Communication in Physical Sciences, 2019, 4(2): 174-187

Green Analytical Methods for Monitoring APIs and Metabolites in Nigerian Wastewater: A Pilot Environmental Risk Study

Faith Osaretin Osabuohien

Received 29th April 2019 /Accepted 9th December 2019/Published online: 30th December 2019

Abstract: *This study presents the development and implementation of green analytical chemistry (GAC) methods for monitoring active pharmaceutical ingredients (APIs) and their metabolites in Nigerian wastewater effluents, with a focus on environmental risk mitigation. The growing prevalence of pharmaceutical residues in aquatic environments poses significant ecological and public health challenges, particularly in regions with inadequate wastewater treatment infrastructure such as Nigeria. Influent and effluent samples were collected from selected wastewater treatment plants in southwestern Nigeria and analyzed using environmentally friendly sample preparation and chromatographic techniques. Specifically, solid-phase microextraction (SPME), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) were employed to reduce solvent use, minimize hazardous waste, and improve the sustainability of the analytical process. Common APIs, including amoxicillin, ciprofloxacin, paracetamol, and artemether, were detected in varying concentrations across the sampled locations. Method validation demonstrated high recovery rates, excellent linearity, and good precision. The greenness of the developed methods was evaluated using the Analytical GREEnness (AGREE) metric, yielding scores between 0.76 and 0.85—indicating strong compliance with the 12 principles of GAC. Environmental risk was assessed using the Risk Quotient (RQ) approach, revealing that several APIs, especially antibiotics, present moderate to high ecological risks. These findings underscore the importance of integrating GAC techniques into routine environmental monitoring programs. The study recommends the adoption of sustainable analytical practices, infrastructural upgrades, regulatory reform, and capacity-building initiatives to address pharmaceutical pollution in Nigeria effectively.*

Keywords: *Green chemistry, APIs, wastewater, Nigeria, environmental risk, sustainable monitoring.*

Faith Osaretin Osabuohien
Department of Chemistry,
Ambrose Alli University, Ekpoma, Edo State, Nigeria.
Email: **ofaith768@gmail.com**
Orcid id:

1.0 INTRODUCTION

The increasing detection of pharmaceutical active ingredients (APIs) and their metabolites in aquatic environments has raised global concerns due to their persistence, bioactivity, and potential for bioaccumulation (Boxall *et al*., 2014). These compounds are introduced into the environment mainly through human and veterinary usage, with excreted unmetabolized drugs and their transformation products eventually reaching wastewater systems (Gros *et al*., 2013). In developing countries like Nigeria, this problem is exacerbated by inadequate wastewater treatment infrastructure and lax regulations regarding pharmaceutical waste disposal (Daughton, 2014).

Recent studies have shown that wastewater treatment plants (WWTPs) are often inefficient in completely removing APIs from effluents, leading to their continuous discharge into receiving water bodies (Michael *et al*., 2013). In Nigeria, many treatment facilities are either non-functional or operate below capacity, allowing untreated or poorly treated wastewater to contaminate surface and groundwater sources (Adesuyi *et al*., 2018). Consequently, pharmaceuticals such as antibiotics, analgesics, anti-

inflammatories, and hormone regulators have been detected in various environmental compartments, posing risks to aquatic ecosystems and possibly affecting human health through water reuse and consumption of contaminated aquatic organisms.

Moreover, the environmental presence of APIs has been linked to ecological disturbances, including endocrine disruption in fish, the development of antibiotic-resistant bacteria, and alteration of microbial community structures in sediment and soil (Mutiyar & Mittal, 2014; K'Oreje *et al*., 2016). In particular, antibiotic residues are a major concern, as they can contribute to the proliferation of antimicrobial resistance (AMR), now recognized as a major global public health threat (WHO, 2015).

Traditional analytical methods for detecting APIs in wastewater rely on techniques such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), which, although accurate, often involve toxic solvents, high energy consumption, and generation of hazardous waste (Armenta *et al*., 2015). These methods are also cost-prohibitive and less accessible to many developing countries. In this context, the development of green analytical chemistry (GAC) methods represents a sustainable alternative. GAC promotes environmentally friendly approaches that minimize chemical use, reduce energy demands, and favor safer reagents without compromising analytical performance (Koel & Kaljurand, 2013).

In recent years, researchers have explored a variety of GAC approaches, such as miniaturized extraction techniques, solid-phase microextraction (SPME), and solvent-free methods like supercritical fluid extraction, to monitor pharmaceutical pollutants with reduced environmental footprint (Khan *et al*., 2017). However, their adoption remains limited in sub-Saharan Africa due to lack of awareness, capacity, and supportive regulatory frameworks.

In Nigeria, the problem is further compounded by poor data availability and a lack of environmental monitoring policies focused on emerging contaminants like pharmaceuticals. Most environmental monitoring programs prioritize traditional pollutants (e.g., heavy metals, nutrients, and pathogens) while ignoring APIs and their metabolites, despite growing evidence of their ecological relevance (Ebele *et al*., 2017). As a result, there is an urgent need to develop locally adaptable, green analytical strategies for effective detection and quantification of pharmaceutical residues in wastewater, particularly as part of integrated risk assessment and mitigation efforts.

The implementation of green analytical methods tailored to the Nigerian context could significantly enhance environmental monitoring, improve public health protection, and align with sustainable development principles. This study, therefore, proposes to bridge this gap by designing and applying green analytical chemistry tools for the detection of APIs and their metabolites in selected Nigerian wastewater effluents.

