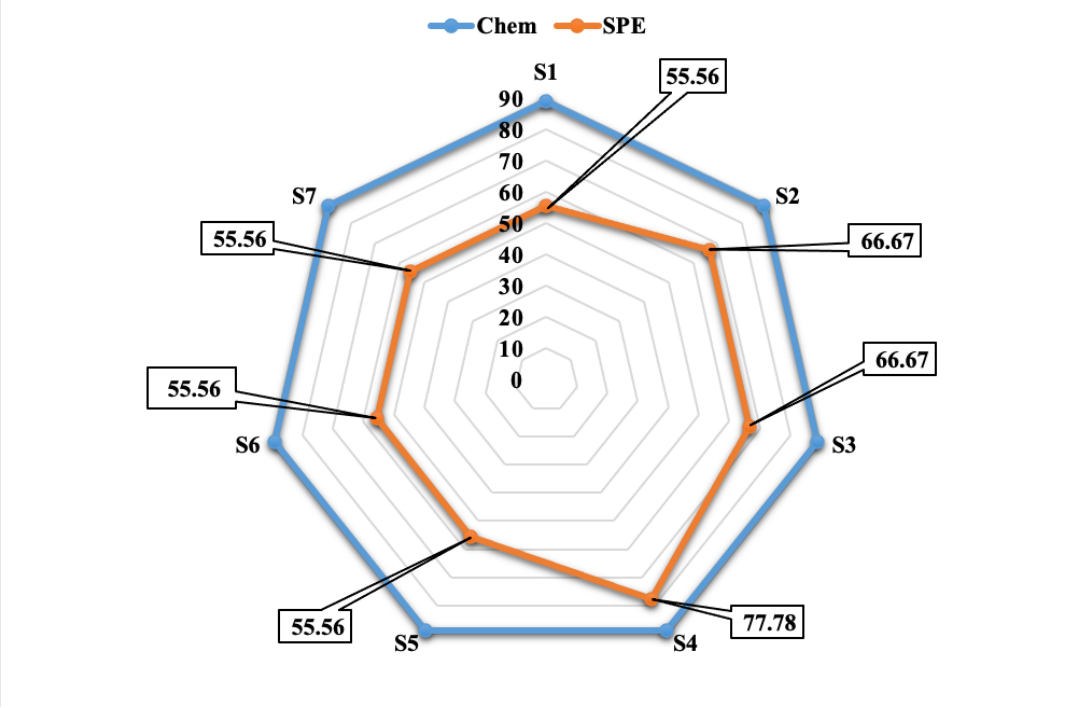

Fig. 3.
Percentage detection frequency of pharmaceuticals in Chemcatcher and SPE extracts for all sites

S1, S5, S6, S7 showed the lowest SPE detection frequency (55.56%), while S4 showed the highest SPE detection frequency (77.78%). This was anticipated for S1 and S5 as they are located furthest away from the sewer outlet, but near the open sea. However, this was not anticipated for S7, which is located closest to the sewer outlet which is a potential source of pharmaceutical contamination in the Durban marina. The detection frequency data for SPE deviating from observations that were anticipated can be explained by homogeneity of the sampling medium, as seen with the low variability in salinity (**Table 3**) across sites (standard deviation = 0.34 PSU).

The pharmaceutical concentrations detected in SPE and Chemcatcher extracts are summarized in **Table 4**. Etilefrine was not detected at any site using either sampling technique. In contrast, metformin was consistently detected across all sites with both methods. Carbamazepine was not detected in SPE extracts but it was present in all Chemcatcher extracts, with concentrations ranging from 13.37 ± 1.50 to 30.33 ± 2.27 ng L^{-1}. Dexamethasone and methocarbamol were found at high concentrations in Chemcatcher extracts across all sites, ranging from 303.02 ± 5.74 to 1667.47 ± 13.26 ng L^{-1}. The presence of these compounds in concentrations reaching 1667.47 ± 13.26 µg L^{-1} suggests an episodic release event in the Durban Marina that was likely missed by grab sampling. This is further supported by their significantly lower concentrations in SPE extracts (13.55 ± 3.97 to 26.70 ± 6.74 ng L^{-1}).

Nevirapine and venlafaxine were consistently detected at all sites in both Chemcatcher and SPE extracts. Nevirapine concentrations were lowest at S6 (1.87 ± 0.49 ng L^{-1}) and highest at S2 (31.88 ± 3.89 ng L^{-1}), while Chemcatcher concentrations ranged from 18.74 ± 0.61 to 318.83 ± 3.84 ng L^{-1}. Although venlafaxine was detected at all sites in both SPE and Chemcatcher extracts, the SPE concentrations were below the quantification limit (0.15 ng L^{-1}) at S6 and S7. Generally, venlafaxine concentrations in SPE extracts were low (<MLOQ – 1.21 ng L^{-1}), except for S2, where it reached 24.03 ± 0.81 ng L^{-1}. In contrast, Chemcatcher extracts

showed significantly higher venlafaxine concentrations, with the lowest value at S3 exceeding 180 ng L^{-1}, and the highest at S1, just below 350 ng L^{-1}. Trimethoprim was sporadically detected in SPE extracts but consistently found in Chemcatcher extracts. It was only present in SPE extracts from S2, S3, and S4, with concentrations ranging from 5.63 ± 0.58 to 10.1 ± 1.19 ng L^{-1}, and was absent from S1, S5, S6, and S7. In Chemcatcher extracts, trimethoprim was detected at its lowest concentration at S7 (41.96 ± 0.44 ng L^{-1}) and its highest at S2 (294.59 ± 5.88 ng L^{-1}).

Table 4.
Pharmaceutical concentrations in SPE and Chemcatcher 1 mL extracts of the samples (ng L^{-1})

Compound	S1		S2		S3		S4		S5		S6		S7	
	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE
Carbamazepine	30.33 ±2.27	<MLOD	39.6 ±5.61	<MLOD	14.97 ±4.32	<MLOD	18.77 ±2.15	<MLOD	20.17 ±1.73	<MLOD	13.37 ±1.50	<MLOD	17.27 ±2.09	<MLOD
Dexamethasone	918.07 ±8.73	17.84 ±3.11	1123.8 ±11.06	17.76 ±3.39	676.03 ±6.12	20.23 ±5.43	1667.47 ±13.26	26.70 ±6.74	1557.2 ±9.01	16.46 ±4.23	1065.43 ±6.06	26.07 ±2.51	1318.51 ±11.26	17.3 ±3.11
Etilefrine	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
Lopinavir	39.89 ±1.63	ND	35.37 ±1.87	<MLOD	27.81 ±1.55	<MLOD	15.29 ±1.16	<MLOQ	13.07 ±1.73	<MLOD	43.94 ±2.47	ND	41.52 ±1.63	ND
Metformin	D	D	D	D	D	D	D	D	D	D	D	D	D	D
Methocarbamol	539.8 ±2.02	13.55 ±3.97	450.26 ±3.95	14.36 ±2.61	303.02 ±5.74	16.9 ±3.00	328.85 ±2.97	17.01 ±2.55	434.5 ±6.59	18.56 ±1.65	827.91 ±8.52	18.57 ±5.63	466.91 ±8.47	15.87 ±2.39
Nevirapine	258.73 ±2.28	11.45 ±2.51	318.83 ±3.84	14.03 ±3.89	92.97 ±4.41	3.85 ±1.24	137.46 ±2.94	9.81 ±1.74	81.03 ±1.36	10.65 ±0.93	18.74 ±0.61	11.07 ±0.49	56.67 ±1.21	2.90 ±1.40
Trimethoprim	145.8 ±3.57	<MLOD	294.59 ±5.88	9.15 ±2.43	136.87 ±3.25	10.1 ±1.19	120.3 ±2.85	5.63 ±0.58	139.54 ±5.99	<MLOD	56.38 ±2.48	<MLOD	41.96 ±0.44	<MLOD
Venlafaxine	349.39 ±3.02	0.97 ±0.29	312.07 ±2.12	24.03 ±0.81	182.14 ±1.96	1.1 ±0.10	276.77 ±2.81	1.21 ±0.34	266.13 ±5.42	<MLOQ	219.88 ±3.22	<MLOQ	287.61 ±1.74	<MLOQ

∑Concentration	2282.04	43.81	2574.52	79.33	824.81	52.18	2564.91	60.36	2511.64	45.67	2245.61	55.71	2230.45	36.07

Chem – Chemcatcher sampler, D – Detected, but not quantified, ∑ – Sum, Concentration (±RSD, n=3), concentrations (<MLOD and MLOQ) not included in ∑concentration

5.3.4.2 Chemcatcher time-weighted average concentrations and grab water sample concentrations

The time-weighted average (TWA) concentrations of the target pharmaceuticals accumulated in the Chemcatcher sampler were calculated using laboratory-determined sampling rates (**Table 2**), as presented in (**Table 5**). These TWA concentrations were then compared to the concentrations obtained from the 200 mL water samples, representing the concentrations in the sampling medium. For trimethoprim, a literature-based sampling rate (0.346 L day^{-1}) was applied to calculate the TWA concentration (Bayen et al., 2014). For lopinavir, a sampling rate (0.124 L day^{-1}) for the compound in fresh water was used (Domínguez-García et al., 2025). Metformin TWA concentrations were not calculated due to poor adsorption to HLB sorbents, linked to its high lipophilicity (LogKow < 0).

The comparison of TWA concentrations of pharmaceuticals between SPE and Chemcatcher sampling techniques reveals significant differences in detection limits, concentration levels, and sensitivity across all sampling sites. Overall, Chemcatcher consistently detected higher concentrations of pharmaceuticals compared to SPE. Majority of the compounds, including carbamazepine, etilefrine, and trimethoprim, were not detected in all SPE samples, while Chemcatcher was able to capture their presence. This highlights passive sampling's superior ability to detect pharmaceuticals in the aquatic environment, especially for compounds that may be present in low concentrations or in episodic bursts.

For instance, carbamazepine was not detected in any of the SPE samples, whereas Chemcatcher concentrations ranged from 1.99 ± 1.50 ng L^{-1} at S6 to 5.89 ± 5.61 ng L^{-1} at S2. Similarly, dexamethasone concentrations were significantly higher in Chemcatcher samples, ranging from 125.97 ± 6.12 ng L^{-1} at S3 to 310.72 ± 13.26 ng L^{-1} at S4, while SPE detected only very low concentrations, with a maximum of 0.1335 ng L^{-1}. This indicates that

Chemcatcher was more effective in capturing episodic contamination events that might have been missed by the grab sampling technique.

Lopinavir, methocarbamol, and nevirapine also showed considerable differences in concentrations between the two sampling methods. While SPE detected very low concentrations or none at all, Chemcatcher consistently recorded significantly higher levels. For example, methocarbamol was detected at concentrations as high as 132.67 ± 8.52 ng L^{-1} in Chemcatcher samples, compared to much lower levels in SPE, where concentrations did not exceed 0.0929 ng L^{-1}. Similarly, Chemcatcher detected venlafaxine at concentrations ranging from 26.98 ± 1.96 ng L^{-1} at S3 to 51.76 ± 3.02 ng L^{-1} at S1, whereas SPE recorded values mostly below the quantification limit, with concentrations as low as 0.00225 ng L^{-1}.

In the case of trimethoprim, SPE detected the compound only at a few sites and at very low concentrations, whereas Chemcatcher consistently detected trimethoprim at all sites, with concentrations ranging from 7.58 ± 0.44 ng L^{-1} at S7 to 53.21 ± 5.88 ng L^{-1} at S2. This difference further emphasizes Chemcatcher's greater sensitivity and reliability in monitoring pharmaceuticals in water systems.

Overall, Chemcatcher proved to be more reliable for detecting a wider range of pharmaceuticals, capturing both continuous and episodic contamination events effectively. In contrast, SPE often underestimated the presence of pharmaceuticals, particularly those present at low concentrations or those exhibiting episodic spikes, such as dexamethasone and methocarbamol. This highlights importance of using passive sampling as a complementary approach to grab sampling in environmental pollution monitoring studies. While grab sampling provides a snapshot of contaminant concentrations at a specific moment, passive sampling offers time-integrated measurements, capturing variations in pollutant levels over extended periods. This combined approach enhances the accuracy and comprehensiveness of environmental assessments, especially for detecting episodic pollution events or contaminants

present at low but persistent concentrations. Although a concentration gradient was anticipated, with higher pharmaceutical concentrations expected at S6 and S7 (near the sewer outlet) and lower concentrations at S1 and S4 (further away), no such trend was observed, likely due to tidal activity homogenizing the water between the sampling sites, as indicated by the uniform physiochemical parameters.

Table 5.
Comparison of time–weighted average concentrations of the target pharmaceuticals per site in Chemcatcher and concentrations in grab water samples

Compound	S1		S2		S3		S4		S5		S6		S7	
	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE
Carbamazepine	4.51 ±2.27	<MLOD	5.89 ±5.61	<MLOD	2.28 ±4.32	<MLOD	2.79 ±2.15	<MLOD	3.02 ±1.73	<MLOD	1.99 ±1.50	<MLOD	2.57 ±2.09	<MLOD
Dexamethasone	171.08 ±8.73	0.0892 ±3.11	209.41 ±11.06	0.0888 ±3.39	125.97 ±6.12	0.10115 ±5.43	310.72 ±13.26	0.1335 ±6.74	290.18 ±9.01	0.0823 ±4.23	198.53 ±6.06	0.13035 ±2.41	245.70 ±11.26	0.0865 ±3.51
Etilefrine	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD	<MLOD
Lopinavir	20.10 ±1.63	<MLOD	17.83 ±1.87	<MLOD	17.02 ±1.55	<MLOD	7.71 ±1.16	<MLOQ ±0.62	6.59 ±1.73	<MLOD	22.15 ±2.47	<MLOD	20.93 ±1.63	<MLOD
Metformin	D	D	D	D	D	D	D	D	D	D	D	D	D	D
Methocarbamol	86.51 ±2.02	0.0678 ±3.97	72.17 ±3.95	0.0718 ±2.61	48.56 ±5.74	0.0845 ±3.00	52.70 ±2.97	0.08505 ±2.55	69.63 ±6.59	0.0928 ±1.65	132.67 ±8.52	0.09285 ±5.63	74.83 ±8.47	0.07935 ±2.39
Nevirapine	39.83 ±2.28	0.1294 ±2.51	49.08 ±3.84	0.1594 ±3.89	14.31 ±4.41	0.0465 ±1.24	21.16 ±2.94	0.06875 ±1.72	12.47 ±1.36	0.0405 ±0.93	2.88 ±0.61	0.00935 ±0.49	8.27 ±1.21	0.02835 ±1.40
Trimethoprim	26.34 ± 3.57	<MLOD	53.21 ±5.88	0.04575 ±2.42	24.72 ±3.25	0.0505 ±1.19	21.70 ±2.85	0.02815 ±0.58	25.21 ±5.99	<MLOD	10.18 ±2.48	<MLOD	7.58 ±0.44	<MLOD
Venlafaxine	51.76 ±3.02	0.00485 ±0.29	46.21 ±2.12	0.12015 ±0.81	26.98 ±1.96	0.0055 ±0.10	41.00 ±2.81	0.00605 ±0.34	39.42 ±5.42	<MLOQ	32.57 ±3.22	<MLOQ	42.61 ±1.74	<MLOQ

Min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Max	86.51 ±2.02	0.1294 ±2.51	209.41 ±11.06	0.1594 ±3.89	125.97 ±6.12	0.10115 ±5.43	310.72 ±13.26	0.1335 ±6.74	290.18 ±9.01	0.0928 ±1.65	198.53 ±6.06	0.13035 ±2.41	245.70 ±11.26	0.0865 ±3.51

D – detected, MLOD – Method Limit of Detection, MLOQ – Method Limit of Quantification, Min – Minimum, Max – Maximum, TWA concentrations in ng L^{-1}

Note: All concentrations are in ng L^{-1}

5.3.4.3 Comparative assessment of concentrations of target pharmaceuticals detected with SPE and chemcatcher, and their comparison with other studies

Table 6 compares minimum, maximum and mean concentrations of analytes in SPE extracts and compares them with other studies. Etilefrine was not detected at any of the seven sampling sites using either method. Carbamazepine, a known persistent environmental contaminant, was undetected in all SPE samples, which is unexpected given its well-documented persistence in aquatic environments (Matongo *et al.*, 2015). This persistence is attributed to its continuous input, low biodegradability, and inefficient removal in wastewater treatment processes (K'oreje et al., 2012). In contrast, carbamazepine was detected in all Chemcatcher samples, with concentrations ranging between 133.70 and 396.01 ng L^{-1}. Its absence in SPE samples could be due to its presence in concentrations below the detection limit at the time of sample collection.

Dexamethasone, methocarbamol, nevirapine, and venlafaxine were consistently detected at all sampling sites using both SPE and Chemcatcher. Dexamethasone concentrations ranged from 16.46 to 26.70 ng L^{-1} in SPE, and from 67.60 to 166.75 ng L^{-1} in Chemcatcher samples. Methocarbamol concentrations were found between 13.55 and 18.57 ng L^{-1} in SPE, and from 30.30 to 82.79 ng L^{-1} in Chemcatcher samples. To the best of our knowledge, no previous studies have reported concentrations of dexamethasone or methocarbamol in saline water, limiting direct comparisons. Nevirapine concentrations ranged from 29.03 to 140.32 ng L^{-1} in SPE, and from 1.87 to 31.88 ng L^{-1} in Chemcatcher samples. These values are lower than those reported in Kenyan coastal waters, where concentrations up to 1018 ng L^{-1} have been observed, and attributed to poor wastewater treatment and continuous domestic and industrial discharge (Wanjeri et al., 2023). Concentrations for the antidepressant venlafaxine were measured between 1.77 and 24.26 ng L^{-1} in SPE, and between 182.14 and 349.39 ng L^{-1} in Chemcatcher samples. These values are lower than those reported for the Spanish coast (up to 6 ng L^{-1}) (Čelić

et al., 2021), but comparable to those reported in Portuguese coastal waters (ND–357 ng L^{-1}) (Sousa et al., 2020). Venlafaxine is frequently detected in the aquatic environment, with concerns about its persistence and bioaccumulation in marine organisms such as bivalves and fish(del Carmen Gómez-Regalado et al., 2023; Grabicova et al., 2017; Qu et al., 2019).

Lopinavir was detected at sites S2, S3, S4, and S5 using SPE, and at all sites using Chemcatcher. Concentrations ranged from ND to 6.4 ng L^{-1} in SPE, and from 13.07 to 43.94 ng L^{-1} in Chemcatcher samples. Lopinavir, which was also used in the treatment of COVID-19 (Cao et al., 2020) has been detected in marine environments, with predicted environmental concentrations reaching up to 1143 ng L^{-1} in Santos Bay, Brazil (Cid et al., 2021). These models offer an estimation of the concentration of a substance in the environment, by taking into account the initial amount released into the environment in terms of its fate, transformation and removal, either by artificial or natural means (Ncube et al., 2018). Concentrations observed in this study were lower than these predictions. The detection of this compound in the Mediterranean Sea was reported, however, it was no quantified (Rilievo et al., 2024a).

Trimethoprim, a drug used in conjunction with Sulfamethoxazole to treat various bacterial infections. This drug has been reported to be misused in developing countries due to the ease of access from unregulated vendors (Kamere et al., 2022). As such, it has emerged as one of the most commonly detected API's in aquatic environments. Trimethoprim was detected at sites S2, S3, and S4 with SPE, and at all sites with Chemcatcher. SPE concentrations ranged from ND to 100.98 ng L^{-1}. These values are higher than those reported in Spanish coastal waters (up to 5 ng L^{-1}) (Biel-Maeso et al., 2018; Moreno-González et al., 2015), the Baltic Sea (up to 14.2 ng L^{-1}) (Borecka et al., 2013), coastal California waters (up to 2.1 ng L^{-1}) (Vidal-Dorsch et al., 2012), and Quenera and Gonubie River estuaries in the Eastern Cape, South Africa (up to 4 ng L^{-1}) (Netshithothole & Madikizela, 2024), but comparable to concentrations detected in Bohai Bay, China (up to 120 ng L^{-1}) (Zou et al., 2011).