1.1 Green Analytical Chemistry in Developing Countries

Green Analytical Chemistry (GAC) represents a sustainable approach to chemical analysis that seeks to minimize the environmental and health impacts of analytical procedures by reducing solvent use, lowering energy demands, and enhancing safety throughout the analytical process. In developing countries, where environmental pollution and resource constraints coexist, the implementation of GAC holds significant promise for bridging environmental protection with public health and economic development (Gałuszka, Migaszewski, Konieczka, & Namieśnik, 2013).

Despite its global relevance, GAC remains underutilized in many developing nations due to several factors. These include limited access to eco-friendly instrumentation, inadequate funding for research infrastructure, and a lack of technical training on green analytical techniques (Hernando, Mezcua, Fernández-Alba, & Barceló, 2014). Furthermore, regulatory frameworks in these regions often lag behind global standards,

providing limited incentives for transitioning from conventional, often hazardous, analytical protocols to greener alternatives.

Nevertheless, recent advancements indicate a growing recognition of GAC's value in the Global South. Analytical methods using green solvents such as ethanol and water-rich mixtures have been tested with promising results in Africa and Asia, demonstrating that affordable and scalable green techniques can be applied effectively in resource-limited settings (Płotka-Wasylka, Rutkowska, Tobiszewski, & Namieśnik, 2014). Solid Phase Microextraction (SPME) and Ultrasound-Assisted Extraction (UAE), for instance, have been adapted for environmental monitoring of pharmaceuticals and pesticides, offering solvent-free or low-solvent alternatives suited to complex environmental matrices.

Moreover, the environmental burden from pharmaceutical residues, industrial waste, and agrochemicals is notably higher in developing countries, where waste management and treatment technologies are often rudimentary. The use of GAC in monitoring such pollutants provides a dual benefit—accurate assessment of environmental contaminants and a reduction in the ecological impact of the analysis itself (Hughes, Kay, & Brown, 2013).

The adoption of green metrics such as the Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), and Analytical GREEnness (AGREE) tool has begun to inform the selection and optimization of methods in these contexts. These tools help standardize what constitutes a "green" method, allowing for objective assessment and comparison (Gałuszka *et al*., 2013). This is particularly important for national laboratories and universities in developing countries where environmental policies are increasingly emphasizing sustainable development goals.

Ultimately, integrating GAC into environmental monitoring in developing countries is not merely a technological shift but a strategic necessity. It fosters local innovation, reduces reliance on imported hazardous reagents, and promotes safer working environments while enabling the accurate surveillance of environmental pollutants that disproportionately affect vulnerable populations.

1.2 Statement of the Problem

Pharmaceutical residues are increasingly detected in Nigerian surface waters, yet there is no established national framework or standardized green analytical methodology for monitoring these contaminants. Conventional analytical approaches are often resource-intensive and not aligned with Nigeria's sustainability goals. In addition, untreated or partially treated wastewater effluents containing APIs are directly discharged into the environment, raising concerns about long-term ecological and human health impacts (Ojemaye & Petrik, 2018).

The lack of reliable, cost-effective, and environmentally friendly analytical tools for API detection limits the country's capacity to assess pharmaceutical pollution and implement adequate mitigation strategies. Furthermore, the absence of baseline data on APIs and their metabolites in local wastewater effluents poses a significant barrier to risk assessment and policy development.

1.3 Objectives of the Study

The main objective of this study is to develop and implement green analytical chemistry methods for monitoring APIs and their metabolites in Nigerian wastewater effluents. The specific objectives include:

1. To design and validate eco-friendly analytical methods for detecting selected pharmaceutical compounds in wastewater.
2. To determine the occurrence and concentration levels of APIs and their metabolites in wastewater effluents from selected Nigerian locations.
3. To assess the environmental risks associated with the presence of APIs in wastewater.
4. To recommend sustainable strategies for reducing pharmaceutical pollution

through green chemistry and policy interventions.

1.4 Research Questions

1. What are the most effective green analytical methods for monitoring APIs in wastewater?
2. What levels of APIs and their metabolites are present in selected Nigerian wastewater effluents?
3. What are the potential environmental and health risks posed by these contaminants?
4. How can green analytical approaches support sustainable wastewater management in Nigeria?

1.5 Justification of the Study

With increasing pharmaceutical use in Nigeria, the presence of APIs in wastewater is inevitable. However, the reliance on conventional, environmentally detrimental analytical methods is not sustainable. By adopting GAC principles, this study provides an innovative framework for environmentally responsible monitoring of pharmaceutical pollution.

Moreover, data generated will contribute to national environmental monitoring efforts, promote evidence-based policy-making, and foster compliance with international environmental protection agreements. The study also supports the broader goals of the Sustainable Development Goals (SDGs), particularly SDG 6 (clean water and sanitation) and SDG 12 (responsible consumption and production).

1.6 Scope and Significance of the Study

This study is a pilot project focusing on the development and implementation of GAC-based methods to detect selected APIs and their metabolites in wastewater effluents from urban and peri-urban areas in Nigeria. The study will involve method development, sample collection and analysis, data interpretation, and environmental risk assessment.

This research has the potential to serve as a foundational model for green monitoring of pharmaceutical contaminants in developing countries. It bridges the gap between analytical chemistry and environmental sustainability and highlights the importance of green technologies in achieving environmental protection. Furthermore, it provides scientific data that can be used by environmental regulators, health agencies, and policymakers in Nigeria.

2.0 LITERATURE REVIEW

Pharmaceutical residues, particularly active pharmaceutical ingredients (APIs) and their metabolites, are emerging as critical environmental contaminants. Their persistence, bioactivity at low concentrations, and resistance to conventional wastewater treatment methods make them significant pollutants in aquatic systems. This literature review examines the global and regional occurrence of APIs in wastewater, the environmental and health risks associated with these contaminants, the limitations of traditional analytical methods, and the growing relevance of green analytical chemistry (GAC) in the context of environmental sustainability. It also explores the current status of pharmaceutical monitoring in Nigeria and reviews international case studies that offer practical models for adaptation. Finally, the section identifies key research gaps and provides a justification for the present study.

2.1 Occurrence and Sources of APIs in Wastewater

Pharmaceutical compounds enter aquatic environments primarily through human and veterinary excretion, as many drugs are only partially metabolized in the body. These compounds, along with their metabolites, are released into municipal wastewater systems and subsequently discharged into natural water bodies. Additional sources include effluents from hospitals, pharmaceutical manufacturing plants, and agricultural activities involving veterinary drugs (Gros et al., 2013; Verlicchi et al., 2012).

Multiple studies have confirmed the widespread occurrence of APIs in various environmental compartments. For instance, pharmaceuticals such as ibuprofen,

carbamazepine, sulfamethoxazole, diclofenac, and paracetamol have been detected in surface water, groundwater, sediments, and even drinking water in regions across Europe, North America, and Asia (aus der Beek et al., 2016; Hughes et al., 2013). In developing countries like Nigeria, these issues are magnified due to weak environmental regulations, poor waste disposal practices, and inadequate infrastructure for sewage treatment (Adesuyi et al., 2018).

2.2 Environmental and Health Risks of APIs and Their Metabolites

Pharmaceutical residues in aquatic environments have been associated with a range of ecological and health hazards. Certain classes of APIs, including hormones, antidepressants, and antibiotics, are known to interfere with the endocrine systems of aquatic organisms. Studies have documented feminization of male fish, altered reproductive behavior, and developmental defects in species exposed to endocrine-disrupting compounds (Kidd et al., 2014; Jobling et al., 2006).

The presence of antibiotics in water bodies is particularly troubling due to their role in fostering antibiotic resistance. The continuous low-level exposure of microorganisms to antibiotics can select for resistant strains, contributing to the global health threat of antimicrobial resistance (AMR) as recognized by the World Health Organization (WHO, 2015; Kümmerer, Dionysiou, Olsson, & Fatta-Kassinos, 2018). In addition to ecological risks, APIs may pose human health risks through direct and indirect exposure pathways, including the consumption of contaminated water or aquatic organisms and dermal contact during recreational activities. Although the effects of chronic low-dose exposure in humans remain under-researched, the potential for cumulative harm cannot be ignored.

2.3 Analytical Methods for Detecting APIs in the Environment

Monitoring APIs in the environment requires analytical methods that are both sensitive and selective. Conventional techniques such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), and liquid chromatography–tandem mass spectrometry (LC-MS/MS) are widely employed for this purpose due to their high accuracy and specificity (Gros et al., 2013). However, these techniques often involve time-consuming sample preparation, require expensive instrumentation, and depend on large quantities of hazardous organic solvents (Płotka-Wasylka, 2018).

Sample preparation methods such as solid-phase extraction (SPE), liquid-liquid extraction (LLE), and solid-phase microextraction (SPME) are commonly used but often generate considerable chemical waste and increase operational costs (Armenta et al., 2015). These drawbacks make traditional methods less sustainable and less feasible in resource-limited settings, such as those found in many developing countries.

2.4 Principles and Application of Green Analytical Chemistry (GAC)

Green Analytical Chemistry (GAC) has emerged as a paradigm shift in analytical science, aimed at reducing the environmental footprint of chemical analysis. The 12 principles of GAC, adapted from green chemistry principles, advocate for minimal sample handling, the use of safer reagents, lower energy consumption, direct analysis where feasible, and waste minimization (Gałuszka et al., 2013). These principles guide the design of methods that are not only analytically robust but also environmentally and economically sustainable.

GAC-oriented techniques include solventless methods such as SPME, miniaturized extraction procedures like microextraction by packed sorbent (MEPS), and the use of green solvents such as ethanol and water (Koel & Kaljurand, 2013). Supercritical fluid chromatography and direct spectrometric screening techniques are also increasingly employed. Recent advances have shown that green methods can be as effective as conventional ones in detecting pharmaceuticals in environmental matrices,

including water, food, and soil (Khan et al., 2017; Horstkotte et al., 2014; Płotka-Wasylka et al., 2017).

In developing countries, where financial, infrastructural, and safety constraints exist, the application of GAC is particularly relevant. Green methods not only reduce operational costs but also promote safer working conditions and improve accessibility to routine environmental monitoring.

2.5 Status of Pharmaceutical Monitoring in Nigeria

In Nigeria, research on the occurrence and monitoring of pharmaceutical residues remains limited. Few studies have evaluated the levels of APIs in aquatic systems such as wastewater, rivers, and lagoons. Investigations conducted in Lagos Lagoon and other urban water bodies have reported the presence of pharmaceuticals like ciprofloxacin, paracetamol, and ibuprofen (Adesuyi et al., 2018). Despite these findings, there is no standardized national protocol for monitoring pharmaceutical pollutants in the environment.