Metformin, a widely prescribed antidiabetic drug, was detected at all sampling sites using both SPE and Chemcatcher. Its detection was anticipated, given the high prescribed dosages, which range from 0.5 to 2.5 g per patient (Reitman & Schadt, 2007). Metformin could not be quantified due to limitation of its sorption on to the HLB sorbent used.

Table 6.
Minimum, maximum and mean concentrations of analytes in SPE samples and comparison with other studies

Compound	Current study			Literature	
	Min	Max	mean	Concentration	Reference
Carbamazepine	<MLOD	<MLOD	0	257 (estuary), 475 (sea)	(Sousa et al., 2020)
Dexamethasone	172.70	266.97	203.37	<0.05 – 4.76	(Omar et al., 2019)
				<1.00 – 1.51	(Ismail et al., 2019)
Etilefrine	<MLOD	<MLOD	–	–	–
Lopinavir	ND	6.40	1.83	D	(Rilievo et al., 2024b)
Metformin	D	D	–	<LOQ – 31 ± 1.3	(Rilievo et al., 2024b)
Methocarbamol	143.57	135.51	164.03	–	–
Nevirapine	29.03	140.32	91.23	1018	(Wanjeri et al., 2023)
Trimethoprim	<MLOD	100.98	35.54	0.6 ± 0.4 – 3.4 ± 0.4	(Borecka et al., 2013)
				3 ± 0.311 – 4 ± 0.290	(Netshithothole & Madikizela, 2024)
				20.4 (estuary), 55.8 (sea)	
				120	(Sousa et al., 2020)
					(Zou et al., 2011)
Venlafaxine	<MLOQ	240.26	41.02	6.00	(Čelić et al., 2021)
				16.70	(Sousa et al., 2020)

Min – Minimum concentration, Max – Maximum concentration, Mean –Average concentration, MLOD – Method Limit of Detection, MLOQ – Method Limit of Quantification (n = 7), D – Detected, All concentration values in ng L^{-1}

5.4 Conclusions

This study aimed to evaluate the occurrence and distribution of pharmaceutical compounds in the urban estuarine waters of Durban Marina using two sampling methods: SPE and passive sampling (Chemcatcher). The primary objective was to assess the effectiveness of both sampling techniques in detecting a range of pharmaceutical contaminants under the dynamic conditions of an estuarine environment.

The findings demonstrated that Chemcatcher outperformed SPE in detecting target pharmaceuticals, particularly for persistent contaminants such as carbamazepine, which was undetected in SPE samples but found in all Chemcatcher samples with concentrations ranging from 133.70 to 396.01 ng L^{-1}. Etilefrine was not detected at any sampling sites by either method. Dexamethasone, methocarbamol, nevirapine, and venlafaxine were consistently detected at all sampling sites with both methods, but concentrations were notably higher in Chemcatcher samples, particularly for dexamethasone (67.60–166.75 ng L^{-1}) and methocarbamol (30.30–82.79 ng L^{-1}).

Key findings indicate that Chemcatcher provided a more comprehensive picture of pharmaceutical contamination in the estuarine water due to its ability to accumulate contaminants over time. In contrast, SPE, which captures contaminants at a specific point in time, showed limitations in detecting pharmaceuticals present at low concentrations or those with variable temporal distributions. Additionally, no clear concentration gradient was observed across the sampling sites, likely due to tidal mixing and water exchange between the sites.

Passive sampling, with its limitations proved to be a more sensitive and reliable method for monitoring pharmaceutical pollutants in estuarine environments. These findings highlight the need for integrating passive sampling techniques into routine water quality assessments, particularly in environments with complex hydrodynamics. Further studies should focus on

long-term monitoring to better understand the ecological risks posed by the presence and potential persistence of pharmaceuticals in urban estuarine ecosystems.

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REFERENCES

Adams, J. *et al.* (2020). Nutrient enrichment as a threat to the ecological resilience and health of South African microtidal estuaries. *Afr. J. Aquat. Sci."*, *45*(1–2), 23–40. doi.org/10.2989/16085914.2019.1677212

Al-Kubaish, A. *et al.* (2024). Study of Total Dissolved Solids (TDS) Concentrations Factor of SWCC Al-Khobar Plant Seawater Intakes. *CWEEE*, *13*(01), 1–12. doi.org/10.4236/cweee.2024.131001

Bäuerlein, P. S. *et al.* (2012). Sorption Behavior of Charged and Neutral Polar Organic Compounds on Solid Phase Extraction Materials: Which Functional Group Governs Sorption? *Environ. Sci. Tech.*, *46*(2), 954–961. doi.org/10.1021/es203404x

Bayen, S. *et al.* (2014). Application of Polar Organic Chemical Integrative Sampler (POCIS) to monitor emerging contaminants in tropical waters. *Sci. Total Environ.*, *482–483*, 15–22. doi.org/10.1016/j.scitotenv.2014.02.082

Biel-Maeso, M. *et al.* (2018). Occurrence, distribution and environmental risk of pharmaceutically active compounds (PhACs) in coastal and ocean waters from the Gulf of Cadiz (SW Spain). *Sci. Total Environ.*, *612*, 649–659. doi.org/10.1016/j.scitotenv.2017.08.279

Blair, B. D. *et al.* (2013). Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern. *Chemosphere*, *93*(9), 2116–2123. doi.org/10.1016/j.chemosphere.2013.07.057

Borecka, M. *et al.* (2013). A new approach for the estimation of expanded uncertainty of results of an analytical method developed for determining antibiotics in seawater using solid-phase extraction disks and liquid chromatography coupled with tandem mass spectrometry technique. *J. Chromatogr. A*, *1304*, 138–146. doi.org/10.1016/j.chroma.2013.07.018

Branchet, P. *et al.*. (2021). Pharmaceuticals in the marine environment: What are the present challenges in their monitoring? *Sci. Total Environ.*, *766*, 142644. doi.org/10.1016/j.scitotenv.2020.142644

Buah-Kwofie, A., & Humphries, M. S. (2017). The distribution of organochlorine pesticides in sediments from iSimangaliso Wetland Park: Ecological risks and implications for conservation in a biodiversity hotspot. *Environ. Pollut.*, *229*, 715–723. doi.org/10.1016/j.envpol.2017.07.031

Cantwell, M. G. *et al.* (2017). Temporal and spatial behavior of pharmaceuticals in Narragansett Bay, Rhode Island, United States. *Environ. Toxicol. Chem.*, *36*(7), 1846–1855. doi.org/10.1002/etc.3710

Cao, B. *et al.* (2020). A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. *N. Engl. J. Med.*, *382*(19), 1787–1799. doi.org/10.1056/NEJMoa2001282

Castle, G. D. *et al.* (2018). Calibration and field evaluation of the Chemcatcher® passive sampler for monitoring metaldehyde in surface water. *Talanta*, *179*, 57–63. doi.org/10.1016/j.talanta.2017.10.053

Čelić, M. *et al.* (2021). Extended suspect screening to identify contaminants of emerging concern in riverine and coastal ecosystems and assessment of environmental risks. *J. Hazard. Mater.*, *404*, 124102. doi.org/10.1016/j.jhazmat.2020.124102

Cid, R. S. *et al.* (2021). Toxicity of Antiretrovirals on the Sea Urchin Echinometra lucunter and Its Predicted Environmental Concentration in Seawater from Santos Bay (Brazilian Coastal Zone). *Resour.*, *10*(11), 114. doi.org/10.3390/resources10110114

del Carmen Gómez-Regalado, M. *et al.* (2023). Accumulation and metabolization of the antidepressant venlafaxine and its main metabolite o-desmethylvenlafaxine in non-target marine organisms Holothuria tubulosa, Anemonia sulcata and Actinia equina. *Mar. Pollut. Bull.*, *192*, 115055. doi.org/10.1016/j.marpolbul.2023.115055

Domínguez-García, P. *et al.* (2025). A POCIS-based approach for the monitoring of pharmaceuticals in wastewater treatment plants: Calibration and deployment challenges. *Environ. Pollut.*, *367*, 125641. doi.org/10.1016/j.envpol.2025.125641

Fernandes, M., & Adams, J. (2016). Quantifying the loss of and changes in estuary habitats in the uMkhomazi and Mvoti estuaries, South Africa. *S. Afr. J. Bot.*, *107*, 179–187. doi.org/10.1016/j.sajb.2016.04.009

Grabicova, K. *et al.* (2017). Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. *Water Res.*, *124*, 654–662. doi.org/10.1016/j.watres.2017.08.018

Griffiths, C. L. *et al.* (2010). Marine Biodiversity in South Africa: An Evaluation of Current States of Knowledge. *PLoS ONE*, *5*(8), e12008. doi.org/10.1371/journal.pone.0012008

Guibal, R., Lissalde, S., & Guibaud, G. (2020). Experimental Estimation of 44 Pharmaceutical Polar Organic Chemical Integrative Sampler Sampling Rates in an Artificial River under Various Flow Conditions. *Environ. Toxicol. Chem.*, *39*(6), 1186–1195. doi.org/10.1002/etc.4717

Gyesi, J. N. *et al.* (2022). Occurrence of Pharmaceutical Residues and Antibiotic-Resistant Bacteria in Water and Sediments from Major Reservoirs (Owabi and Barekese Dams) in Ghana. *J. Chem.*, *2022*, 1–14. doi.org/10.1155/2022/1802204

Hartnett, M. *et al.* (2011). Irish estuaries: Water quality status and monitoring implications under the water framework directive. *Mar. Policy.*, *35*(6), 810–818. doi.org/10.1016/j.marpol.2011.01.010

Huysman, S. *et al.* (2017). Development and validation of an ultra-high performance liquid chromatographic high resolution Q-Orbitrap mass spectrometric method for the simultaneous determination of steroidal endocrine disrupting compounds in aquatic matrices. *Anal. Chim. Acta*, *984*, 140–150. doi.org/10.1016/j.aca.2017.07.001

Ismail, N. A. H. *et al.* (2019). Quantification of multi-classes of endocrine-disrupting compounds in estuarine water. *Environ. Pollut.*, *249*, 1019–1028. doi.org/10.1016/j.envpol.2019.03.089

Jones, L. *et al.* (2015). Emerging priority substances in the aquatic environment: a role for passive sampling in supporting WFD monitoring and compliance. *Anal. Methods.*, *7*(19), 7976–7984. doi.org/10.1039/C5AY01059D

Kamere, N. *et al.* (2022). Scoping Review of National Antimicrobial Stewardship Activities in Eight African Countries and Adaptable Recommendations. *J. Antibiot*, *11*(9), 1149. doi.org/10.3390/antibiotics11091149

Kaserzon, S. L. *et al.* (2014). Characterisation and comparison of the uptake of ionizable and polar pesticides, pharmaceuticals and personal care products by POCIS and Chemcatchers. *Environ. Sci.: Processes Impacts*, *16*(11), 2517–2526. doi.org/10.1039/C4EM00392F

Kim, H., & Homan, M. (2020). Evaluation of pharmaceuticals and personal care products (PPCPs) in drinking water originating from Lake Erie. *J. Great Lakes Res.*, *46*(5), 1321–1330. doi.org/10.1016/j.jglr.2020.06.019

Kingston, J. K. *et al.* 2000). Development of a novel passive sampling system for the time-averaged measurement of a range of organic pollutants in aquatic environments. *J. Environ. Monit.*, *2*(5), 487–495. doi.org/10.1039/b003532g

Kolmonen, M. *et al.* (2007). A general screening method for doping agents in human urine by solid phase extraction and liquid chromatography/time-of-flight mass spectrometry. *Anal. Chim. Acta.*, *585*(1), 94–102. doi.org/10.1016/j.aca.2006.12.028

K'oreje, K. O. *et al.* (2012). From multi-residue screening to target analysis of pharmaceuticals in water: Development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya. *Sci. Total Environ.*,, *437*, 153–164. doi.org/10.1016/j.scitotenv.2012.07.052

Kötke, D. *et al.* (2019). Prioritised pharmaceuticals in German estuaries and coastal waters: Occurrence and environmental risk assessment. *Environ. Pollut.*, *255*, 113161. doi.org/10.1016/j.envpol.2019.113161

Krauss, K. W. *et al.* (2018). The Role of the Upper Tidal Estuary in Wetland Blue Carbon Storage and Flux. *Global Biogeochem. Cycles*, *32*(5), 817–839. doi.org/10.1029/2018GB005897

Li, H. *et al.* (2010). Sampling in the Great Lakes for pharmaceuticals, personal care products, and endocrine-disrupting substances using the passive polar organic chemical integrative sampler. *Environ. Toxicol. Chem.*, *29*(4), 751–762. doi.org/10.1002/etc.104

Li, H. *et al.* (2011). The effects of dissolved organic matter and pH on sampling rates for polar organic chemical integrative samplers (POCIS). *Chemosphere*, *83*(3), 271–280. doi.org/10.1016/j.chemosphere.2010.12.071

Loos, R. *et al.* (2013). Analysis of polar organic contaminants in surface water of the northern Adriatic Sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography–QTRAP® MS using a hybrid triple-quadrupole linear ion trap instrument. *Anal. Bioanal. Chem.*, *405*(18), 5875–5885. doi.org/10.1007/s00216-013-6944-8

MacLeod, S. L. *et al.* (2007). Laboratory calibration and field deployment of the Polar organic chemical integrative sampler for pharmaceuticals and personal care products in wastewater and surface water. *Environ. Toxicol. Chem.*, *26*(12), 2517–2529. doi.org/10.1897/07-238.1

Marion, G. M. *et al.* (2011). pH of seawater. *Mar. Chem.*, *126*(1–4), 89–96. doi.org/10.1016/j.marchem.2011.04.002

Martínez Bueno, M. J. *et al.* (2016). POCIS passive samplers as a monitoring tool for pharmaceutical residues and their transformation products in marine environment. *Environ. Sci. Pollut. Res.*, *23*(6), 5019–5029. doi.org/10.1007/s11356-014-3796-5

Meire, P. *et al.* (2005). The Scheldt estuary: a description of a changing ecosystem. *Hydrobiologia*, *540*(1–3), 1–11. doi.org/10.1007/s10750-005-0896-8

Mohapatra, D. P. *et al.* (2014). Analysis and advanced oxidation treatment of a persistent pharmaceutical compound in wastewater and wastewater sludge-carbamazepine. *Sci. Total Environ.*, *470–471*, 58–75. doi.org/10.1016/j.scitotenv.2013.09.034

Moreno-González, R. *et al.* (2015). Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain). *Environmental Res.*, *138*, 326–344. doi.org/10.1016/j.envres.2015.02.016

Morin, N. *et al.* (2012). Chemical calibration, performance, validation and applications of the polar organic chemical integrative sampler (POCIS) in aquatic environments. *TrAC, Trends Anal. Chem.*, *36*, 144–175. doi.org/10.1016/j.trac.2012.01.007

Mutavdžić Pavlović, D. *et al.* (2010). Development and optimization of the SPE procedure for determination of pharmaceuticals in water samples by HPLC-diode array detection. *J. Sep. Sci.*, *33*(2), 258–267. doi.org/10.1002/jssc.200900571

Naidoo, T., Glassom, D., & Smit, A. J. (2015). Plastic pollution in five urban estuaries of KwaZulu-Natal, South Africa. *Mar. Pollut. Bull.*, *101*(1), 473–480. doi.org/10.1016/j.marpolbul.2015.09.044

Ncube, S. *et al.* (2018). Environmental fate and ecotoxicological effects of antiretrovirals: A current global status and future perspectives. *Water Res.*, *145*, 231–247. doi.org/10.1016/j.watres.2018.08.017

Nel, M. A. *et al.* (2023). Heavy metal compartmentalisation in salt marsh and seagrass of the urbanised Swartkops estuary, South Africa. *Mar. Pollut. Bull.*, *192*, 115007. doi.org/10.1016/j.marpolbul.2023.115007

Netshithothole, R., & Madikizela, L. M. (2024). Occurrence of Selected Pharmaceuticals in the East London Coastline Encompassing Major Rivers, Estuaries, and Seawater in the Eastern Cape Province of South Africa. *ACS meas. sci. au*, *4*(3), 283–293. doi.org/10.1021/acsmeasuresciau.4c00004

Newman, B. K. *et al.* (2024). Occurrence and potential hazard posed by pharmaceutically active compounds in coastal waters in Cape Town, South Africa. *Sci. Total Environ.*, *949*, 174800. doi.org/10.1016/j.scitotenv.2024.174800

Ngubane, N. P. *et al.* (2019). Determination of naproxen, diclofenac and ibuprofen in Umgeni estuary and seawater: A case of northern Durban in KwaZulu–Natal Province of South Africa. *Reg. Stud. Mar. Sci.*, *29*, 100675. doi.org/10.1016/j.rsma.2019.100675

Ohoro, C. R. *et al.* (2021). Occurrence and distribution of pharmaceuticals in surface water and sediment of Buffalo and Sundays River estuaries, South Africa and their ecological risk assessment. *Emerg. Contam., 7*, 187–195. doi.org/10.1016/j.emcon.2021.09.002

Ojemaye, C. Y., & Petrik, L. (2022). Pharmaceuticals and Personal Care Products in the Marine Environment Around False Bay, Cape Town, South Africa: Occurrence and Risk-Assessment Study. *Environ. Toxicol. Chem.*, *41*(3), 614–634. doi.org/10.1002/etc.5053

Olisah, C. *et al.*. (2021). The state of persistent organic pollutants in South African estuaries: A review of environmental exposure and sources. *Ecotoxicol. Environ. Saf.*, *219*, 112316. doi.org/10.1016/j.ecoenv.2021.112316

Olisah, C. *et al.* (2019). Occurrence and risk evaluation of organochlorine contaminants in surface water along the course of Swartkops and Sundays River Estuaries, Eastern Cape Province, South Africa. *Environ. Geochem. Health.*, *41*(6), 2777–2801. doi.org/10.1007/s10653-019-00336-0

Olisah, C. *et al.* (2019). Distribution of organochlorine pesticides in fresh fish carcasses from selected estuaries in Eastern Cape Province, South Africa, and the associated health risk assessment. *Mar. Pollut. Bull.*, *149*, 110605. doi.org/10.1016/j.marpolbul.2019.110605

Oliveira, V. H. *et al.* (2024). Restoration of degraded estuarine and marine ecosystems: A systematic review of rehabilitation methods in Europe. *J. Hazard. Mater.*, *469*, 133863. doi.org/10.1016/j.jhazmat.2024.133863

Omar, T. F. T. *et al.* (2019). Risk assessment of pharmaceutically active compounds (PhACs) in the Klang River estuary, Malaysia. *Environ. Geochem. Health.*, *41*(1), 211–223. doi.org/10.1007/s10653-018-0157-1

Pavlović, D. M. *et al.* (2013). Development and optimization of the determination of pharmaceuticals in water samples by SPE and HPLC with diode-array detection. *J. Sep. Sci.*, *36*(18), 3042–3049. doi.org/10.1002/jssc.201300486

Potter, I. C. *et al.* (1993). The fish fauna of a seasonally closed Australian estuary. Is the prevalence of estuarine-spawning species high? *Mar. Biol.*, *116*(1), 19–30. doi.org/10.1007/BF00350727

Qu, H. *et al.* (2019). Enantiospecific toxicity, distribution and bioaccumulation of chiral antidepressant venlafaxine and its metabolite in loach (Misgurnus anguillicaudatus) co-exposed to microplastic and the drugs. *J. Hazard. Mater.*, *370*, 203–211. doi.org/10.1016/j.jhazmat.2018.04.041

Ranjan, N. *et al.* (2022). Pharmaceuticals in water as emerging pollutants for river health: A critical review under Indian conditions. *Ecotoxicol. Environ. Saf.*, *247*, 114220. doi.org/10.1016/j.ecoenv.2022.114220

Reddering, J., & Rust, I. (1990). Historical changes and sedimentary characteristics of southern African estuaries. *S. Afr. J. Sci.*, *86*(7), 425.

Reig, M. *et al.* (2006). A chromatography method for the screening and confirmatory detection of dexamethasone. *Meat Sci.*, *74*(4), 676–680. doi.org/10.1016/j.meatsci.2006.05.018

Reitman, M. L., & Schadt, E. E. (2007). Pharmacogenetics of metformin response: a step in the path toward personalized medicine. *J. Clin. Investig.*, *117*(5), 1226–1229. doi.org/10.1172/JCI32133

Rilievo, G. *et al.* (2024). From a validated targeted method to a retrospective UHPLC-HRMS non-targeted analysis unveiling COVID-19-related contaminants in clams. Have we bias in marine model organisms for ecotoxicological studies? *Chemosphere*, *364*, 142994. doi.org/10.1016/j.chemosphere.2024.142994

Sousa, J. C. G. *et al.* (2020). Distribution of micropollutants in estuarine and sea water along the Portuguese coast. *Mar. Pollut. Bull.*, *154*, 111120. doi.org/10.1016/j.marpolbul.2020.111120

Tyler, R. H. *et al.* (2017). Electrical conductivity of the global ocean. *Earth Planets Space*, *69*(1), 156. doi.org/10.1186/s40623-017-0739-7

Vidal-Dorsch, D. E. *et al.*. (2012). Contaminants of emerging concern in municipal wastewater effluents and marine receiving water. *Environ. Toxicol. Chem*, *31*(12), 2674–2682. doi.org/10.1002/etc.2004

Vogt, T. *et al.* (2018). PAHs, OCPs and PCBs in sediments from three catchments in Durban, South Africa. *Afr. J. Aquat. Sci.*, *43*(1), 35–49. doi.org/10.2989/16085914.2018.1445616

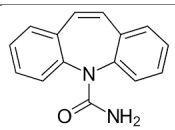
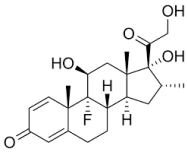
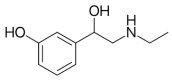
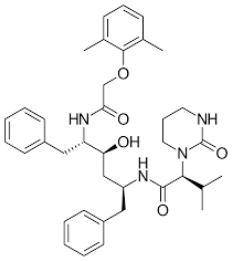
Wanjeri, V. O. W. *et al.* (2023). Occurrence, distribution, and environmental risk of pharmaceutical residues in Mombasa peri-urban creeks, Kenya. *Chemosphere*, *311*, 137144. doi.org/10.1016/j.chemosphere.2022.137144

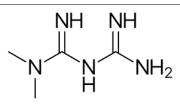
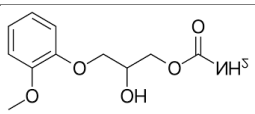
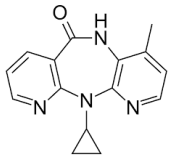
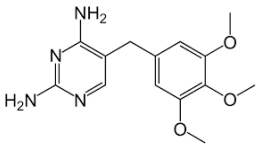
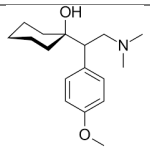
Yahaya, A. *et al.* (2017). Occurrences of Organochlorine Pesticides along the Course of the Buffalo River in the Eastern Cape of South Africa and Its Health Implications. *Int. J. Environ. Res. Public Health*, *14*(11), 1372. doi.org/10.3390/ijerph14111372

Zou, S. *et al.* (2011). Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: Impacts of river discharge and aquaculture activities. *Environ. Pollut.*, *159*(10), 2913–2920. doi.org/10.1016/j.envpol.2011.04.037

Appendix 2

Table 1S.
Properties of target pharmaceutical compounds

Target compound	Therapeutic class	Structure	Empirical formula	CAS no.	$LogK_{ow}$	Molecular weight $g.mol^{-1}$	Solubility in water $mg\ L^{-1}$
Carbamazepine	Anticonvulsant		C15H12N2O	298-46-4	2.3	236.27	238.64
Dexamethasone	Corticosteroid		C22H29FO5	50-02-2	1.83	392.46	89.0
Etilefrine	Adrenergic agonist		C10H15NO2	709-55-7		181.23	18.9
Lopinavir	Anti-retroviral		C37H48N4O5	192725-17-0	5.94	628.814	1.92×10^3

Metformin	Antidiabetic agent		C4H11N5	657-24-9	-1.43	129.164	300.5
Methocarbamol	Muscle relaxant		C11H15NO5	532-03-6	0.61	241.24	29.9
Nevirapine	Non-nucleoside reverse transcriptase inhibitors		C15H14N4O	129618-40-2	3.89	266.21	100
Trimethoprim	Antibiotic		C14H18N4O3	738-70-5	0.91	290.32	0.4
Venlafaxine	Selective serotonin and norepinephrine reuptake inhibitors (SNRIs)		C17H27NO2	93413-69-5	3.20	277.40	572

CHAPTER 6 MANUSCRIPT 3

The transport and distribution of pharmaceutical contaminants in the Crocodile River system, South Africa

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ABSTRACT

Pharmaceutical contaminants in aquatic systems pose environmental risks due to their bioactivity and persistence as a result of their continuous discharge. This study assessed the spatial distribution and transport of nine pharmaceutical compounds in the Crocodile River system, South Africa, using Chemcatcher passive samplers and solid–phase extraction (SPE). Chemcatcher exhibited higher detection frequencies (100% at S2 and S3) than SPE (44% at S5 and 88% at S2 and S3), highlighting its enhanced sensitivity for low concentration contaminants. The highest sum of pharmaceutical concentrations were recorded at S2 (1191.05 ng L^{-1}) and S1 (1028.27 ng L^{-1}), primarily driven by etilefrine (668.8 ng L^{-1}) and methocarbamol (406.61 ng L^{-1}), respectively. S3 and S5 exhibited the lowest concentration sums (447.05 ng L^{-1} and 388.60 ng L^{-1}, respectively), despite S3 having the highest detection frequency. Time-weighted average (TWA) concentrations confirmed methocarbamol as the most persistent compound (68.34 ng L^{-1} at S1), followed by trimethoprim (29.52 ng L^{-1} at S3) and lopinavir (30.13 ng L^{-1} at S1). Contaminant transport was influenced by anthropogenic inputs, dilution, and tributary contributions, particularly from the Gwatlhe, Hex and Elands Rivers, which increased contaminant loads at S7 (518.10 ng L^{-1}). The results highlight the complexity of pharmaceutical dispersion within a river system influenced by multiple tributaries and highlights the need for extensive spatial and temporal monitoring to identify contamination sources and inform mitigation strategies.

Keywords: Pharmaceutical pollution; Crocodile River; Hartbeespoort Dam, Chemcatcher; Passive Sampling; Solid–phase extraction

6.1 Introduction

Active pharmaceutical ingredients (APIs) are increasingly detected as environmental contaminants in surface waters (Löffler and Ternes, 2003; Li, Helm and Metcalfe, 2010; Brodin *et al.*, 2014; Madikizela, Tavengwa and Chimuka, 2017; Rimayi *et al.*, 2018, 2019; Sammut Bartolo, Azzopardi and Serracino-Inglott, 2021). This is primarily due to their widespread use in human and veterinary medicine. The usage of pharmaceuticals as well as the introduction of new pharmaceutical products continues to increase as the world changes. Factors such as climate change have contributed toward changes in disease patterns and subsequently the need for the development of new pharmaceutical products to address the issue (Löffler *et al.*, 2005). These compounds enter aquatic environments from various sources, which include the discharge of treated and untreated wastewater, agricultural runoff, and industrial effluents (Hu and Cheng, 2013; Schoeman, Dlamini and Okonkwo, 2017; Vaudreuil *et al.*, 2022). Once in the aquatic environment, the fate and transport of APIs is influenced by several processes, which determine their persistence, distribution, and potential impacts on aquatic ecosystems (Bavumiragira, Ge and Yin, 2022).

Processes such as transformation, degradation and sorption of these compounds govern their fate in the environment. Transformation can occur as APIs are metabolised into their respective metabolites or through chemical reactions between the functional groups they bear with reactive species present in the matrices they end up in. Degradation can occur via biodegradation, photodegradation, and hydrolysis (Waterman *et al.*, 2002; Joss *et al.*, 2006; Kawabata *et al.*, 2013). However, many pharmaceuticals are designed to be biologically stable, leading to limited degradation in the environment (Yoshioka and Aso, 2007). Sorption onto sediments and suspended particulate matter also plays a significant role in determining the mobility and bioavailability of pharmaceuticals in aquatic ecosystems, potentially facilitating

their accumulation in benthic habitats or acting as a temporary sink before remobilisation (Löffler *et al.*, 2005; Koba *et al.*, 2018).

The transport of pharmaceuticals in river systems is controlled by hydrological factors such as flow rate, dilution, and mixing, as well as the physical and chemical properties of the pharmaceuticals, including solubility, molecular weight, and affinity for organic matter (García-Santiago *et al.*, 2017). These factors influence whether pharmaceuticals remain in the water column, adhere to particulates, or undergo transformation into metabolites or degradation products (Zuehlke, Duennbier and Heberer, 2007; Benotti and Brownawell, 2009; Lahti and Oikari, 2011; Zhang *et al.*, 2017).

Understanding the fate and transport of pharmaceuticals in aquatic ecosystems is essential for assessing their potential ecological risks. This is because pharmaceuticals have been shown to affect aquatic organisms, even at low concentrations, potentially leading to disruptions in reproductive, developmental, and behavioural processes (Watanabe *et al.*, 2016; Grabicova *et al.*, 2017; Ncube *et al.*, 2018; Rilievo *et al.*, 2024). As a result, addressing the presence of pharmaceuticals in the environment is critical for effective water quality management and the protection of aquatic ecosystems. There remains significant uncertainty regarding whether the loss of pharmaceuticals from surface waters is driven by temporal or spatial processes, and whether degradation or sorption is the dominant pathway for their removal (Löffler *et al.*, 2005; Sui *et al.*, 2015; Archer *et al.*, 2017; Bavumiragira, Ge and Yin, 2022).

This study aims to build upon previous research that reported the targeted quantification of five pharmaceutical compounds and the detection of 61 pharmaceuticals and 25 pesticides in the Hennops River and its catchment area (Hartbeespoort Dam) (Mapetla *et al.*, no date). The current research aims to investigate the transport and distribution of pharmaceutical contaminants in an urban river systems of South Africa, specifically focusing on their presence

in the Crocodile River after it exits Hartbeespoort Dam and flows through the Northwest Province towards Limpopo Province.

6.2 Materials and Methods

6.2.1 Reagents and chemicals

Analytical grade nitric acid, HPLC Grades acetonitrile, methanol and formic acid were purchased from Merck (Johannesburg, South Africa). Analytical grades lopinavir, metformin, trimethoprim, venlafaxine hydrochloride, methocarbamol, etilefrine hydrochloride, nevirapine, and carbamazepine and dexamethasone were from Merck (Johannesburg, South Africa). AttractSPE^{TM} hydrophilic-lipophilic balance (HLB) disks (90 mm) and EXPRESS PLUS polyether sulphone (PES) membranes (0.45 µm, diameter 90 mm) were purchased from Anatech Pty Ltd (Johannesburg, South Africa). The SPE setup consisted of Supel-Select HLB cartridges with 500 mg sorbent material purchased from Merck (Johannesburg, South Africa). Ultrapure water was produced inhouse using the Millipore DirectQ 3 UV water purification system from Millipore (Massachsetts, USA). All reagents and chemicals were used as received unless specified. A 1000 mg L^{-1} stock solution mixture of the nine pharmaceutical standards was prepared by dissolving 10 mg each of the standards in 10 mL of methanol. This was kept refrigerated at 4 ºC and renewed every week. The nine calibration solutions were prepared in ultrapure water/methanol, 90:10, v/v with concentrations ranging from 0.1 to 500 µg L^{-1}. The physical-chemical properties of the analytes of interest are shown in (Table S1).

6.2.2. Instrumentation

pH, conductivity and total dissolved solids (TDS) of the grab water samples were determined in the laboratory using a calibrated YSI multi-parameter meter (Model 556, YSI Inc., Yellow Springs, OH, USA). The bacteriological assessments for this study were carried

out at the Agricultural Research Council (ARC), Centurion, South Africa. The grab water samples were assessed for total aerobic count, coliform count and *E.coli* detection and count. The SPE vacuum manifold was from Pall Corporation (Fribourg, Switzerland). High resolution LC-MS analysis was performed using a Thermo Scientific Vanquish UHPLC^{+} Q Exactive Focus Orbitrap (Anatech, Johannesburg, South Africa).

6.2.3. Study area

The Crocodile River is one of the major rivers in South Africa, with a course that spans several provinces. The course of the river can be divided into 3 parts which are the upper, middle and lower course. The upper course makes up where the river's course begins in the Witwatersrand, Gauteng Province. As it flows through this area, it receives runoff from mostly urban areas. The river's flow continues until it enters the Hartbeespoort Dam, Northwest Province. Two major tributaries of the Crocodile River before it enters Hartbeespoort Dam are Hennops and Jukskei Rivers. These rivers have been reported to be heavily polluted (Schoeman, 1979; 'Integrated water quality management: getting people involved in the Jukskei River', 1995; Rimayi *et al.*, 2019; Hoorzook *et al.*, 2021). The upper course was the focus of the study this work aims to build on (Mapetla *et al.*, no date).

The middle course begins as the river exits the dam. From the dam, the Crocodile River flows through the Northwest Province, receiving additional water from tributaries like the Magalies, Hex, Elands and Gwatlhe Rivers. In the middle course, the river passes through agricultural, suburban and rural areas. The present study focuses on the middle course of the river (Fig. 1.).

Eventually, the river enters the Limpopo Province, where it becomes a tributary of the larger Limpopo River. Along its lower course, the Crocodile River forms part of the Limpopo

River Basin, which serves as an important water resource for both South Africa and neighbouring countries like Botswana and Zimbabwe.

The specific sampling site locations are shown in Fig. 1. S1 is situated along the riverbank of Mount Amanzi Resort, where the Crocodile River exits Hartbeespoort Dam. S2 is located along a canalized stream of the Crocodile River that flows through Crocodile River Mine. S3 is positioned downstream, along the Crocodile River after it passes through the town of Brits. Further downstream, S4 is located at the entrance of Rooikoppies Dam, where the Gwatlhe River also enters before both rivers merge and exit the dam. S5 is situated on the Elands River at the outflow of Vaalkop Dam, which is fed by the Elands and Hex Rivers. The Elands River is one of the major tributaries of the Crocodile River, and Vaalkop Dam is located near the Magalies Water – Vaalkop Water Treatment Plant. S6 represents a canalized section of the Elands River, primarily used for irrigation. Finally, S7 is located along the Crocodile River after it exits Rooikoppies Dam but before its confluence with the Elands River at S5

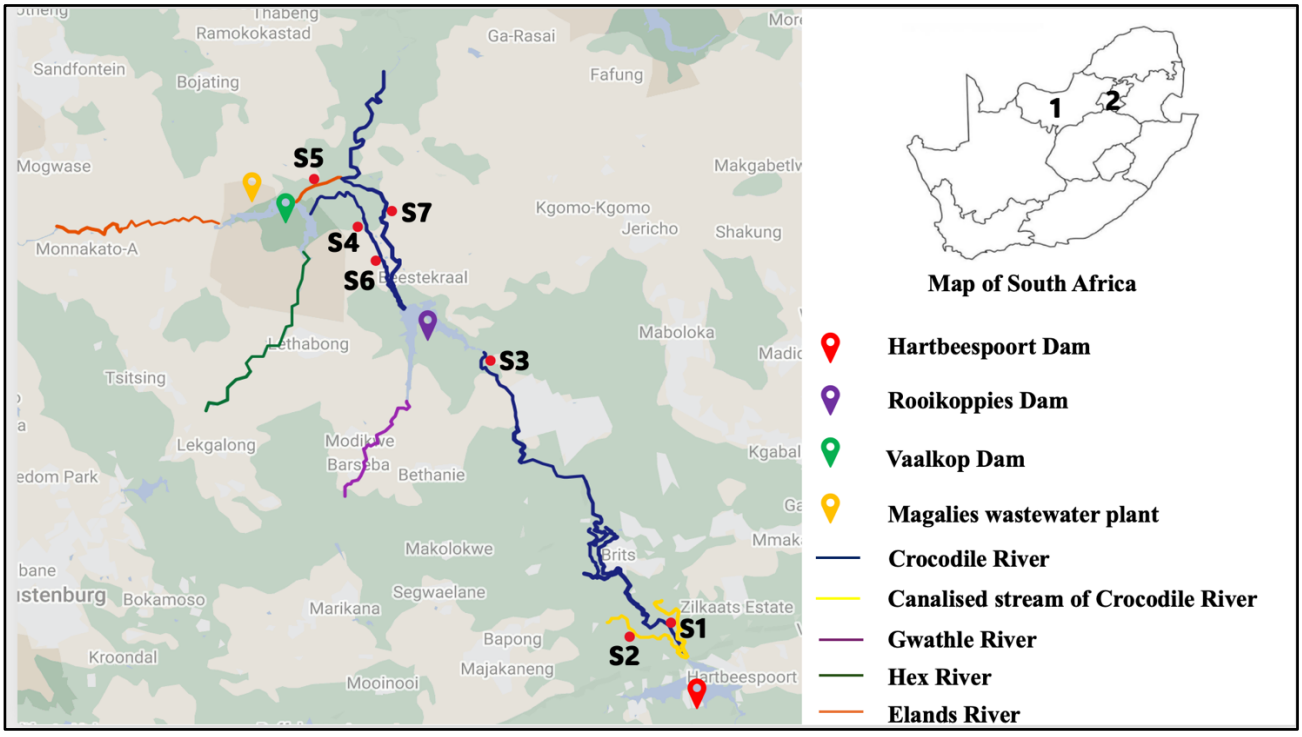

Fig. 1.
Google my maps map showing the locations of the seven sampling sites, (1) Northwest Province, (2) Gauteng Province

6.2.4 Assembly and extraction of Chemcatcher sampler

Three component polytetrafluoroethylene (PTFE) Chemcatcher bodies (external diameter 6.6 cm, internal depth 0.8 cm and internal diameter 5.4 cm) were manufactured by the University of the Witwatersrand Physics workshop (Johannesburg, South Africa). This was similar to a design reported by (Castle *et al.*, 2018). The sampler holders were soaked in 10% nitric acid solution and rinsed with ultrapure water, which was followed by acetone before use. HLB receiving phase disks, and PES membranes were cut to the internal diameter of the sample holder and assembled. These were assembled by exposing the rough side of the PES membrane on the sample holder's support plate. This was followed by placing the HLB disks, after which the sample holder lid was placed in such a manner that the HLB disks was sandwiched between the polyether sulfone disk membranes and sample holder lid. The assembled passive sampling devices were soaked in MeOH overnight to activate the HLB disks, after which they were soaked in ultrapure water until use to prevent drying.

Sampling rates obtained from a previous Chemcatcher passive sampler calibration experiment were used to calculate TWA concentrations. The Chemcatcher passive sampler calibration experiments were conducted in ultrapure water using the static calibration method as described in our previous report (Mapetla *et al.*, no date). Briefly, ten Chemcatcher samplers were suspended vertically in a 5L glass tank spiked to 0.4 µg L^{-1} each of the target compounds. The experiment was conducted at a stirring rate of 100 rpm, at room temperature over 14 days. Two Chemcatcher samplers were retrieved after two, five, eight, eleven and sixteen days. Upon retrieval, the passive samplers were wrapped in aluminium foil and stored at – 4 °C until ready for extraction and analysis.

The Chemcatcher samplers were extracted using a modified procedure based on literature (Glanzmann *et al.*, 2023). Briefly, passive samplers were rinsed with ultrapure water to remove debris. Following disassembly, the HLB sorbent was transferred into a clean 50 mL HDPE

centrifuge tube and allowed to air dry for five hours. Extraction was performed by adding 40 mL of methanol to the tube, followed by vortexing for five minutes. The sorbent material was then removed, and the solvent was filtered through a 0.22 µm syringe filter into a 50 mL HDPE centrifuge tube. The filtrate volume was reduced to 1 mL under a stream of nitrogen gas, after which the extracts were transferred to glass vials and stored at –20 °C until analysis. Sampling rates were determined based on linear uptake kinetics, as described by (Vrana *et al.*, 2005).