Nigeria faces several challenges in addressing pharmaceutical pollution. These include the absence of regulatory guidelines for pharmaceuticals and personal care products (PPCPs), poor coordination among regulatory agencies, inadequate laboratory infrastructure, and limited expertise in green analytical techniques. Additionally, awareness of pharmaceutical pollution among stakeholders remains low, resulting in fragmented data and weak policy responses.

2.6 Case Studies from Other Countries

International experience offers valuable lessons for Nigeria. In India and Kenya, for example, SPME combined with LC-MS has been successfully applied to detect analgesics and antibiotics in municipal wastewater and rivers (Mutiyar & Mittal, 2014; K'Oreje et al., 2016). In European countries, the use of solvent-free and miniaturized analytical techniques is becoming standard in environmental monitoring, particularly for detecting trace concentrations of endocrine-disrupting compounds in effluents (Horstkotte et al., 2014; Płotka-Wasylka et al., 2017). These examples demonstrate the practicality and adaptability of green methods in diverse socioeconomic and environmental contexts.

2.7 Research Gaps and Justification for the Study

While there is a growing body of literature on pharmaceutical pollution globally, significant gaps remain in sub-Saharan Africa, especially concerning the development and application of green analytical methods. Existing studies in Nigeria have primarily focused on occurrence and distribution, with little emphasis on method validation, environmental risk assessment, or alignment with GAC principles.

The current study seeks to address these gaps by developing green, cost-effective, and locally adaptable methods for monitoring APIs and their metabolites in Nigerian wastewater. It also aims to generate baseline data essential for policy formulation and environmental risk mitigation. Furthermore, the study supports broader sustainability objectives by integrating green chemistry into environmental surveillance efforts.

3.0 MATERIALS AND METHODS

3.1 Research Design

This study employed an experimental research design to develop and implement green analytical chemistry (GAC) methods for detecting and quantifying selected active pharmaceutical ingredients (APIs) and their metabolites in wastewater effluents from Nigerian urban settings. The methodology emphasized environmental sustainability, analytical precision, and reproducibility, in accordance with the 12 principles of GAC (Gałuszka et al., 2013).

3.2 Study Area

Sampling was carried out in wastewater treatment plants (WWTPs) located in four states in southwestern and southern Nigeria: Lagos, Ogun, Edo, and Delta. The selection of these sites was based on population density, pharmaceutical consumption patterns, urbanization levels, and the availability of operational wastewater

infrastructure. Lagos State, the most urbanized and industrialized state in Nigeria, was chosen for its large population and high pharmaceutical usage, with functional WWTPs serving as ideal sites for environmental monitoring. Ogun State was included due to its proximity to Lagos and its concentration of pharmaceutical manufacturers and hospitals. Delta and Edo States, located in the Niger Delta region, were selected based on evidence of increasing drug usage, rapid urbanization, persistent environmental pollution, and the emergence of municipal treatment systems, particularly in Benin City. These sites offered a representative distribution of wastewater effluent characteristics across different ecological and infrastructural settings in Nigeria.

3.3 Sample Collection

Influent and effluent wastewater samples were collected from selected WWTPs using a 24-hour composite sampling technique to capture diurnal variations in pharmaceutical concentrations. One-liter samples were collected at each sampling point using pre-cleaned amber glass bottles. Immediately after collection, samples were preserved in ice at approximately 4°C and transported to the laboratory within six hours. Where immediate analysis was not possible, samples were stored at −20°C to maintain analyte integrity.

3.4 Target Analytes

The pharmaceutical compounds selected for analysis included amoxicillin and ciprofloxacin (antibiotics), paracetamol and ibuprofen (analgesics), and artemether and lumefantrine (antimalarials). These APIs were chosen based on their widespread use in Nigeria and frequent detection in previous environmental monitoring studies within the region.

3.5 Sample Preparation

Green analytical sample preparation techniques were applied to ensure minimal solvent usage and reduced environmental impact. Solid-phase microextraction (SPME), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) were employed based on their compatibility with the physicochemical characteristics of the target APIs.

SPME was used for semi-volatile APIs and involved the use of biodegradable polymeric fibers fabricated in-house using cellulose acetate and chitosan crosslinked with eco-friendly agents. These fibers exhibited high adsorption efficiency and mechanical stability compared to conventional silica-based materials.

MAE was selected for sludge samples, leveraging microwave energy to enhance analyte recovery from complex solids using low volumes of solvent. UAE was utilized for liquid samples, applying ultrasonic waves to facilitate desorption of analytes from the matrix. The combination of these green extraction techniques ensured effective isolation of pharmaceutical residues while minimizing hazardous waste and energy consumption.

3.6 Analytical Techniques

Quantitative analysis of APIs and their metabolites was performed using high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection. HPLC was selected for its robustness and efficiency, and green solvents such as ethanol–water mixtures were used as mobile phases to align with GAC principles by reducing toxicity and flammability.

For confirmatory analysis and detection of trace-level metabolites, liquid chromatography–tandem mass spectrometry (LC-MS/MS) was employed. A triple quadrupole mass spectrometer provided the sensitivity and selectivity required for complex wastewater matrices. This dual-analytical approach ensured both broad

detection capability and high analytical precision.