6.2.5 Field deployment of Chemcatcher samplers

Field deployed Chemcatcher samplers were housed in PVC cages (25 mm × 15 cm) with 2 cm diameter perforations on the sides to allow water flow. The samplers were secured within the cages using cable ties, while a ski rope was used to anchor the cages at the deployment sites. The cages were fully submerged at a depth of approximately 30 cm throughout the 14-day deployment period during the spring season (October 2024). Three Chemcatcher samplers were deployed per site. Upon retrieval, the Chemcatcher samplers were detached from the cages, gently rinsed with ultrapure water, and sealed with Chemcatcher lids. They were then transported to the laboratory in a cooler and stored at –20 °C until extraction.

6.2.6 Grab sampling and SPE procedures

On the day of Chemcatcher sampler deployment, 1000 mL grab water samples were collected at each site. Prior to collection, PTFE bottles were rinsed three times with river water to minimize contamination. The bottles were then filled to the neck, securely sealed, and placed in a cooler for transportation to the laboratory to preserve sample integrity. Additional water samples were collected for bacteriological analysis at the Agricultural Research Council (ARC) in Irene, Centurion, South Africa.

For SPE, 1000 mL grab water samples were filtered through 0.45 µm filter paper using a Büchner funnel, and the filtrate was collected in clean glass bottles. The SPE procedure was

adapted from (Mostafa *et al.*, 2023). HLB cartridges were mounted on an SPE manifold connected to a vacuum pump. Pre-conditioning was performed by passing 2 × 6 mL methanol at a rate of one drop per second, followed by 6 mL ultrapure water. A 200 mL aliquot of the filtered sample was then passed through the HLB cartridges at the same flow rate. After percolation, the cartridges were vacuum-dried, and the retained analytes were eluted with 6 mL of HPLC-grade methanol. Extracts were collected in clean 15 mL HDPE centrifuge tubes, and the solvent volume was reduced to 1 mL under a gentle stream of nitrogen gas. The final extracts were transferred to glass vials for analysis.

For the analyte recovery study, 200 mL of ultrapure and river water samples were spiked with a pharmaceutical standard mixture to achieve a concentration of 10 µg L^{-1}. The same extraction protocol was applied, using 6 mL of HPLC-grade methanol for elution. Each extraction, including both grab water samples and recovery study samples, was performed in triplicate to assess reproducibility.

6.2.7 UHPLC-Q Orbitrap MS analysis for targeted analysis

Analysis was performed using a Thermo Scientific Vanquish UHPLC+ Q Exactive Focus Orbitrap system (Anatech, Cape Town, South Africa) equipped with a Phenomenex Luna Omega 1.6 µm C18 (100 Å, 100 × 2.1 mm) column (Anatech, Johannesburg, South Africa). Instrument data acquisition was conducted using Thermo Scientific Chromeleon™ Chromatography Data System (CDS) software.

The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), both acidified with 0.1% formic acid. A constant flow rate of 0.400 mL min^{-1} was maintained throughout the 15-minute run. The initial equilibration was set at 5% solvent B for 1 minute, followed by a gradient increase to 55% over 5 minutes (curve 3). This composition was held for 0.5 minutes before increasing to 65% over 1.5 minutes (curve 3). Solvent B was then

ramped to 100% over 3 minutes (curve 3), maintained for 3 minutes, and subsequently reduced to 5% over 3 minutes. A final equilibration at 5% solvent B was maintained for 2 minutes. The sample injection volume was 5 µL.

Tandem mass spectrometry (MS/MS) was performed using a heated electrospray ionization (HESI) source in positive mode. The optimized MS parameters included a sheath gas flow rate of 40 aU, an auxiliary gas flow rate of 10 aU, a spray voltage of 3.20 kV, an S-lens RF level of 55.0, a capillary temperature of 300 °C, and an auxiliary gas heater temperature of 400 °C. Full scan acquisition was conducted over the mass range of 120–1000 m/z at a resolution of 70,000. Data-dependent acquisition (dd–MS^2) was performed in positive mode at a resolution of 35,000, with an AGC target of 1×10^6. The optimized MS parameters are summarized in Table 1.

6.2.8 Quality assurance

A field blank was prepared for each deployment to monitor potential contamination during sampling. The field blank consisted of a Chemcatcher sampler exposed to ambient air during the deployment process. This was done while assembling the Chemcatcher samplers into cages at each field site. The field blank was opened at the beginning of deployment and remained open until the deployment process was complete. Afterward, it was sealed with a Chemcatcher lid and transported to the laboratory in a cooler box for storage at –20°C until extraction.

To assess contamination during sample extraction, procedural blanks were processed alongside Chemcatcher and SPE samples from the field. Procedural blanks were prepared for both Chemcatcher samplers and HLB cartridges used in SPE. For Chemcatcher procedural blanks, a sampler was assembled and subsequently disassembled. The PES membrane and HLB sorbent were transferred to 50 mL HDPE centrifuge tubes, followed by the addition of 40 mL methanol. The tubes were vortexed for 5 min, after which the PES membrane and HLB

sorbent were removed. The extract was then filtered through a 0.22 µm syringe filter into clean 50 mL HDPE centrifuge tube. The filtrate volume was reduced to 1 mL under a gentle stream of nitrogen gas before being transferred to glass vials and stored at –20°C until analysis.

For SPE procedural blanks, 200 mL of ultrapure water was collected in clean PTFE bottles and filtered through a 0.45 µm membrane using a Büchner funnel. The filtrate was collected in clean 250 mL conical flasks and then passed through pre-conditioned Oasis HLB cartridges mounted on an SPE manifold connected to a vacuum pump at a flow rate of 1 drop per second. The HLB cartridges were pre-conditioned by sequentially passing 6 mL methanol and 6 mL ultrapure water. After percolation, the cartridges were vacuum dried, and analytes were eluted using 6 mL HPLC-grade methanol. The eluates were collected in 15 mL HDPE centrifuge tubes, concentrated to precisely 1 mL under a gentle stream of nitrogen gas, and then transferred to glass vials for storage at –20°C until analysis.

To ensure instrument performance and analytical precision, quality control (QC) checks were included for every five samples analysed. These QC checks consisted of a standard mixture of nine pharmaceutical compounds prepared in methanol at a concentration of 10 µg L^{-1}. This procedure was implemented to account for potential sensitivity deviations of the instrument during the analytical run. Additionally, for each triplicate sample analysed, a solvent blank was included to prevent analyte carryover. The solvent blank consisted of pure methanol and was injected between sample runs to ensure no residual carryover between analyses.

SPE % recoveries were determined to assess the accuracy of the method and to quantify how efficiently the target analytes were extracted from the matrix. Potential interferences from the sample matrix were evaluated to ensure that the presence of co-extracted substances did not impact the analytical results.

Samples were injected in triplicates, and the relative standard deviation (RSD) was calculated to check the precision and reproducibility of the method.

Calibration curve regression coefficients (R^2 values) were used to assess the linearity of the method over the concentration range tested, ensuring accurate quantification of the analytes. Sampling rates used to calculate TWA concentrations for carbamazepine, dexamethasone, methocarbamol, nevirapine and venlafaxine were obtained from the work done in Chapter 4.

6.3. Results and Discussion

6.3.1 Quality assurance

In the field and procedural blanks for both Chemcatcher and SPE, the analytes of interest were not detected. This suggests that contamination between samples did not occur in the field nor during sample preparation and extraction in the laboratory. Representative chromatograms and calibration curves are shown in Appendix 4 (Figures 1–14). The analytical method's calibration regression coefficients, LOD, LOQ and SPE % recoveries are presented in Table 1. The analytical method demonstrated excellent sensitivity and precision, as indicated by the acceptable regression coefficients ($R^2 > 0.97$) for all pharmaceutical compounds analysed using UHPLC-Q Orbitrap MS. The method LOD's ranged from 0.15 to 2.03 ng L^{-1}, while the LOQ's were between 0.49 and 6.75 ng L^{-1}, ensuring the accurate detection of trace level pharmaceuticals in the environmental samples analysed.

The efficiency of the SPE process was evaluated through percentage recoveries obtained for river and ultrapure water samples. The recoveries ranged from 64.96% (etilefrine) to 92.64% (trimethoprim), demonstrating acceptable performance for most compounds. However, etilefrine exhibited lower recovery values, which may be attributed to adsorption losses or incomplete elution from the SPE cartridges. The RSD values remained below 5% for all analytes, indicating good reproducibility of the extraction method.

Table 1.
UHPLC-Q Orbitrap MS calibration regression coefficients, MLOD, MLOQ and SPE % recoveries ± %RSD

Compound	R^2	MLOD (ng L^{-1})	MLOQ (ng L^{-1})	Water type	SPE recovery	
					% recovery $\pm RSD$	% matrix effect
Carbamazepine	0.9884	0.21	0.75	River	86.38 ±3.21	–4.73
				Ultrapure	82.29 ±2.87	–
Dexamethasone	0.9988	1.43	4.75	River	80.26 ±2.50	1.81
				Ultrapure	81.71 ±2.87	–
Etilefrine	0.9921	1.83	6.10	River	64.96 ±1.93	–4.46
				Ultrapure	62.06 ±3.48	–
Lopinavir	0.9843	0.48	1.60	River	85.08 ±2.46	2.94
				Ultrapure	87.58 ±1.96	–
Metformin	0.9851	_	_	River	_	_
				Ultrapure	_	_
Methocarbamol	0.9952	2.03	6.75	River	87.96 ±4.60	–5.73
				Ultrapure	83.19 ±2.76	–
Nevirapine	0.9704	0.38	1.28	River	88.62 ±2.49	–4.22
				Ultrapure	85.03 ±2.72	–
Trimethoprim	0.9931	0.34	1.16	River	92.64 +2.53	–2.23
				Ultrapure	90.63 ±1.82	–
Venlafaxine	0.9884	0.15	0.49	River	77.94 ±2.65	3.20
				Ultrapure	80.52 ±2.83	–

R^2– calibration curve regression coefficient, MLOD– method limit of detection, MLOQ– method limit of quantification, RSD– relative standard deviation

Matrix effects were assessed by comparing signal responses in river water to those in ultrapure water. The results revealed both signal enhancement and suppression, with matrix

effect values ranging from –5.73% for methocarbamol to +3.20% for venlafaxine. The negative matrix effects observed for carbamazepine, etilefrine, methocarbamol, nevirapine and trimethoprim suggest ion suppression likely due to the presence of co-eluting matrix components in the river water. Conversely, lopinavir, venlafaxine and dexamethasone exhibited slight ion enhancement, which could be attributed to competitive ionization effects in the river water. The matrix effects were then used to correct for the concentrations of target analytes in the sampling medium.

6.3.2 Physical–chemical parameters and bacteriological analysis of water samples

The physical-chemical and microbiological analysis of water samples from the sampling sites provides critical insight into water quality and potential health risks. The physiochemical and bacteriological data from the seven sites are summarized in Table 2, where they are compared to drinking and surface water standards set by South Africa (RSA) and the world health organization (WHO) (Forestry, 1996; World health organization, 2016). The pH values ranged from 7.319 for S3 to 7.992 for S4, falling within the acceptable limits set by South Africa and WHO drinking and surface water standards (5–9.7 and 6.5–9.5, respectively) (Forestry, 1996; World health organization, 2016). This indicates that the water from the sites is neutral, which is crucial for maintaining ecosystem balance.

Conductivity values ranged 188.5 (S3) and 381.4 (S4) µS cm^{-1}, with a mean of 270.7 µS cm^{-1}. Although RSA does not specify a surface water standard for conductivity, all values were below the WHO drinking water guideline of 400 µS cm^{-1}, suggesting a relatively low dissolved ion content. TDS concentrations ranged from 96.65 (S3) to 193.4 (S4) ppt, with a mean value of 148.2 ppt. These values are well within both the RSA and WHO drinking water standards, ≤1200 ppm and ≤300 ppm, respectively (Forestry, 1996; World health organization, 2016). This implies that the water has a relatively low level of dissolved inorganic and organic matter.

Table 2.
Physicochemical parameters and bacteriological analysis of water samples from the sampling sites

Sample ID	pH	Conductivity ($\mu S.cm^{-1}$)	TDS (ppt)	Total aerobic count ($CFU\ mL^{-1}$)	Coliform count ($CFU\ mL^{-1}$)	*E.coli* count ($CFU\ mL^{-1}$)
S1	7.638	315.1	155.2	10150	101	0
S2	7.849	381.4	193.4	12967	1737	3
S3	7.368	188.5	96.65	15267	2200	0
S4	7.992	261.3	184.7	88000	2450	0
S5	7.319	308.4	158.0	87000	435	0
S6	7.986	193.7	97.32	7433	1223	0
S7	7.539	246.3	151.8	5700	950	0
Mean	7.670	270.7	148.2	32359.57	1299.43	0.43
Minimum	7.319	188.5	96.65	5700	101	0
Maximum	7.992	261.3	184.7	88000	2450	3
RSA drinking water standard	5 – 9.7	≤170	≤1200 (ppm)	ND	≤10	ND
RSA surface water standard	4 – 11	_	_	_	_	_
WHO drinking water standard	6.5 – 8.5	≤ 400	≤300 (ppm)	ND	<1	ND
WHO surface water standard:	6.5 – 9.5	_	≤600 (ppm)	_	_	235

ND– not detected, CFU– colony forming units, ppt– parts per trillion, mL– millilitre

Microbiological contamination varied significantly across the sites. The total aerobic count ranged from 5700 (S7) to 88000 (S4) CFU mL^{-1}, with a mean of 32359.57 CFU mL^{-1}. This high bacterial count indicates potential organic contamination, likely due to agricultural runoff and wastewater discharge. Coliform counts, which serve as an indicator of fecal

contamination, were detected at all sites, with values ranging from 101 (S1) to 2450 (S4) CFU mL^{-1}. This suggests contamination from poor functioning sewage systems and wastewater discharge. S5, which is located near the Magalies water treatment plant was anticipated to show the highest coliform count, however, water from this site had the second lowest coliform count (435).

The presence of *E. coli* was observed in only S2 (3 CFU mL^{-1}), exceeding the permissible limits for drinking water according to both RSA and WHO standards (ND) (Forestry, 1996; World health organization, 2016). The detection of *E. coli* suggests fecal contamination and a possible risk of waterborne diseases. The variability in microbial contamination across sites suggests differing levels of pollution, which is linked to land use patterns and proximity to contamination sources. Sites with elevated coliform and *E. coli* counts may be influenced by wastewater discharge, surface runoff, or poor sanitation infrastructure. Although some sites met microbiological drinking water standards, the presence of coliforms in all samples and *E. coli* in one site is concerning.

6.3.3 Analysis of Chemcatcher and SPE extracts from the Crocodile River system

6.3.3.1 Detection and distribution of pharmaceutical contaminants in the Crocodile River system

Fig. 2. presents the detection frequency of pharmaceutical compounds across the seven sampling sites, and the individual pharmaceutical concentrations in Chemcatcher and SPE extracts are listed in Table 2S. Chemcatcher extracts from S4 and S6 were not analysed; therefore, a direct comparison between Chemcatcher and SPE for these sites was not possible.

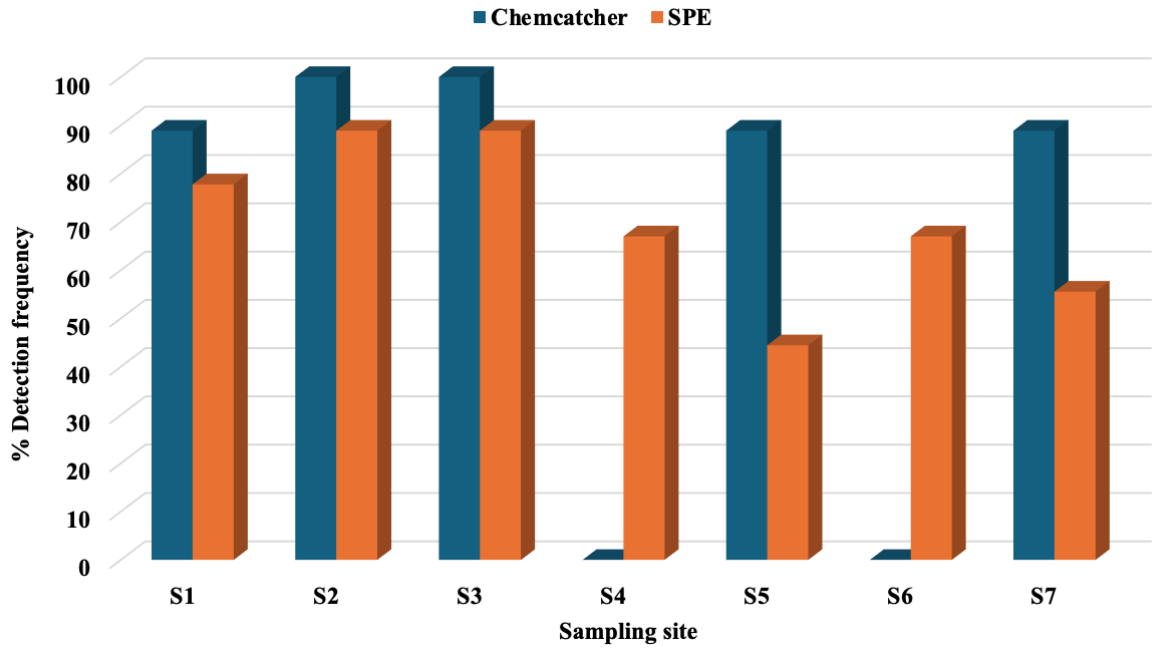

Fig. 2.
Detection frequency of pharmaceutical compounds from Chemcatcher and SPE extracts.

Note: Chemcatcher samples from S4 and S6 were not analysed

Chemcatcher consistently demonstrated a higher detection frequency, reaching 100% at S2 and S3, whereas SPE exhibited greater variability, with detection frequencies ranging from 44.44% (S5) to 88.89% (S2 & S3). The highest mean detection frequencies were observed for S2 and S3 (94%), indicating these sites as potential contamination hotspots. This may be attributed to runoff and their proximity to human activities, which likely contribute to the elevated presence of pharmaceutical compounds. In contrast, S5 and S7 exhibited the lowest mean detection frequencies (67% and 72%, respectively). The lower detection in SPE samples (44% at S5 and 56% at S7) suggests that contaminants are present but at concentrations below instrumental detection limits. This interpretation aligns with the higher detection frequencies observed in Chemcatcher (88% at both sites), which likely captures low level contaminants more effectively.

For S4 and S6, the detection frequencies were both 67%, which is higher than those observed for S5 and S7 but lower than those at S1, S2, and S3. This suggests that S4 and S6

represent moderate contamination hotspots, with pharmaceutical concentrations falling between those of the highly contaminated sites (S2, S3) and the lower contamination sites (S5, S7). Overall, the data indicates that S2 and S3 are the most contaminated sites, while S5 and S7 exhibit lower contamination levels, potentially due to dilution, degradation, or reduced pharmaceutical input. The discrepancies between Chemcatcher and SPE further highlight the importance of passive sampling in detecting low concentration contaminants that may go undetected with SPE.

In Chemcatcher extracts from S1, eight out of the nine target compounds were detected, with the exception of the anti-diabetic drug metformin. However, metformin was detected in S2 and S3. S2 is a canalized stream of the Crocodile River, while S3 is located along the Crocodile River after it passes through the town of Brits. Potential sources of metformin include human activities form the Crocodile River Mine near S2 and domestic wastewater from Brits. This compound was also detected in extracts from S5, a site located along the Elands River after it flows through Vaalkop Dam. The Elands River, a tributary of the Crocodile River, traverses several townships before reaching the dam, which may contribute to metformin contamination. In contrast, metformin was not detected in Chemcatcher extracts from S7, likely due to dilution in Rooikoppies Dam before the Crocodile River reaches this site.