The environmental sustainability of each analytical protocol was assessed using AGREE (Analytical GREEnness Metric) and GAPI (Green Analytical Procedure Index). AGREE provided a quantitative score (ranging from 0 to 1) reflecting compliance with the 12 principles of GAC, while GAPI offered a visual color-coded representation of environmental impact across the analytical workflow.

3.7 Method Validation

Validation of the developed analytical methods was performed according to International Council for Harmonisation (ICH) guidelines and United States Environmental Protection Agency (US EPA) protocols. Validation parameters included linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, recovery, and matrix effects. Calibration curves were generated using standard solutions prepared in matrix-matched conditions, and replicate analyses were conducted to assess repeatability and reproducibility.

3.8 Environmental Risk Assessment

The environmental risk associated with detected APIs was evaluated using the Risk Quotient (RQ) approach. RQ was calculated as the ratio of the measured environmental concentration (MEC) to the predicted no-effect concentration (PNEC), expressed as:

$$RQ = \frac{MEC}{PNEC} \quad (1)$$

Risk levels were interpreted using standard thresholds: RQ < 0.1 indicated low risk, 0.1 ≤ RQ < 1 indicated medium risk, and RQ ≥ 1 indicated high ecological risk. This assessment framework enabled prioritization of contaminants based on their potential environmental impact.

3.9 Data Analysis

Chromatographic data were processed using MassHunter and ChemStation software for quantification. Statistical analyses were carried out using SPSS version 26.0 and R software. Descriptive statistics, analysis of variance (ANOVA), and principal component analysis (PCA) were applied to identify trends and assess variability. Correlations between API concentrations in effluents and WWTP operational parameters were also examined to evaluate treatment efficiency.

3.10 Ethical and Environmental Considerations

All procedures involving the collection, transport, and analysis of wastewater adhered to international environmental ethics and biosafety standards. Waste generated from analytical procedures was neutralized and disposed of in accordance with laboratory safety protocols and environmental regulations. Permissions were obtained from the relevant regulatory authorities and facility managers prior to sample collection to ensure compliance with institutional and governmental guidelines.

4.0 RESULTS AND DISCUSSION

This section presents and interprets the experimental results obtained from the development and implementation of green analytical chemistry (GAC) methods for monitoring selected pharmaceutical active ingredients (APIs) and their metabolites in Nigerian wastewater effluents. The findings are organized into five core areas: method validation, API concentration profiles in wastewater, environmental risk assessment, greenness assessment of analytical methods, and overall discussion in relation to global standards and previous literature.

4.1 Method Validation of Green Analytical Protocols

Before application to environmental samples, the developed green analytical methods were validated in accordance with the International Council for Harmonisation (ICH) Q2(R1) guidelines. Parameters assessed include linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, and relative standard deviation (RSD).

Table 1 presents the summary of the validation metrics for each targeted API.

Table 1: Summary of Method Validation Parameters for Selected APIs

API	R^2 (Linearity)	LOD (µg/L)	LOQ (µg/L)	Recovery (%)	RSD (%)
Amoxicillin	0.998	0.13	0.42	87.2	4.1
Ciprofloxacin	0.996	0.08	0.25	90.4	3.6
Paracetamol	0.999	0.05	0.15	93.5	2.9
Ibuprofen	0.997	0.07	0.21	88.7	3.4
Artemether	0.995	0.10	0.31	85.1	4.6
Lumefantrine	0.994	0.12	0.38	84.9	4.9

All compounds demonstrated excellent linearity (R^2 > 0.994), indicating accurate quantification across a range of concentrations. Recovery values were all above 84%, satisfying international analytical performance requirements. The low RSD values (≤ 5%) confirm the precision and repeatability of the GAC methods. These findings align with those of Tobiszewski (2016) and Gałuszka et al. (2018), who highlighted that GAC protocols can maintain analytical robustness when properly optimized, even under eco-friendly constraints such as reduced solvent volumes and biodegradable sorbents.

4.2 Concentration Profiles of APIs in Wastewater

Effluent and influent samples from wastewater treatment plants (WWTPs) in Lagos, Ogun, Delta, and Edo States were analyzed to determine the concentrations of selected APIs. Results are presented in Table 2**.**

Table 2: Detected Concentrations of APIs in Influent and Effluent Samples (µg/L)

API	Influent (Mean ± SD)	Effluent (Mean ± SD)
Amoxicillin	7.3 ± 0.5	2.1 ± 0.3
Ciprofloxacin	4.6 ± 0.7	1.4 ± 0.2
Paracetamol	11.2 ± 1.1	3.8 ± 0.6
Ibuprofen	5.4 ± 0.6	2.0 ± 0.4
Artemether	3.1 ± 0.4	0.9 ± 0.1
Lumefantrine	2.7 ± 0.3	0.8 ± 0.1

The influent samples showed notable concentrations of all six APIs, with paracetamol being the most abundant (11.2 µg/L), followed by amoxicillin (7.3 µg/L). Post-treatment effluent values were significantly lower, but none of the APIs were entirely removed. Ciprofloxacin and amoxicillin, in particular, were still present above 1 µg/L in treated water. These results highlight the inability of conventional WWTPs to completely eliminate pharmaceutical pollutants, consistent with the findings of Fekadu et al. (2019) and aus der Beek et al. (2016), who documented similar inefficiencies globally, especially in regions with underdeveloped tertiary treatment systems.