Methocarbamol, nevirapine, and carbamazepine were detected in all Chemcatcher and SPE extracts. The highest concentrations were observed at S1, with levels decreasing progressively at S2, S3, S4, and S5, where they were at their lowest. This trend suggests dilution as the river flows downstream from Hartbeespoort Dam and limited additional inputs from farming, mining, and domestic activities before reaching S7. However, in Chemcatcher extracts from S7, the concentrations of these compounds were elevated to levels similar to those in S2. This increase may be attributed to tributaries such as the Gwatlhe River, which flows through the village of Modikwe before entering Rooikoppies Dam.

Etilefrine, trimethoprim, venlafaxine, and dexamethasone were detected in all Chemcatcher extracts but not in all SPE extracts. Venlafaxine and trimethoprim followed a pattern where concentrations were highest at S1, decreasing progressively downstream to S7. However, etilefrine exhibited peak concentrations in S2, while trimethoprim peaked in S3. These variations suggest that mining and farming activities near S2 and domestic wastewater inputs from Brits before S3 may be sources of these contaminants.

An exception to the expected correlation between passive sampling and SPE grab sampling was observed for lopinavir. While lopinavir was detected in all SPE extracts, it was not found in all Chemcatcher extracts, specifically at S5. This suggests an episodic release of lopinavir at S5, which was captured by grab sampling but missed by passive sampling.

6.3.3.2 Pharmaceutical contaminant concentrations and distribution across sampling sites: Chemcatcher vs. SPE extracts

Fig. 3. shows the concentrations of pharmaceutical compounds in Chemcatcher and SPE extracts across the seven sites. The individual pharmaceutical concentrations in Chemcatcher and SPE extracts are listed in Table 2S. Due to the unavailability of Chemcatcher extracts for S4 and S6, direct comparisons for these sites were not possible.

Among the analysed sites, S2 exhibited the highest pharmaceutical concentration sum (1191.05 ng L^{-1}), which aligns with its high detection frequency. The primary contributors were etilefrine (668.8 ng L^{-1}) and methocarbamol (200.77 ng L^{-1}) in Chemcatcher extracts. S1 followed with a total concentration of 1028.27 ng L^{-1}, 14.68% lower than S2, with methocarbamol (406.61 ng L^{-1}) and etilefrine (141.71 ng L^{-1}) being the dominant compounds. S7 ranked third in total pharmaceutical concentration (518.10 ng L^{-1}), 66% and 79% lower than S1 and S2, respectively. The major contributors were methocarbamol (190.05 ng L^{-1}) and etilefrine (101.36 ng L^{-1}). Notably, S7 exhibited the fourth-highest mean detection frequency

(72%) but the third-highest total concentration, suggesting that while fewer compounds were detected at this site, those present were in relatively high concentrations. The lowest total concentrations were recorded at S3 (447.05 ng L^{-1}) and S5 (388.60 ng L^{-1}). In S3, methocarbamol, etilefrine, and trimethoprim were the predominant compounds, whereas in S5, methocarbamol and etilefrine were the primary contributors. S3 deviated from the detection frequency trend, as it exhibited the highest detection frequency but the second-lowest total concentration. Conversely, S5 showed consistency, with both the lowest detection frequency and the lowest total concentration, indicating minimal pharmaceutical contamination at this site.

As anticipated, SPE concentrations were lower than those obtained with Chemcatchers, ranging from ND to 24.93 ng L^{-1} (carbamazepine, S1). Overall, SPE concentrations were low, with the highest total pharmaceutical concentrations observed at S1 (90.97 ng L^{-1}), followed by S2 (76.33 ng L^{-1}) and S3 (67.23 ng L^{-1}). This trend contradicts the detection frequency results, where S2 and S3 exhibited the highest pharmaceutical detection frequencies (100%), while S1 followed with 88%. Additionally, the SPE concentration sum pattern does not align with the Chemcatcher trend (S2 > S1 > S7). The elevated SPE concentration sum at S1 may be attributed to the Hartbeespoort Dam, which receives inflow from two of the most polluted rivers in Gauteng province.

Etilefrine concentrations ranged from ND to 1.87 ng L^{-1}, lower than those reported in Orlando Dam, South Africa (8.17–12.88 ng L^{-1}) using a molecularly imprinted polymer-based passive sampler (Khulu *et al.*, 2022) Metformin was not detected at any site. Trimethoprim was found at three sites (S1, S2, and S3) at concentrations ranging from 2.55 to 10.43 ng L^{-1}, lower than those reported in the Jiulong River, China (up to 55 ng L^{-1}), and the Leça River, Portugal (up to 110 ng L^{-1}) (Hong *et al.*, 2021) (Fernandes *et al.*, 2020). Venlafaxine was detected in samples from S1, S2, S3 and S4 in concentration ranges from 2.06 to 5.86 ng L^{-1}. Which are

lower than those reported (641 ng L^{-1}) in Leça River, Portugal, and Porto (22.1 to 70.4 ng L^{-1}) but similar to those reported in Paarl and West Rivers (3.17 and 1.21 ng L^{-1}) (Fernandes *et al.*, 2020; Paíga and Delerue-Matos, 2024). Carbamazepine was detected at all sites in concentration ranges 7.16 to 24.93 ng L^{-1}. These are similar to those reported in Paarl River (up to 17.11 ng L^{-1}) and Porto (Fernandes *et al.*, 2020; Paíga and Delerue-Matos, 2024)(11.4 to 150 ng L^{-1}), but lower than those reported in Leça River, Portugal (up to 354 ng L^{-1}).

Methocarbamol, Nevirapine and lopinavir were detected in SPE samples from all sites. Methocarbamol concentrations ranged from 2.81 to 21.68 ng L^{-1}. These are lower than those reported in Orlando Dam, south Africa (16.99 – 72.99 ng L^{-1}). Nevirapine concentrations ranged from 2.55 (S7) to 22.57 (S1) ng L^{-1}. These are lower than those reported in Umbilo, Umhlathuzane, and Amazimtoti Rivers, South Africa (28.5 – 52.3 ng L^{-1}) as well as those reported for rivers in Gauteng Province (0.08 – 0.5 µg L^{-1}) (Horn *et al.*, 2020; Ngwenya and Mahlambi, 2023). Lopinavir was detected in concentrations ranging from 1.56 – 4.34 ng L^{-1}. These are lower than those reported in Olifantsfontein, Sunderland, Vlakplaats and Welgedacht, Gauteng South Africa, which ranged from 0.9 –39 µg L^{-1} (Horn *et al.*, 2020). Dexamethasone was detected in SPE extracts from S2, S3, S4 and S6 in concentrations ranging from 4.42 – 9.62 ng L^{-1}. These levels are lower than those reported in the Danube River, Budapest (0.1–0.7 ng L^{-1}) (Tölgyesi *et al.*, 2010).

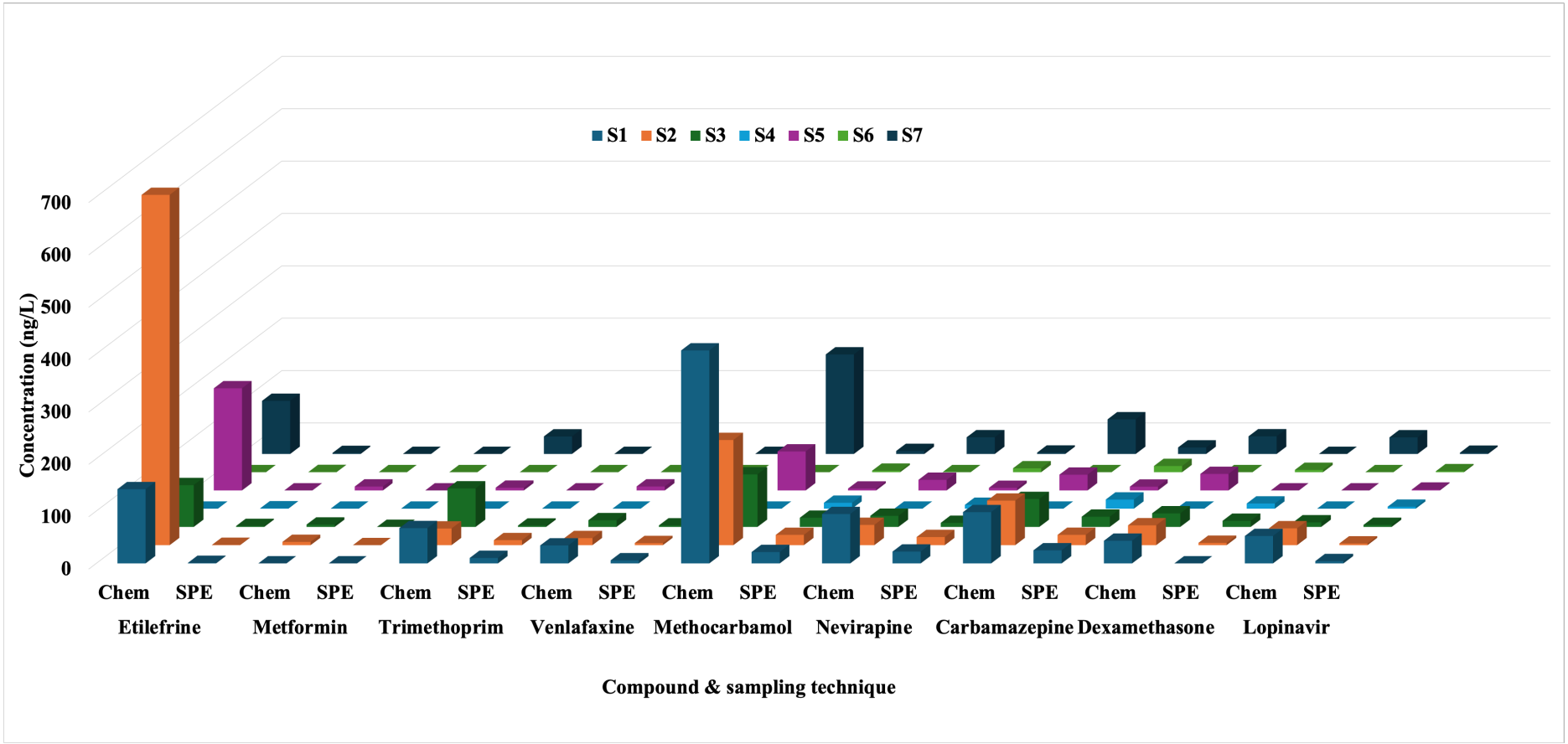

Fig. 3.
Comparison of pharmaceutical concentrations in 1ml extracts of Chemcatcher and SPE

6.3.3.3 TWA concentrations of pharmaceutical contaminants in the Crocodile River system and their comparison with other studies

Table 3 presents the TWA concentrations of pharmaceuticals across the sampling sites, as well as the grab water sample concentrations, which represent pharmaceutical concentrations in the sampling medium. The presence of these compounds in the environment highlights potential contamination from pharmaceutical residues, likely introduced through wastewater discharge or run-off. The TWA concentrations for carbamazepine, methocarbamol, dexamethasone, nevirapine, and venlafaxine were calculated using sampling rates from a previous study (Mapetla *et al.*, no date). However, the TWA concentration for etilefrine could not be determined due to its poor stability in solution. A previous calibration experiment showed linear accumulation for etilefrine until day eight, after which the accumulation became non-linear. Similarly, the TWA concentration for metformin was not calculated due to its poor adsorption onto HLB disks. Consequently, these two compounds were reported as either detected or not detected. In a study done in lake Ontario, the quantification of metformin using a POCIS sampler was reported. Although the study did not apply sampling rates to determine the TWA concentrations of the pharmaceutical compounds reported, the masses of the analytes accumulated in the sampler were averaged for the deployment period (ng day^{-1}). Metformin was present in concentrations ranging 1.18 to 2.96 ng day^{-1}. For trimethoprim and lopinavir, literature sampling rates were used to compute their TWA concentrations (Domínguez-García, Almirall and Gómez-Canela, 2025a).

Methocarbamol had the highest recorded TWA concentration (68.34 ng L^{-1}) at S1, followed by 33.74 ng L^{-1} at S2. Concentrations ranging from 0.32 to 3.98 ng day^{-1} were reported for methocarbamol using a POCIS sampler in lake Ontario. The antibiotic trimethoprim peaked at 29.52 ng L^{-1} at S3, with significant concentrations also observed at S1

(27.01 ng L^{-1}). The lake Ontario study reported concentrations up to 2.91 ng L^{-1} for the antibiotic drug, trimethoprim (Wang *et al.*, 2022). Lopinavir was notable at S1 (30.13 ng L^{-1}) and S2 (18.55 ng L^{-1}). Venlafaxine and dexamethasone were consistently detected but at relatively low concentrations across all sites, 0.88 – 5.68 and 5.05 – 8.44 ng L^{-1}, respectively. In the lake Ontario study, venlafaxine was detected in concentrations ranging from 0.25 – 3.96 ng day^{-1}. In river Itchen, venlafaxine TWA average concentrations up to 30.4 ng L-1 were reported (Robinson *et al.*, 2024). Another study reported the calibration and application of a POCIS-based sampler for the monitoring of 49 pharmaceutical contaminants in WWTPs, four of which are reported in the present study (dexamethasone, lopinavir, trimethoprim and venlafaxine). In the study, lopinavir, dexamethasone and trimethoprim were below the method LOD, and venlafaxine was below the method LOQ (Domínguez-García, Almirall and Gómez-Canela, 2025b). This was the first ever study to report the calibration of a POCIS–based sampler for the uptake of lopinavir. To the best of our knowledge, no other study has reported the uptake of lopinavir using POCIS nor Chemcatcher. In river Itchen, trimethoprim concentrations ranged from 0.2 to 4.3 ng L^{-1}, and these are lower than those recorded in the present study (Robinson *et al.*, 2024).

Carbamazepine was detected in moderate concentrations, peaking at 15.64 ng L^{-1} at S1. These are lower than the TWA concentrations (up to 68.4 ng L^{-1}) reported in River Itchen (Robinson *et al.*, 2024). Nevirapine exhibited declining concentrations from S1 (15.33 ng L^{-1}) to S3 (3.44 ng L^{-1}). Overall, S1 exhibited the highest concentration of most pharmaceuticals, followed by S2 and S7. S3 and S5 showed the lowest contaminant concentrations. Sources of contamination at individual sites were discussed in detail in the previous sections.

Table 3.
Time-weighted average (TWA) concentrations of pharmaceuticals (ng L^{-1}) across sampling sites

Compound	Sampling site													
	S1		S2		S3		S4		S5		S6		S7	
	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE
Etilefrine	D	<MLOD	D	<MLOD	D	0.087	–	0.0325	D	<MLOD	–	<MLOD	D	0.094
Metformin	<MLOD	<MLOD	D	<MLOD	D	<MLOD	–	<MLOD	D	<MLOD	–	<MLOD	<MLOD	<MLOD
Trimethoprim	27.01	0.522	12.74	0.477	29.52	0.128	–	<MLOD	2.07	<MLOD	–	<MLOD	13.41	<MLOD
Venlafaxine	5.68	0.293	2.23	0.197	2.11	0.141	–	0.103	1.25	<MLOD	–	<MLOD	0.880	<MLOD
Methocarbamol	68.34	1.083	33.74	0.979	16.84	0.872	–	0.562	12.51	<MLOQ	–	<MLOQ	31.94	<MLOQ
Nevirapine	15.33	1.129	6.29	0.774	3.44	0.387	–	0.363	3.29	0.246	–	0.372	5.180	0.128
Carbamazepine	15.64	1.247	13.58	0.982	8.52	0.974	–	0.872	4.8	0.358	–	0.592	10.57	0.617
Dexamethasone	8.440	<MLOD	7.390	<MLOQ	5.05	0.581	–	0.492	6.15	<MLOD	–	<MLOQ	6.570	<MLOD
Lopinavir	30.13	0.217	18.55	0.163	5.02	0.193	–	0.185	0	<MLOQ	–	0.089	18.25	0.114

Chem – Chemcatcher, D – detected, MLOD – method limit of detection, MLOQ – method limit of quantification, SPE – solid-phase extraction

Note: All SPE concentrations are a factor of 1×10^{-2}

6.4 Conclusions

The transport and distribution of pharmaceutical contaminants in the Crocodile River system exhibited spatial variability influenced by multiple factors, including anthropogenic inputs, hydrological processes, and the complexity of tributary networks. The highest contamination levels were observed at S2 and S3, likely due to wastewater discharge, runoff, and proximity to urban and industrial areas. Chemcatcher demonstrated consistently higher detection frequencies than SPE, underscoring the importance of passive sampling in detecting low-concentration contaminants that may be missed by grab sampling. Downstream, dilution, degradation, and potential sorption processes contributed to lower pharmaceutical concentrations at S5 and S7. However, S7 showed an unexpected increase in contaminant levels, likely due to additional inputs from tributaries such as the Gwatlhe River. The multiple tributaries feeding into the Crocodile River add complexity to the transport and distribution of contaminants, as they introduce variable pollution loads from diverse land-use activities, including agriculture, mining, and domestic wastewater discharge. This interconnected system necessitates a broader spatial and temporal monitoring strategy to fully understand contamination sources, movement, and fate. The findings emphasize the need for expansive and continuous sampling campaigns that integrate both passive and grab sampling techniques. Such efforts are essential for accurately assessing the impact of tributaries on pharmaceutical pollution, identifying critical contamination hotspots, and informing effective water quality management strategies. Given the persistence of contaminants such as methocarbamol, nevirapine, and carbamazepine, targeted mitigation measures are required to minimize their long-range transport and ecological impact within the river system.

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REFERENCES

Archer, E. *et al.* (2017) 'The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters', *Chemosphere*, 174, 437–446. doi.org/10.1016/j.chemosphere.2017.01.101.

Bavumiragira, J.P. *et al.* (2022) 'Fate and transport of pharmaceuticals in water systems: A processes review', *Sci. Total Environ.*, 823, 153635. doi.org/10.1016/j.scitotenv.2022.153635.

Benotti, M.J. and Brownawell, B.J. (2009) 'Microbial degradation of pharmaceuticals in estuarine and coastal seawater', *Environ. Pollut.*, 157(3), 994–1002. doi.org/10.1016/j.envpol.2008.10.009.

Brodin, T. *et al.* (2014) 'Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioural alterations', *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 369(1656), 20130580. doi.org/10.1098/rstb.2013.0580.

Castle, G.D. *et al.* (2018) 'Calibration and field evaluation of the Chemcatcher® passive sampler for monitoring metaldehyde in surface water', *Talanta*, 179, 57–63. doi.org/10.1016/j.talanta.2017.10.053.

Domínguez-García, P. *et al.* (2025) 'A POCIS-based approach for the monitoring of pharmaceuticals in wastewater treatment plants: Calibration and deployment challenges', *Environ. Pollut.*, 367, 125641. doi.org/10.1016/j.envpol.2025.125641.

Fernandes, M.J. *et al.* (2020) 'Antibiotics and antidepressants occurrence in surface waters and sediments collected in the north of Portugal', *Chemosphere*, 239, 124729. doi.org/10.1016/j.chemosphere.2019.124729.

Forestry, D. of W.A. and (1996) 'South African Water Quality Guidelines: aquatic ecosystems', 7.

García-Santiago, X. *et al.* (2017) 'Fate of pharmaceuticals in soil after application of STPs products: Influence of physicochemical properties and modelling approach', *Chemosphere*, 182, 406–415. doi.org/10.1016/j.chemosphere.2017.05.049.

Glanzmann, V. *et al.* (2023) 'An improved Chemcatcher-based method for the integrative passive sampling of 44 hydrophilic micropollutants in surface water – Part A: Calibration under four controlled hydrodynamic conditions', *Sci. Total Environ.*, 871, 162037. doi.org/10.1016/j.scitotenv.2023.162037.

Grabicova, K. *et al.* (2017) 'Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream', *Water Res.*, 124, 654–662. doi.org/10.1016/j.watres.2017.08.018.

Hong, B. *et al.* (2021) 'Development of a pH-paralleling approach of quantifying six-category pharmaceuticals in surface water using SPE-HPLC-MS/MS', *Watershed Ecol. Environ.*, 3, 1–16. doi.org/10.1016/j.wsee.2021.01.001.

Hoorzook, K.B. *et al.* (2021) 'Soul of the Jukskei River: The Extent of Bacterial Contamination in the Jukskei River in Gauteng Province, South Africa', *Int. J. Environ. Res. Public Health*, 18(16), 8537. doi.org/10.3390/ijerph18168537.

Horn, S. *et al.* (2020) 'Impact of Potential COVID-19 Treatment on South African Water Sources Already Threatened by Pharmaceutical Pollution', *Environ. Toxicol. Chem.*, 39(7), 1305–1306. doi.org/10.1002/etc.4734.

Hu, Y. and Cheng, H. (2013) 'Water pollution during China's industrial transition' Environ. Dev., 8, 57–73. doi.org/10.1016/j.envdev.2013.06.001.

'Integrated water quality management: getting people involved in the Jukskei River' (1995) *Water Sci. Technol.*, 32(5–6). doi.org/10.1016/0273-1223(95)00658-3.

Joss, A. *et al.* (2006) 'Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme', *Water Res.*, 40(8), 1686–1696. doi.org/10.1016/j.watres.2006.02.014.

Kawabata, K. *et al.* (2013) 'Photodegradation of pharmaceuticals in the aquatic environment by sunlight and UV-A, -B and -C irradiation', *J. Toxicol. Sci.*, 38(2), 215–223. doi.org/10.2131/jts.38.215.

Khulu, S. *et al.* (2022) 'Development and application of a membrane assisted solvent extraction-molecularly imprinted polymer based passive sampler for monitoring of selected pharmaceuticals in surface water', *Water Res.*, 225, 119145. doi.org/10.1016/j.watres.2022.119145.

Koba, O. *et al.* (2018) 'Transport of pharmaceuticals and their metabolites between water and sediments as a further potential exposure for aquatic organisms', *J. Hazard. Mater.*, 342,. 401–407. doi.org/10.1016/j.jhazmat.2017.08.039.

Lahti, M. and Oikari, A. (2011) 'Pharmaceuticals in settleable particulate material in urban and non-urban waters', *Chemosphere*, 85(5), 826–831. doi.org/10.1016/j.chemosphere.2011.06.084.

Li, H. *et al.* (2010) 'Sampling in the Great Lakes for pharmaceuticals, personal care products, and endocrine-disrupting substances using the passive polar organic chemical integrative sampler', *Environ.l Toxicol. Chem.*, 29(4), 751–762. doi.org/10.1002/etc.104.

Löffler, D. *et al.* (2005) 'Environmental Fate of Pharmaceuticals in Water/Sediment Systems', *Environ. Sci. & Technol.y*, 39(14), 5209–5218. doi.org/10.1021/es0484146.

Löffler, D. and Ternes, T.A. (2003) 'Determination of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using liquid chromatography–tandem mass spectrometry', *J. Chromatogr. A.,* 1021(1–2), 133–144. doi.org/10.1016/j.chroma.2003.08.089.

Madikizela, L.M. *et al.* (2017) 'Status of pharmaceuticals in African water bodies: Occurrence, removal and analytical methods', *J. Environ. Manag.*, 193, 211–220. doi.org/10.1016/j.jenvman.2017.02.022.

Mostafa, A. *et al.* (2023) 'Multi-class determination of pharmaceuticals as emerging contaminants in wastewater from Eastern Province, Saudi Arabia using eco-friendly SPE-UHPLC-MS/MS: Occurrence, removal and environmental risk assessment', *Microchem. J.*, 187, 108453doi.org/10.1016/j.microc.2023.108453.

Ncube, S. *et al.* (2018) 'Environmental fate and ecotoxicological effects of antiretrovirals: A current global status and future perspectives', *Water Res.*, 145, 231–247. doi.org/10.1016/j.watres.2018.08.017.

Ngwenya, N. and Mahlambi, P. (2023) 'Methods optimization and application: Solid phase extraction, ultrasonic extraction and Soxhlet extraction for the determination of antiretroviral drugs in river water, wastewater, sludge, soil and sediment', *J. Pharm. Biomed. Anal.*, 230, 115358doi.org/10.1016/j.jpba.2023.115358.

Paíga, P. and Delerue-Matos, C. (2024) 'Tracing Pharmaceuticals in Water Systems: Focus on Neurodegenerative and Psychiatric Treatments', *J. Xenobiot.*, 14(4), 1807–1825. doi.org/10.3390/jox14040096.

Rilievo, G. *et al.* (2024) 'From a validated targeted method to a retrospective UHPLC-HRMS non-targeted analysis unveiling COVID-19-related contaminants in clams. Have we bias in marine model organisms for ecotoxicological studies?', *Chemosphere*, 364, 142994. doi.org/10.1016/j.chemosphere.2024.142994.

Rimayi, C. *et al.* (2018) 'Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa', *Sci. Total Environ.*,, 627, 1008–1017. doi.org/10.1016/j.scitotenv.2018.01.263.

Rimayi, C. *et al.* (2019) 'Use of the Chemcatcher® passive sampler and time-of-flight mass spectrometry to screen for emerging pollutants in rivers in Gauteng Province of South Africa', *Environ. Monit. Assess.*, 191(6), 388. doi.org/10.1007/s10661-019-7515-z.

Robinson, R.F.A. *et al.* (2024) 'Quantification and risk assessment of polar organic contaminants in two chalk streams in Hampshire, UK using the Chemcatcher passive sampler', *Sci. Total Environ.*,, 939, 173316. doi.org/10.1016/j.scitotenv.2024.173316.

Sammut Bartolo, N. *et al.* (2021) 'Pharmaceuticals and the environment', *Early Hum. Dev.*, 155, 105218. doi.org/10.1016/j.earlhumdev.2020.105218.

Schoeman, C. *et al.* (2017) 'The impact of a Wastewater Treatment Works in Southern Gauteng, South Africa on efavirenz and nevirapine discharges into the aquatic environment', *Emerg. Contam.*, 3(2), 95–106. doi.org/10.1016/j.emcon.2017.09.001.

Schoeman, F.R. (1979) 'Diatoms as indicators of water quality in the upper Hennops (Transvaal, South Africa)', *J. Limnol. Soc. South. Afr.*, 5(2), 73–78. doi.org/10.1080/03779688.1979.9633188.

Sui, Q. *et al.* (2015) 'Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: A review', *Emerg. Contam.*, 1(1), 14–24. doi.org/10.1016/j.emcon.2015.07.001.

Tölgyesi, Á. *et al.* (2010) 'Simultaneous determination of corticosteroids, androgens, and progesterone in river water by liquid chromatography–tandem mass spectrometry', *Chemosphere*, 78(8), 972–979. doi.org/10.1016/j.chemosphere.2009.12.025.

Vaudreuil, M.A. *et al.* (2022) 'Pharmaceutical pollution of hospital effluents and municipal wastewaters of Eastern Canada', *Sci. Total Environ.*,, 846, 157353. doi.org/10.1016/j.scitotenv.2022.157353.

Vrana, B. *et al.* (2005) 'Passive sampling techniques for monitoring pollutants in water', *TrAC, Trends Anal. Chem.*, 24(10), 845–868. doi.org/10.1016/j.trac.2005.06.006.

Wang, S. *et al.* (2022) 'Combining Passive Sampling with Suspect and Nontarget Screening to Characterize Organic Micropollutants in Streams Draining Mixed-Use Watersheds', *Environ. Sci. Technol.*, 56(23), 16726–16736. doi.org/10.1021/acs.est.2c02938.

Watanabe, H. *et al.* (2016) 'Chronic toxicity of an environmentally relevant mixture of pharmaceuticals to three aquatic organisms (alga, daphnid, and fish)', *Environ. Toxicol. Chem.*, 35(4), 996–1006. doi.org/10.1002/etc.3285.

Waterman, K.C. *et al.* (2002) 'Hydrolysis in Pharmaceutical Formulations', *Pharm. Dev. Technol.*, 7(2), 113–146. doi.org/10.1081/PDT-120003494.

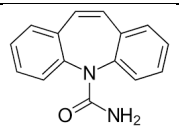
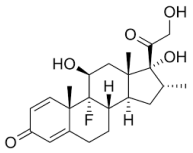
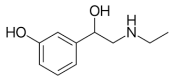
World health organization (2016) 'Protecting surface water for health: identifying, assessing and managing drinking-water quality risks in surface-water catchments', *World health organization* [Preprint].

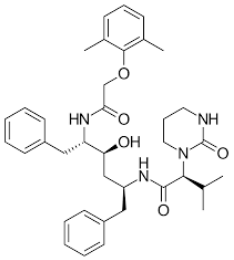
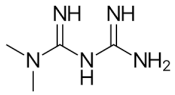
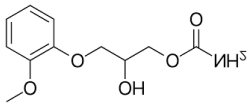
Yoshioka, S. and Aso, Y. (2007) ‘Correlations between Molecular Mobility and Chemical Stability During Storage of Amorphous Pharmaceuticals’, *J. Pharm. Sci.*, 96(5), 960–981. doi.org/10.1002/jps.20926.

Zhang, Q. *et al.* (2017) ‘Recent applications of hydrophilic interaction liquid chromatography in pharmaceutical analysis’, *J. Sep. Sci.*, 40(1), 49–80. doi.org/10.1002/jssc.201600843.

Zuehlke, S. *et al.* (2007) ‘Investigation of the behavior and metabolism of pharmaceutical residues during purification of contaminated ground water used for drinking water supply’, *Chemosphere*, 69(11), 1673–1680. doi.org/10.1016/j.chemosphere.2007.06.020.

Appendix 3
Table 1S
Properties of target pharmaceutical compounds

Target compound	Therapeutic class	Structure	Empirical formula	CAS no.	$LogK_{ow}$	Molecular weight $g.mol^{-1}$	Solubility in water $mg\ L^{-1}$
Carbamazepine	Anticonvulsant		C15H12N2O	298-46-4	2.3	236.27	238.64
Dexamethasone	Corticosteroid		C22H29FO5	50-02-2	1.83	392.46	89.0
Etilefrine	Adrenergic agonist		C10H15NO2	709-55-7		181.23	18.9

Lopinavir	Anti-retroviral		C37H48N4O5	192725-17-0	5.94	628.814	1.92×10^3
Metformin	Antidiabetic agent		C4H11N5	657-24-9	-1.43	129.164	300.5
Methocarbamol	Muscle relaxant		C11H15NO5	532-03-6	0.61	241.24	29.9

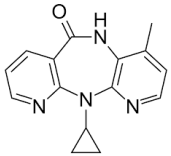
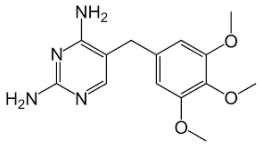
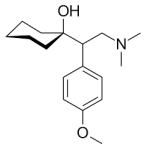
Nevirapine	Non-nucleoside reverse transcriptase inhibitors		C15H14N4O	129618-40-2	3.89	266.21	100
Trimethoprim	Antibiotic		C14H18N4O3	738-70-5	0.91	290.32	0.4
Venlafaxine	Selective serotonin and norepinephrine reuptake inhibitors (SNRIs)		C17H27NO2	93413-69-5	3.20	277.40	572

Table 2S
Concentrations of pharmaceutical contaminants in Chemcatcher and SPE extracts in ng L^{-1} (± %RSD)

Site	Etilefrine		Metformin		Trimethoprim		Venlafaxine		Methocarbamol		Nevirapine		Carbamazepine		Dexamethasone		Lopinavir		Sum
	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	Chem	SPE	
S1	141.71	<MLOD	<MLOD	<MLOD	67.32	10.43	34.27	5.86	406.61	21.65	94.22	22.57	97.86	24.93	43.23	<MLOD	52.3	4.34	1028.27
	(5.20)				(3.23)	(6.48)	(5.17)	(10.48)	(7.26)	(7.10)	(6.29)	(8.81)	(2.54)	(9.90)	(6.48)		(9.07)	(8.61)	
S2	668.8	<MLOD	6.23	<MLOD	31.76	9.54	13.44	3.94	200.77	19.58	38.64	15.47	85.01	19.64	37.87	<MLOQ	32.2	3.25	1191.05
	(2.97)		(6.03)		(3.65)	(7.31)	(3.18)	(4.26)	(5.96)	(11.24)	(4.65)	(9.01)	(3.50)	(9.91)	(9.14)		(6.32)	(7.23)	
S3	79.8	1.74	4.43	<MLOD	73.56	2.55	12.74	2.81	100.22	17.44	21.15	7.74	53.31	19.47	25.89	11.62	8.72	3.86	447.05
	(8.11)	(8.40)	(8.92)		(7.93)	(13.04)	(6.90)	(9.73)	(8.79)	(2.25)	(4.86)	(7.23)	(7.79)	(8.24)	(9.54)	(4.01)	(5.38)	(8.37)	
S4	NA	0.65	NA	<MLOD	NA	<MLOD	NA	2.06	NA	11.24	NA	7.25	NA	17.43	NA	9.84	NA	3.7	52.17
		(8.70)						(6.06)		(7.15)		(8.05)		(4.58)		(7.05)		(5.29)	
S5	195.03	<MLOD	7.45	<MLOD	5.17	<MLOD	7.57	<MLOD	74.41	<MLOQ	20.24	4.91	30.06	7.16	31.53	<MLOD	<MLOD	<MLOQ	388.6
	(1.56)		(2.33)		(7.11)		(6.94)		(6.17)		(4.50)	(10.10)	(9.28)	(6.54)	(9.48)				
S6	NA	<MLOD	NA	<MLOD	NA	<MLOD	NA	<MLOD	NA	<MLOQ	NA	7.44	NA	11.83	NA	<MLOQ	NA	1.78	28.51
												(9.67)		(6.51)				(9.86)	
S7	101.36	1.87	<MLOD	<MLOD	33.42	<MLOD	5.29	<MLOD	190.05	<MLOQ	31.84	2.55	66.13	12.33	33.64	<MLOD	31.69	2.28	518.1
	(3.52)	(3.57)			(6.28)		(7.32)		(7.89)		(8.37)	(8.48)	(4.07)	(5.64)	(5.34)		(6.77)	(8.52)	

NA – not analysed, MLOD – method limit of detection, MLOQ – method limit of quantification, RSD – relative standard deviation

CHAPTER 7 SUMMARY AND RECOMMENDATIONS FOR FUTURE WORK

7.1 Summary

This study investigated the presence, potential sources, transport, and distribution of pharmaceutical residues in South African aquatic ecosystems using a dual sampling approach. That is; grab sampling and passive sampling. The research focused on three key study sites, which are the Hennops River and its catchment Hartbeespoort Dam (Chapter 4), the Durban Marina estuary (Chapter 5), and the Crocodile River system (Chapter 6). Ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-Q-Orbitrap MS) was employed to analyse pharmaceutical residues, providing insights into contamination trends and sampling method efficiencies.

In Chapter 4, the study assessed pharmaceutical pollution in a heavily impacted freshwater system. Targeted analysis with Chemcatcher revealed the presence of carbamazepine, dexamethasone, etilefrine, methocarbamol and nevirapine at all sites with TWA concentrations up to 17.87 µg. L^{-1}. With SPE, only etilefrine, methocarbamol, nevirapine and dexamethasone were detected at all sites, and carbamazepine was only detected at S1, S2 and S3. Their concentrations in the SPE extracts ranged from 4.88 – 84.71 µg L^{-1}. Venlafaxine was not detected at all sites with SPE, while it was detected at all sites, expect S2 with Chemcatcher. This highlighted one advantage of passive sampling to grab sampling. That is the ability of passive samplers to accumulate trace level contaminants to detectable levels, that would otherwise go undetected with grab sapling and SPE processes. Potential sources of these contaminants were identified as run-off, wastewater discharge and improper sewage systems. This was evidenced by the detection of *E-coli* (up to 2250 CFU mL^{-1}), a coliform count up to 195000 CFU mL^{-1} and a total aerobic count up to 400000000 CFU mL^{-1}, which implies the presence of faecal matter contamination. In suspect screening, a total of 25 pesticides and 61 pharmaceutical compounds were detected. Detection frequency was higher with Chemcatcher compared to SPE at all sites. A maximum of 17 pesticides were detected with Chemcatcher at S2, whereas a maximum of 11 pesticides were detected with SPE at S1, S2 and S5. The same detection frequency trend was observed for pharmaceutical compounds where a maximum of 45 compounds were detected with Chemcatcher at S4, while a maximum of 20 compounds were detected SPE. Suspect screening allowed for the detection of compounds for which

certified reference standards were not present. These include all pesticides that were identified, antibiotics (trimethoprim and netilmicin), antihistamines (fexofenadine and terfenadine), beta-blockers (alprenolol, Eplerenone, oxprenolol and pindolol) and metabolites (desmethyl nefopam, enalaprilat, N-Desmethylvenlafaxine, O-Desmethylvenlafaxine and paraxanthine). This chapter emphasized the benefits of passive sampling and the combined use of targeted analysis and suspect screening for comprehensive pollution profiling.

Chapter 5 focused on pharmaceutical contamination in the Durban Marina estuary, a critical transitional environment between freshwater and marine systems. The study was extended to nine pharmaceutical compounds with the addition of lopinavir, metformin and trimethoprim. Metformin was detected across all sites while etilefrine was not detected at any site with both sampling procedures. Carbamazepine was detected across all sites with Chemcatcher, in contrast to going undetected with SPE across all sites. Chemcatcher consistently showed a higher detection frequency (88.89%) across all sampling sites, whereas SPE detection frequencies varied more across sites (55.56 – 77.78%). A maximum pharmaceutical concentration sum of 2574.52 ng L^{-1} was obtained with Chemcatcher at S2, and this was found to be 32 times greater than that obtained with SPE (79.33 ng L^{-1}) at the same site. Converting the extract concentrations to Chemcatcher TWA concentrations and grab water sample concentrations in the sampling medium, a trend similar to that of extract concentrations was observed. That is Chemcatcher TWA concentrations (up to 290.18 ng L^{-1} for dexamethasone at S2) were consistently higher than grab water concentrations in the sampling medium (up to 0.1594 ng L^{-1} for nevirapine at S2). Passive sampling provided a more comprehensive view of pharmaceutical occurrence over time, whereas grab sampling reflected fluctuating contamination levels influenced by tidal dynamics. This chapter emphasized the limitations of point-source sampling in dynamic estuarine environments, and the importance of incorporating passive sampling techniques to better profile the extent of pollution.

In Chapter 6, pharmaceutical transport, potential sources and distribution in the Crocodile River system after it exits Hartbeespoort Dam were examined. This chapter built on Chapter 4, in which the presence of pharmaceutical contaminants along Hennops River and Hartbeespoort Dam was investigated. The Crocodile River system is an intricate system with several tributaries and canalised streams of the main river, which complicates the monitoring of contaminant movement and input sources of contaminants. Nine target pharmaceutical compounds and seven sampling sites were considered. Chemcatcher consistently demonstrated

a higher detection frequency, reaching 100% at S2 and S3, whereas SPE exhibited greater variability, with detection frequencies ranging from 44.44% (S5) to 88.89% (S2 & S3). In both Chemcatcher and SPE extracts, the highest concentrations were observed at S1 (Dam exit), with levels decreasing progressively at S2, S3, S4, and S5, where they were at their lowest. This trend suggested dilution as the river flows downstream from Hartbeespoort Dam and limited additional inputs before reaching S7. An exception was observed in Chemcatcher extracts from S7, where the concentrations of the target compounds were elevated to levels similar to those in S2. This increase was attributed to input from tributaries such as the Gwatlhe River, which flows through the village of Modikwe before entering Rooikoppies Dam before making its way to S7. Microbial counts (total aerobic count: 5700–88,000 CFU mL^{-1}; coliform count: 101–2450 CFU mL^{-1}) pointed to contamination sources such as runoff, wastewater discharge, and inadequate sewage management. Passive samplers consistently captured a broader range of pharmaceuticals than grab sampling, confirming their superiority for long-term pollution monitoring. The study also demonstrated that dilution effects in riverine systems may lead to underestimation of contamination levels when using only grab sampling methods.