4.3 Environmental Risk Assessment of Detected APIs

To assess potential ecological harm, Risk Quotients (RQ) were calculated using measured environmental concentrations (MEC) from effluents and predicted no-effect concentrations (PNEC) sourced from ecotoxicological databases. The results are summarized in Table 3**.**

Four APIs—amoxicillin, ciprofloxacin, lumefantrine, and artemether—had RQ values >1, indicating a high environmental risk, especially to aquatic life. Ciprofloxacin posed the greatest threat (RQ = 7.0), a concern echoed by Danner et al. (2019), who emphasized the role of fluoroquinolones in fostering antibiotic resistance. The moderate

risk levels for paracetamol and ibuprofen also require attention due to their chronic exposure potential and ubiquity in urban waste streams.

Table 3: Risk Quotient (RQ) of APIs in Effluent Samples

API	MEC (µg/L)	PNEC (µg/L)	RQ	Risk Level
Amoxicillin	2.1	0.5	4.2	High
Ciprofloxacin	1.4	0.2	7.0	High
Paracetamol	3.8	10.0	0.38	Medium
Ibuprofen	2.0	4.0	0.50	Medium
Artemether	0.9	0.3	3.0	High
Lumefantrine	0.8	0.2	4.0	High

4.4 Green Analytical Assessment of Developed Methods

To evaluate the sustainability of the analytical protocols, the AGREE metric was used to score the greenness of each method based on the 12 principles of green analytical chemistry. Results are shown in Table 4**.**

Table 4: Green Assessment of Analytical Methods Using AGREE Score

API	AGREE Score (0–1)	Green Category
Amoxicillin	0.82	Excellent
Ciprofloxacin	0.85	Excellent
Paracetamol	0.79	Good
Ibuprofen	0.80	Good
Artemether	0.77	Good
Lumefantrine	0.76	Good

AGREE scores ranged from 0.76 to 0.85, placing all methods within the "good" to "excellent" green category. These scores reflect the use of water-ethanol mobile phases in HPLC, minimal solvent use in extraction techniques (e.g., SPME, UAE), and biodegradable materials in sorbent development. This aligns with recent advancements in GAC highlighted by Armenta et al. (2019), who stressed that modern green methods can match traditional techniques in analytical performance while significantly reducing environmental burden.

4.5 Comprehensive Discussion

This study reinforces the growing evidence that conventional wastewater treatment systems in Nigeria are insufficient in removing pharmaceutical pollutants, particularly antibiotics and antimalarials. The persistence of APIs in effluents may exacerbate antimicrobial resistance and endocrine disruption in aquatic species, consistent with earlier observations by Kümmerer et al. (2014) and Santos et al. (2013). The validated green analytical methods proved to be both technically reliable and environmentally responsible, as demonstrated by high recovery rates, excellent linearity, and strong AGREE scores. The integration of green protocols into pharmaceutical monitoring can enable developing countries like Nigeria to implement sustainable pollution control without the prohibitive cost and environmental drawbacks of conventional methods.

Furthermore, the inclusion of artemether and lumefantrine represents an important addition to pharmaceutical surveillance, given their underrepresentation in global datasets despite their heavy use in malaria-endemic regions (Ebele et al., 2017).

The study successfully applied validated green analytical chemistry techniques to quantify pharmaceutical residues in Nigerian wastewater effluents. All methods showed excellent analytical performance and complied with international standards for

green chemistry. Ciprofloxacin, amoxicillin, artemether, and lumefantrine were identified as high-risk pollutants due to their persistence and bioactivity. The findings underscore the need for enhanced treatment processes, expanded monitoring frameworks, and policy reforms targeting pharmaceutical pollution in Nigeria's aquatic environments.

5.0 CONCLUSION

The findings from this study confirm that multiple pharmaceutical active ingredients (APIs), including amoxicillin, ciprofloxacin, paracetamol, ibuprofen, artemether, and lumefantrine, persist in Nigerian wastewater effluents even after conventional treatment processes. Measured concentrations in effluent samples ranged from 0.8 µg/L to 3.8 µg/L, with particularly high residues observed for paracetamol and amoxicillin. Method validation demonstrated that the developed green analytical protocols based on Solid Phase Microextraction (SPME), Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE) were sensitive, precise, and reproducible, with linearity ($R^2 > 0.99$), recovery rates above 84%, and relative standard deviations under 5%. Risk Quotient (RQ) assessments revealed that several compounds, particularly ciprofloxacin and lumefantrine, pose high ecological risks due to their elevated environmental concentrations relative to their predicted no-effect concentrations (PNEC). The AGREE assessment metric rated all analytical methods as "good" to "excellent," indicating strong alignment with the twelve principles of green analytical chemistry.

In conclusion, the study demonstrates that conventional wastewater treatment technologies currently employed in Nigeria are inadequate for the complete removal of pharmaceutical residues. The persistence of antibiotics and antimalarials in treated effluents represents a significant ecological and public health concern due to their potential contribution to antimicrobial resistance and aquatic toxicity. The green analytical methods developed in this study proved to be both environmentally sustainable and technically effective, offering a viable alternative to conventional protocols for pharmaceutical monitoring.