Overall, the findings highlight the widespread presence of pharmaceutical pollution in South African freshwater and estuarine environments. The study also showed that mainland pollution affects the environment, in that river systems that are connected to estuarine environments carry with contaminants which end up in the sea. It also highlight the advantages of passive sampling over conventional grab sampling in detecting trace contaminants and capturing long-term pollution trends. This study supports with the United Nation's sustainability development goal (SDG 6: Clean Water and Sanitation).

7.2 Recommendations for future work

Based on the findings of this study, the following recommendations are proposed to enhance pharmaceutical pollution monitoring and management in South African aquatic ecosystems:

1. Integration of passive sampling in water quality assessment

Given the demonstrated sensitivity of passive samplers in detecting trace pharmaceuticals, they should be incorporated into national water quality monitoring programs. Combining passive and grab sampling will provide a more comprehensive understanding of pollution trends and contamination dynamics.

2. Expansion of pharmaceutical monitoring efforts

Future studies should extend pharmaceutical pollution assessments to additional rivers, estuaries, and marine environments to develop a broader contamination profile. Complex river networks, such as the Crocodile River system, require multiple sampling sites along the main river, tributaries, and canalized streams to pinpoint pollution sources to aid in the implementation of mitigation strategies.

3. Longitudinal studies for seasonal and long-term pollution trends

Ongoing research should employ both targeted analysis and suspect screening to monitor seasonal and long-term pollution patterns. This approach will offer a more detailed pollution profile and facilitate the identification of emerging contaminants.

4. Assessing the impact of mainland pollution on marine ecosystems

The connection between freshwater and marine systems through estuaries poses a risk of pharmaceutical contamination to marine environments. Future studies should focus on tracking contaminant transport from rivers to estuaries and marine environments to assess their impact on marine organisms and ecosystem health.

5. Upgrade existing sewage networks and wastewater treatment facilities

Many pharmaceuticals persist in aquatic environments due to incomplete removal in WWTPs and sewage leaks. Upgrading WWTPs with advanced treatment technologies and the maintenance of exist sewage networks should be prioritised to enhance pharmaceutical removal efficiency and prevent sewage leaks into aquatic ecosystems. Regulations should mandate pharmaceutical-specific treatment processes in areas that have been identified as being affected by pharmaceutical contamination.

6. Establishing regulatory frameworks for pharmaceutical residues

South Africa currently lacks specific legal thresholds for pharmaceutical residues in water. Establishing ecotoxicologically based regulatory limits is crucial for effective pollution control. Additionally, guidelines should address the proper disposal of expired and unused pharmaceuticals to minimize environmental contamination. Community awareness campaigns should be introduced to raise awareness about the environmental risks of improper pharmaceutical disposal, and pollution as a whole.

7. Investigating bioaccumulation and combined effects of contaminants

Further studies should explore the bioaccumulation of pharmaceuticals in aquatic organisms and their potential impact on human health though the food chain. Research should also investigate the combined effects of pharmaceutical pollution with other contaminants, such as pesticides and industrial chemicals, in South African water systems.

By implementing these recommendations, South Africa can improve pharmaceutical pollution management strategies, enhance water quality monitoring, and protect aquatic ecosystems from long-term contamination risks.

7.3 Limitations of study

The present study, while comprehensive in its dual sampling approach and analytical methodology, is subject to several limitations that should be acknowledged. Firstly, the investigation focused on a select group of pharmaceutical compounds, chosen for their relevance in South African aquatic systems. This limited scope means that other potentially harmful pharmaceuticals and emerging contaminants may have been overlooked, potentially underestimating the full extent of pharmaceutical pollution. Secondly, the sampling strategy, although strengthened by the integration of grab and passive sampling techniques, presented inherent challenges. Grab sampling offers only a snapshot of contaminant levels at a specific point and time, making it susceptible to temporal variability and potentially missing episodic pollution events. Conversely, passive sampling using Chemcatcher devices, while advantageous for detecting time-integrated concentrations, is affected by environmental factors such as water flow rate, temperature, and biofouling, which can influence compound uptake rates and, consequently, the accuracy TWA concentration estimates. Additionally, the absence of PRCs for all target analytes limited the ability to fully correct for these environmental influences. Analytical limitations were also noted. Despite the high resolution and sensitivity of the UHPLC-Q-Orbitrap MS, issues such as matrix effects and ion suppression could have impacted detection and quantification accuracy, particularly in complex environmental matrices. Collectively, these limitations underscore the need for further research involving a broader range of contaminants, refined calibration strategies for passive samplers, and continued development of robust analytical methods to enhance data reliability and environmental risk assessment.

Appendix 4

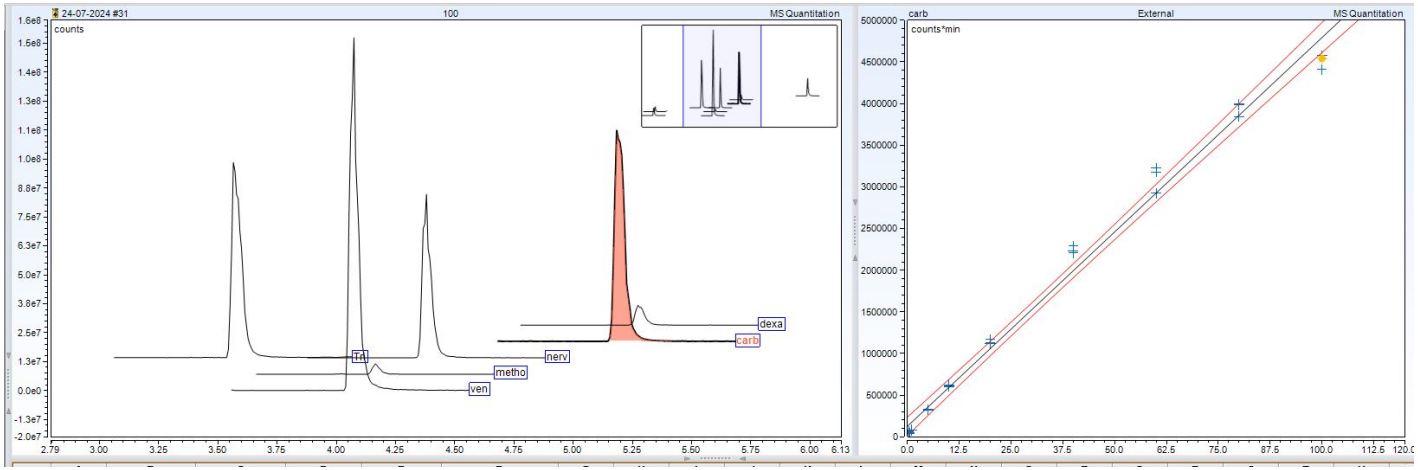


Figure 1 Carbamazepine calibration curve

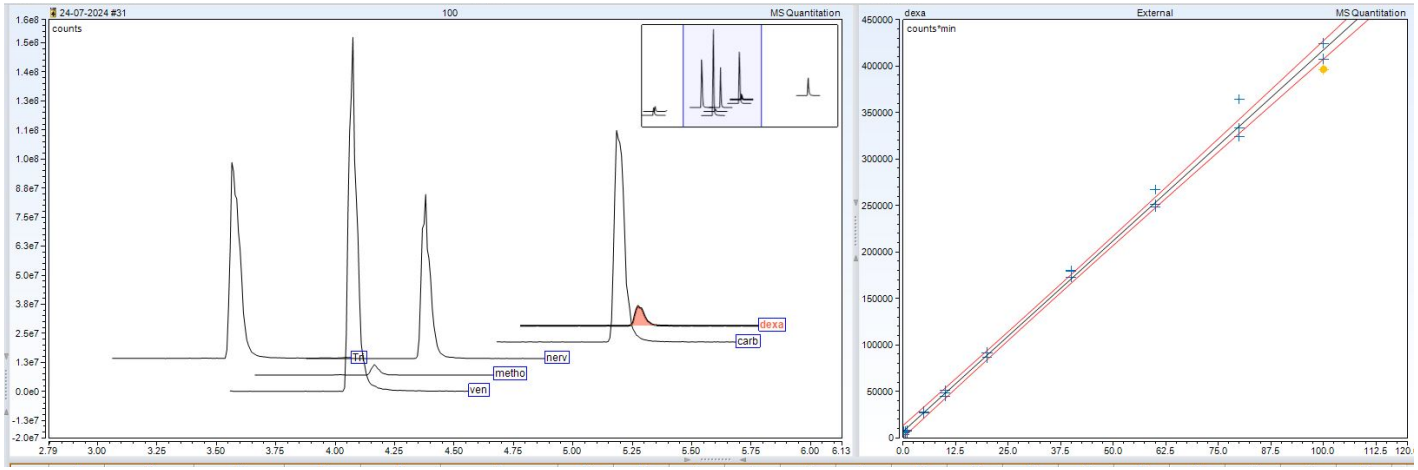


Figure 2 Dexamethasone calibration curve

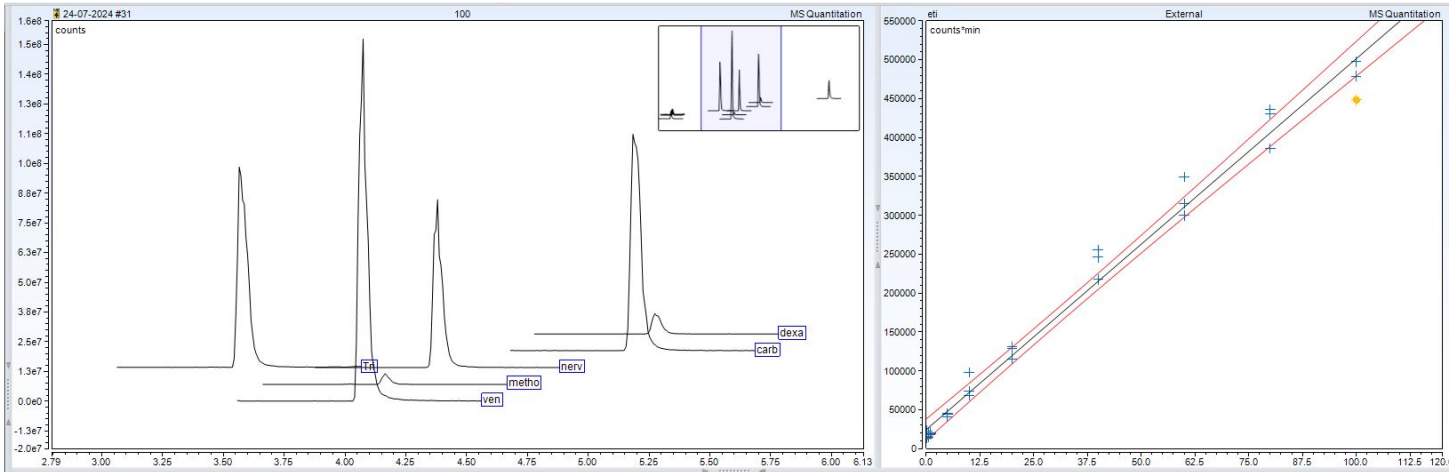


Figure 3 Etilefrine calibration cure

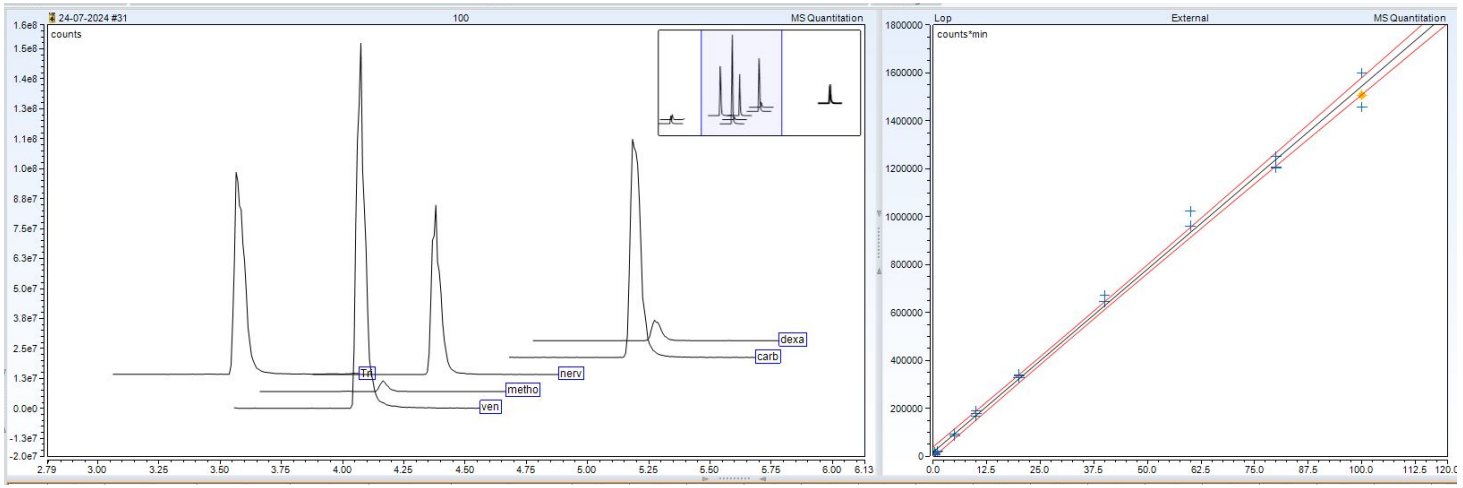


Figure 4 Lopinavir calibration curve

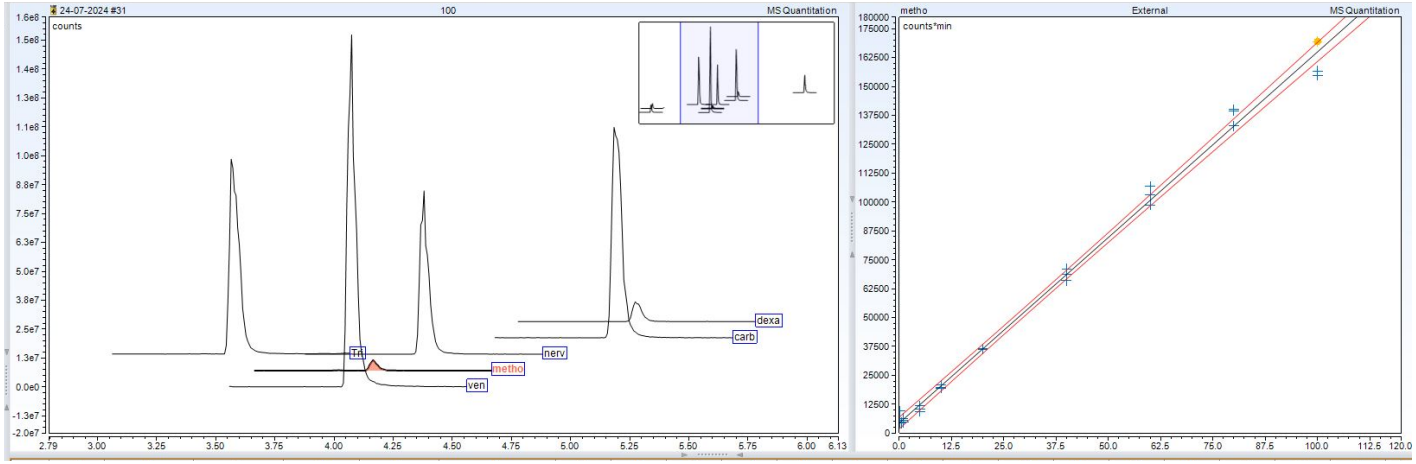


Figure 5 Methocarbamol calibration curve

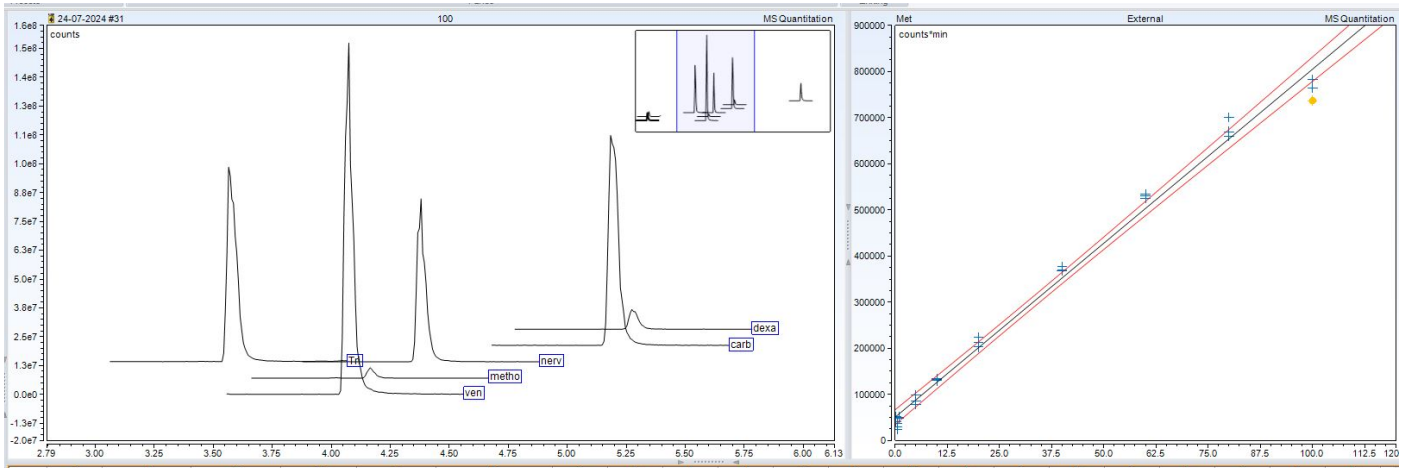


Figure 6 Metformin calibration curve

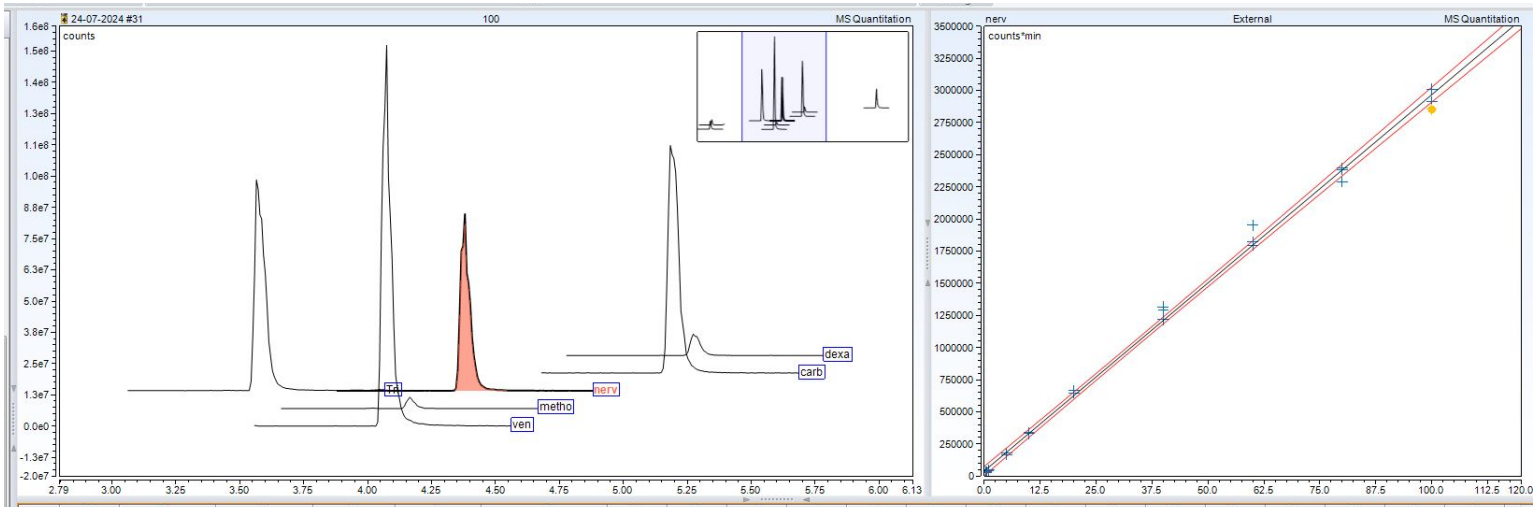


Figure 7 Nevirapine calibration curve

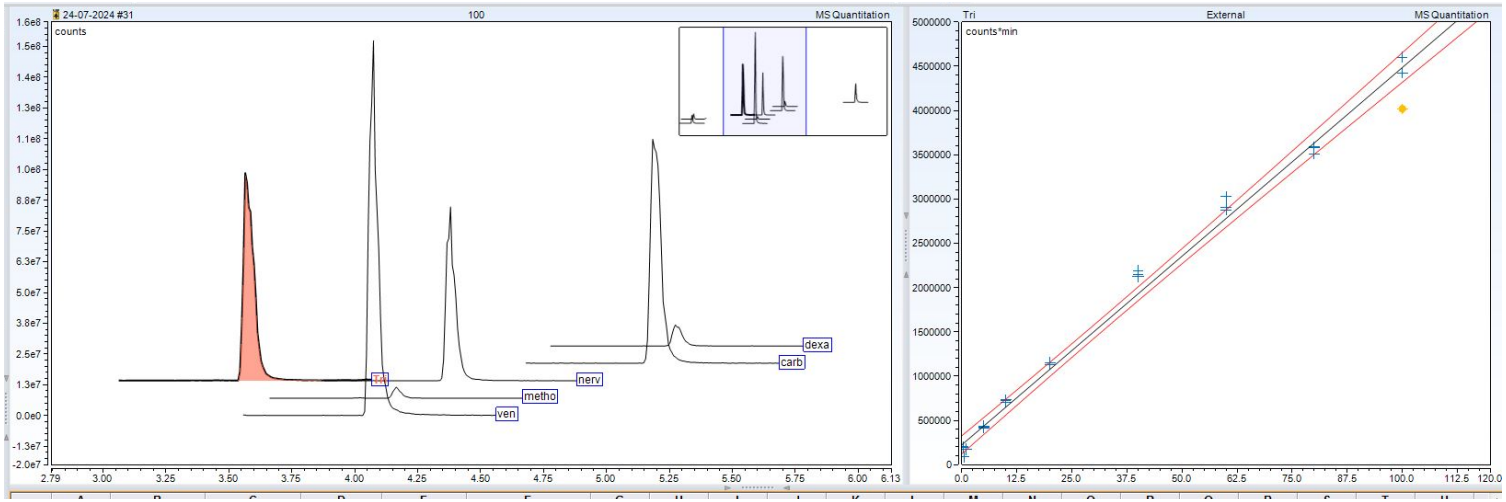


Figure 8 Trimethoprim calibration curve

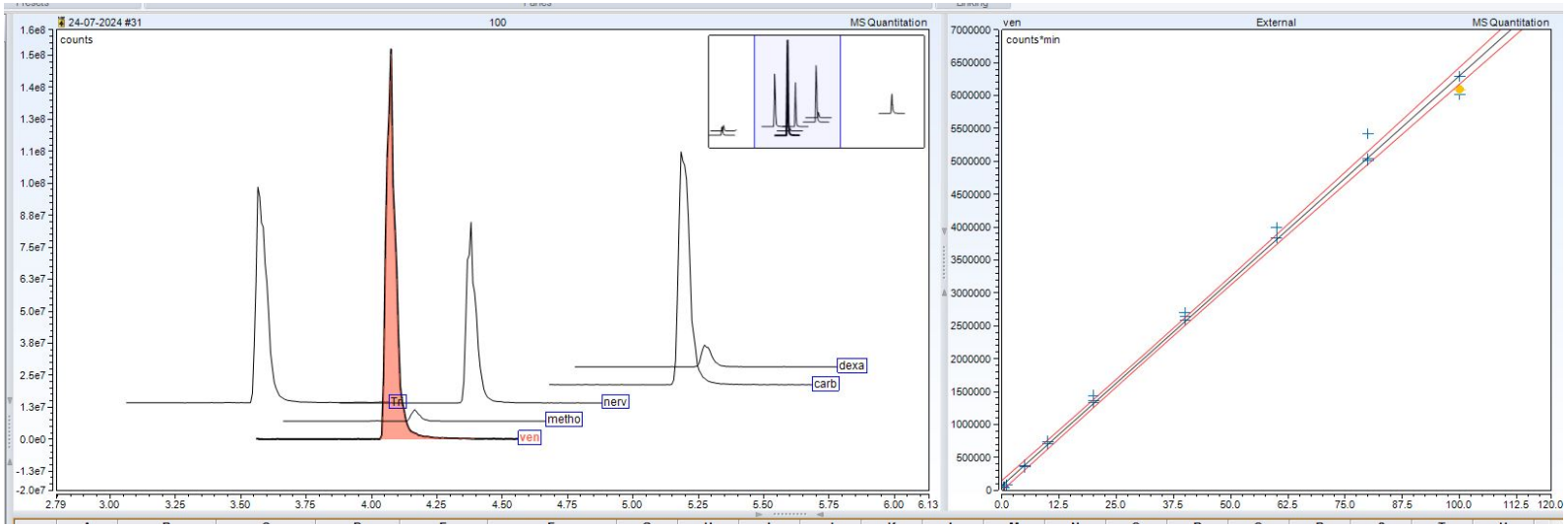


Figure 9 Venlafaxine calibration curve

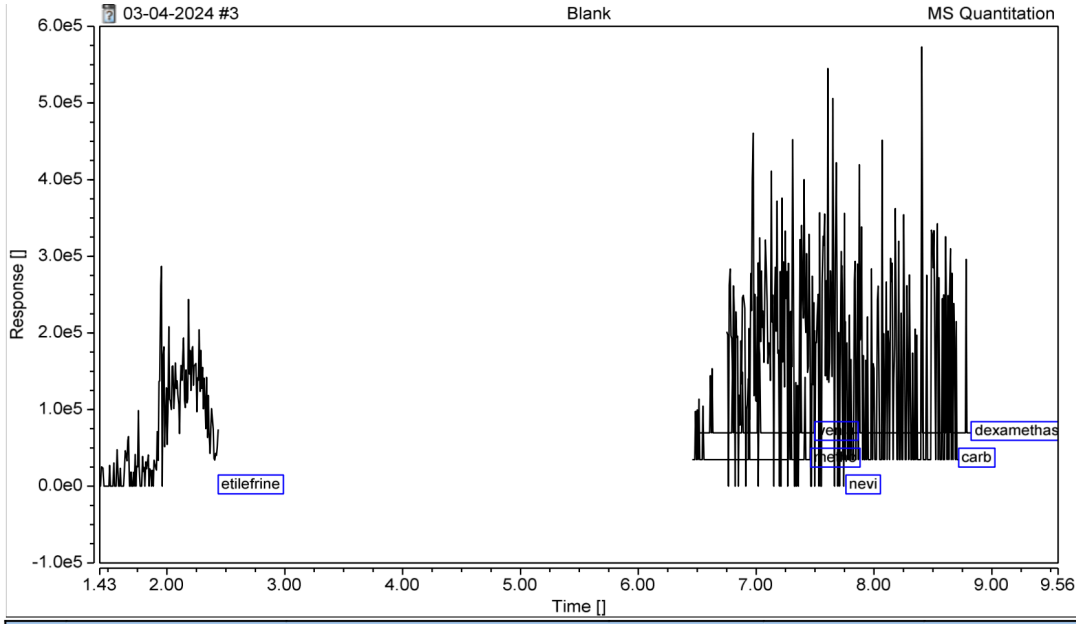


Figure 10 Chemcatcher laboratory blank representative chromatogram

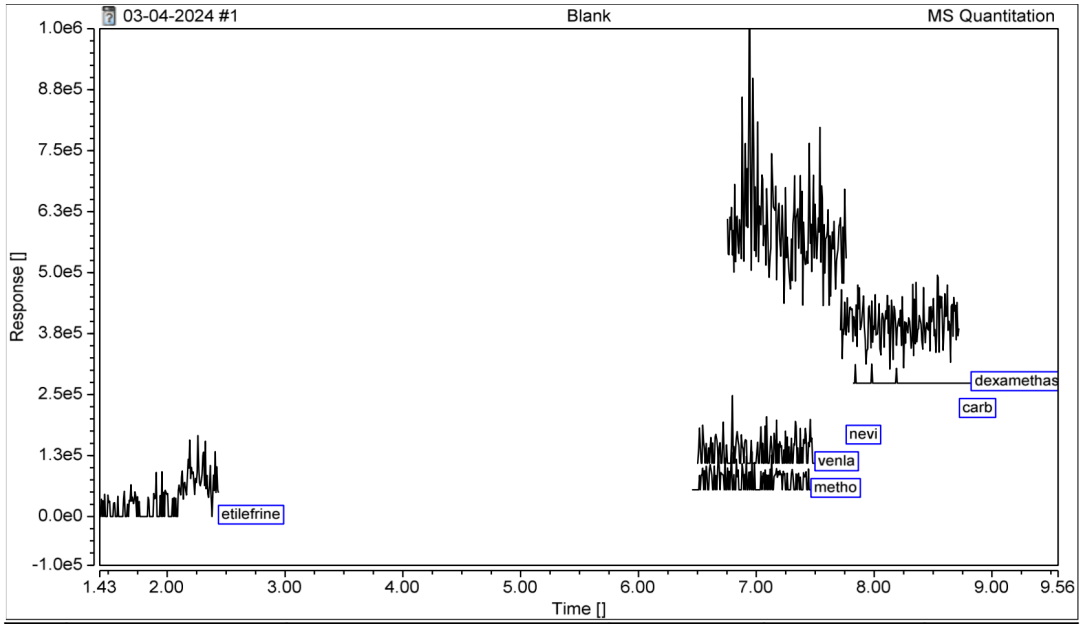


Figure 11 Chemcatcher field blank representative chromatogram

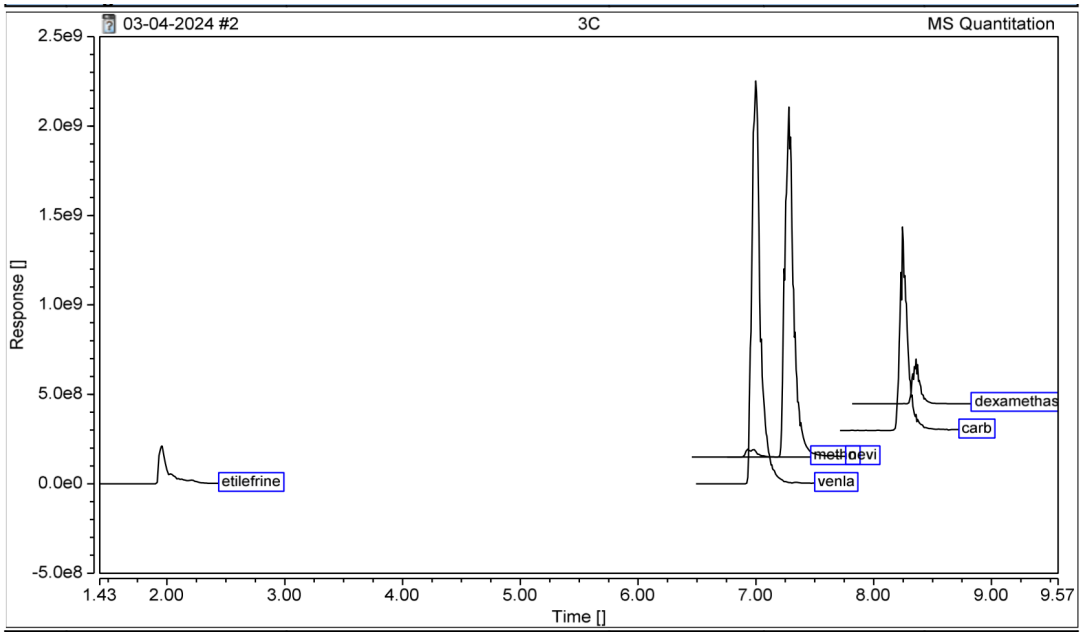


Figure 12 Chemcatcher representative sample chromatogram

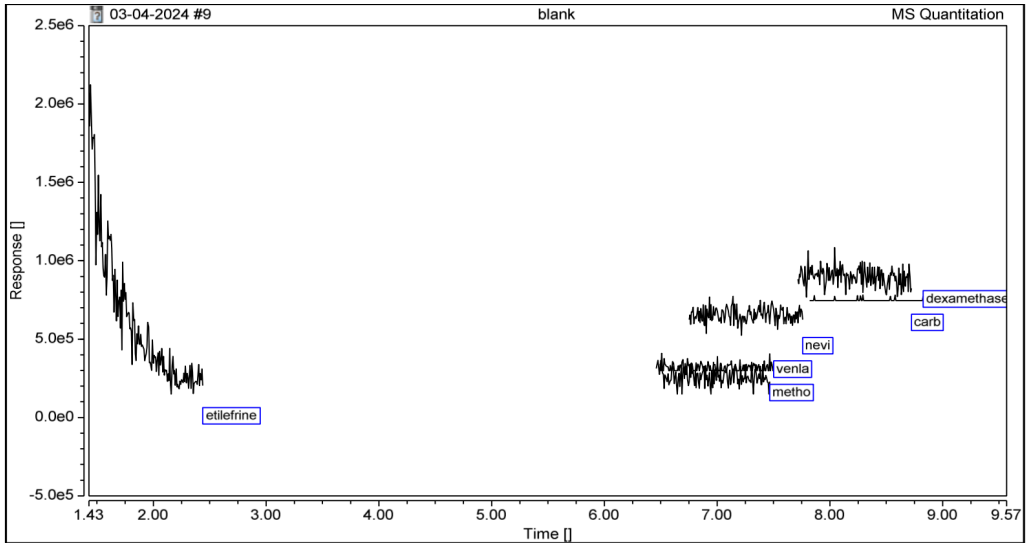


Figure 13 Grab sample with SPE laboratory blank representative chromatogram

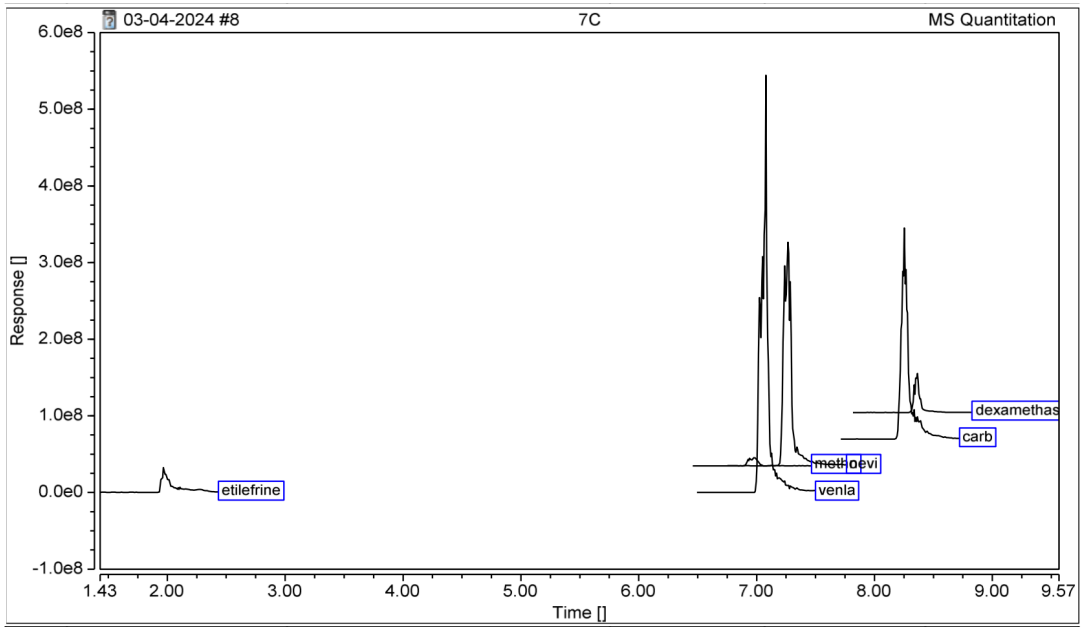


Figure 14 Grab sample with SPE sample representative chromatogram

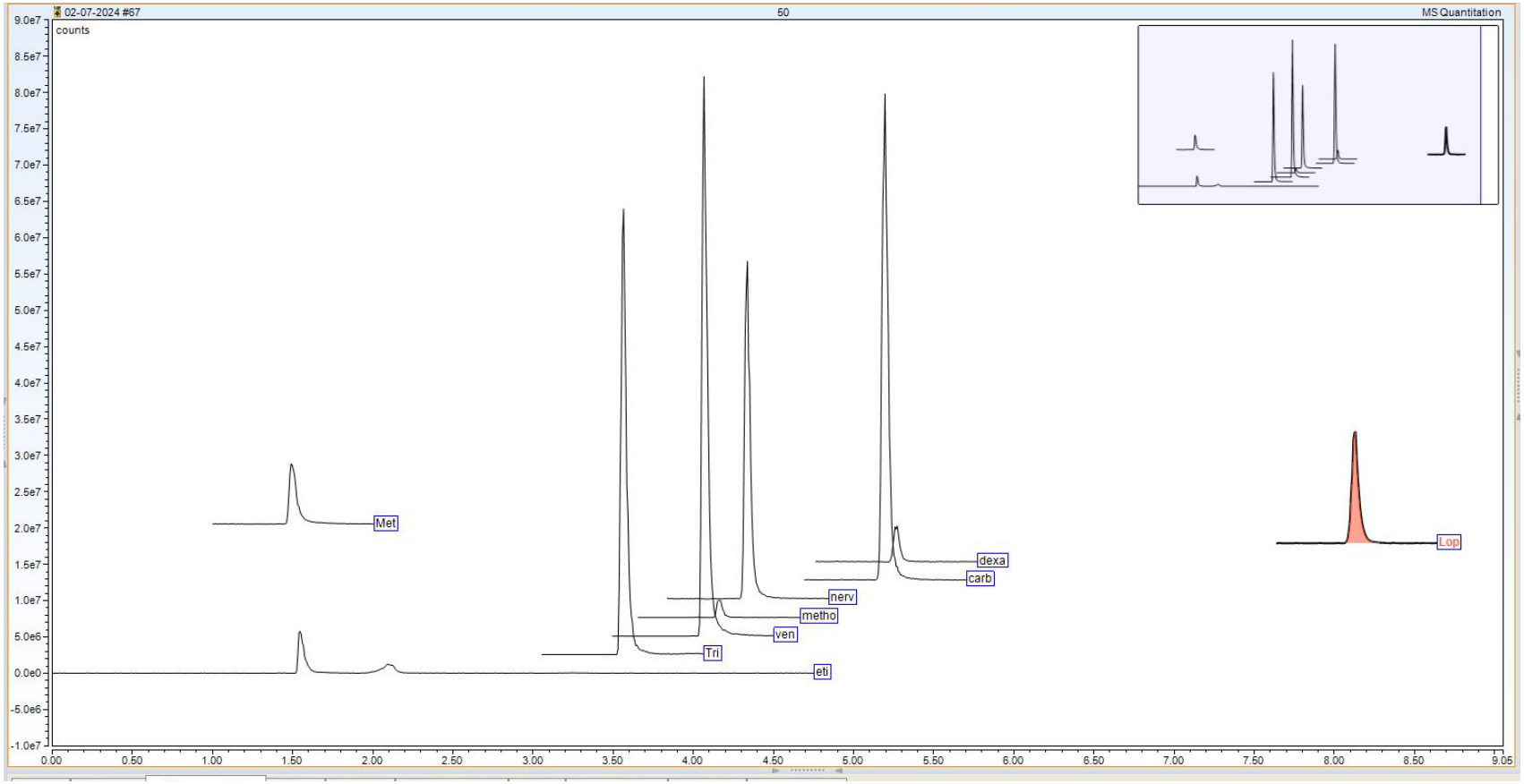


Figure 15 Calibration standards representative chromatogram (Carb – carbamazepine, Dex –dexamethasone, Eti – etilefrine, Lop – lopinavir, Meth – methocarbamol, Met – metformin, Nerv – nevirapine, Tri – trimethoprim, Ven – venlafaxine)