It is recommended that regulatory agencies in Nigeria adopt green analytical chemistry approaches as standard tools for routine environmental monitoring of pharmaceuticals. Upgrading wastewater treatment infrastructure to include tertiary treatment technologies such as advanced oxidation, membrane filtration, or constructed wetlands would enhance removal efficiency. Public awareness campaigns and stricter pharmaceutical waste disposal regulations should also be implemented to reduce contamination at the source. Finally, expanded nationwide surveillance and ecotoxicological studies are essential for developing comprehensive environmental risk management strategies and supporting evidence-based policy reforms.

5.1 Limitations and Future Research

Despite the valuable insights generated by this pilot study, several limitations must be acknowledged. First, the geographic scope was limited to selected WWTPs in Lagos, Ogun, Delta, and Edo States, which may not fully represent national variability in pharmaceutical discharge and treatment efficiency. Broader coverage across Nigeria's geopolitical zones is necessary to develop a more comprehensive national baseline.

Second, the study focused primarily on a select group of widely used APIs (e.g., paracetamol, ciprofloxacin, amoxicillin), potentially overlooking other emerging contaminants such as non-steroidal anti-inflammatory drugs (NSAIDs), antidepressants, or veterinary drugs that may also be prevalent in effluents. Inclusion of these compounds in future monitoring will enhance the risk assessment profile.

Third, while green sample preparation techniques and analytical tools (SPME, MAE, UAE, HPLC, LC-MS/MS) were successfully applied, real-time monitoring approaches such as biosensors or portable field-deployable methods were not explored. Incorporating such innovations could support

more rapid and cost-effective surveillance in low-resource settings.

Lastly, the ecotoxicological impacts of detected pharmaceutical residues on local aquatic organisms were not evaluated. Future studies should integrate bioassays or in vitro models to better link chemical concentrations to ecological outcomes.

Future research should also aim to:

- Investigate advanced treatment options (e.g., ozonation, membrane filtration, constructed wetlands) to improve API removal.
- Develop locally sourced biodegradable SPME fibers for broader environmental application.
- Expand the application of green metrics like AGREE and GAPI to optimize new analytical protocols across different classes of pollutants.

6.0 REFERENCES

Adesuyi, A. A., Njoku, K. L., Akinola, M. O., & Jolaoso, A. O. (2018). Assessment of pharmaceutical residues in surface water of Lagos Lagoon using solid phase extraction method. *Journal of Environmental Protection*, 9, 3, pp. 337–354. https://doi.org/10.4236/jep.2018.93022

Adesuyi, A. A., Njoku, K. L., Akinola, M. O., & Jolaoso, A. O. (2018). Assessment of pharmaceutical residues in surface water of Lagos Lagoon using solid phase extraction method. *Journal of Environmental Protection*, 9, 3, pp. 337–354.

Armenta, S., Garrigues, S., & de la Guardia, M. (2015). The role of green analytical chemistry in ensuring food safety. *TrAC Trends in Analytical Chemistry*, 67, 2–13. https://doi.org/10.1016/j.trac.2014.12.001.

Armenta, S., Garrigues, S., & de la Guardia, M. (2019). Green analytical chemistry. *TrAC Trends in Analytical Chemistry*, 116, pp. 248–260.

Armenta, S., Garrigues, S., & de la Guardia, M. (2019). *Green analytical chemistry* (2nd ed.). Elsevier.

Aus der Beek, T., Weber, F. A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., & Küster, A. (2016). Pharmaceuticals in the environment—Global occurrences and perspectives. *Environmental Toxicology and Chemistry*, 35, 4, pp. 823–835. https://doi.org/10.1002/etc.3339.

Boxall, A. B. A., Rudd, M. A., Brooks, B. W., Caldwell, D. J., Choi, K., Hickmann, S., & Van Der Kraak, G. (2014). Pharmaceuticals and personal care products in the environment: What are the big questions? *Environmental Health Perspectives*, 120, 9, pp. 1221–1229. https://doi.org/10.1289/ehp.1104477.

Danner, M. C., Robertson, A., Behrends, V., & Reiss, J. (2019). Antibiotic pollution in surface fresh waters: Occurrence and effects. *Science of the Total Environment*, 664, pp. 793–804.

Danner, M. C., Robertson, A., Behrends, V., & Reiss, J. (2019). Antibiotic pollution in surface fresh waters: Occurrence and effects. *Science of the Total Environment*, 664, 793–804. https://doi.org/10.1016/j.scitotenv.2019.01.406

Daughton, C. G. (2014). The Matthew Effect and widely prescribed pharmaceuticals lacking environmental monitoring: Case study of an exposure-assessment vulnerability. *Science of the Total Environment*, 466, pp. 315–325. https://doi.org/10.1016/j.scitotenv.2013.07.062.

Ebele, A. J., Abdallah, M. A. E., & Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants*, 3, 1, pp. 1–16. https://doi.org /10.1016/j.emcon.2016.12.004.

Ebele, A. J., Abou-Elwafa Abdallah, M., & Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants*, 3, 1, pp. 1–16. https://doi.org/10.1016/j.emcon.2016.12.004.

Fatta-Kassinos, D., Vasquez, M. I., Schroder, H. F., & Kümmerer, K. (2011). Transformation products of

pharmaceuticals in surface waters and wastewater: Analytical aspects, occurrence, and toxicity. *Environment International*, 37, 2, pp. 357–376.

Fekadu, S., Alemayehu, E., Dewil, R., & Van der Bruggen, B. (2019). Pharmaceuticals in freshwater aquatic environments: A comparison of the African and European challenge. *Science of the Total Environment*, 654, pp. 324–337.

Fekadu, S., Alemayehu, E., Dewil, R., & Van der Bruggen, B. (2019). Pharmaceuticals in freshwater aquatic environments: A comparison of the African and European challenge. *Science of the Total Environment*, 654, pp. 324–337. https://doi.org/10.1016/j.scitotenv.2018.11.072.

Gałuszka, A., Migaszewski, Z. M., & Namieśnik, J. (2013). The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. *TrAC Trends in Analytical Chemistry*, 50, pp. 78–84. https://doi.org/10.1016/j.trac.2013.04.010.

Gałuszka, A., Migaszewski, Z., & Namieśnik, J. (2013). The 12 principles of green analytical chemistry and the SIGNIFICANCE OF GREEN METRICS. *TrAC Trends in Analytical Chemistry*, 50, pp. 78–84.

Gros, M., Petrovic, M., & Barceló, D. (2013). Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography–quadrupole-linear ion trap mass spectrometry. *Analytical Chemistry*, 85, 10, pp. 4822–4830.

Hernando, M. D., Mezcua, M., Fernández-Alba, A. R., & Barceló, D. (2014). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta*, 122, 92–99. https://doi.org/10.1016/j.talanta.2014.01.028.

Horstkotte, B., Giera, M., & Cordero, C. (2014). Green sample preparation strategies in environmental analysis. *Green Chemistry Letters and Reviews*, 7, 4, pp. 282–303.

Hughes, S. R., Kay, P., & Brown, L. E. (2013). Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. *Environmental Science & Technology*, 47(2), 661–677.

Jobling, S., Nolan, M., Tyler, C. R., Brighty, G., & Sumpter, J. P. (2006). Widespread sexual disruption in wild fish. *Environmental Science & Technology*, 40, 23, pp. 7046–7054. https://doi.org/10.1021/es0524245.

Khan, N. A., Khan, S. U., Ahmed, S., & Verpoort, F. (2017). Sustainable detection of pharmaceutical pollutants using green analytical chemistry: A review. *Environmental Science and Pollution Research*, 24, 15, pp. 13410–13424. https://doi.org/10.1007/s11356-017-8911-6.

Kidd, K. A., Paterson, M. J., Rennie, M. D., Podemski, C. L., Findlay, D. L., Blanchfield, P. J., & Liber, K. (2014). Direct and indirect responses of a freshwater food web to a potent synthetic oestrogen. *Philosophical Transactions of the Royal Society B*, 369, 1656, https://doi.org/10.1098/rstb.2013.0578

Koel, M., & Kaljurand, M. (2013). Application of the principles of green chemistry in analytical chemistry. *Pure and Applied Chemistry*, 85, 4, pp. 555–568. https://doi.org/10.1351/PAC-CON-12-10-10.

K'Oreje, K. O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., & Demeestere, K. (2016). Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere*, 149,pp. 238–244. https://doi.org/10.1016/j. chemosphere.2016.01.095.

Kümmerer, K. (2013). Emerging contaminants and their removal technologies. *Environmental Science: Processes & Impacts*, 15, 5, pp. 936–938. https://doi.org/10.1039/c3em90021b.

Kümmerer, K., Dionysiou, D. D., Olsson, O., & Fatta-Kassinos, D. (2018). A path to

clean water. *Science*, 361, pp. 222–224. https://doi.org/10.1126/science .aau2405.

Kümmerer, K., Dionysiou, D. D., Olsson, O., & Fatta-Kassinos, D. (2014). A path to clean water. *Science*, 361, pp. 222–224. https://doi.org/10.1126/science. aav6263.

Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., Dagot, C., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Science of the Total Environment*, 447, pp. 345–360. https://doi.org/10 .1016/j .scitotenv.2013.01.032.

Mutiyar, P. K., & Mittal, A. K. (2014). Occurrences and fate of selected human pharmaceuticals in the aquatic environment of India. *Environmental Monitoring and Assessment*, 186, 1, pp. 535–548. https://doi.org/10.1007/s10661-013-3402-4.

Mutiyar, P. K., & Mittal, A. K. (2014). Occurrences and fate of selected human pharmaceuticals in the aquatic environment of India. *Environmental Monitoring and Assessment*, 186, 1, pp. 535–548.

Ojemaye, C. Y., & Petrik, L. F. (2018). Pharmaceuticals in the marine environment: A review. *Environmental Reviews*, 26, 2, pp, 151–169. https:// doi.org/10.1139/er-2017-0060.

Płotka-Wasylka, J. (2018). A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. *Talanta*, 181, pp. 204–209.

Płotka-Wasylka, J. (2018). A new tool for the evaluation of the analytical procedure: Green Analytical Procedure Index. *Talanta*, 181,pp. 204–209. https://doi.org/ 10.1016/j.talanta.2018.01.013

Płotka-Wasylka, J., Rutkowska, M., Owczarek, K., Tobiszewski, M., & Namieśnik, J. (2017). Extraction with environmentally friendly solvents. *TrAC Trends in Analytical Chemistry*, 91, pp. 12–25. https://doi.org/10.1016/j.trac.201 7.03.006.

Płotka-Wasylka, J., Rutkowska, M., Owczarek, K., Tobiszewski, M., & Namieśnik, J. (2014). Extraction with environmentally friendly solvents. *TrAC Trends in Analytical Chemistry*, 61, pp.88–98. https://doi.org/10.1016/j.trac. 2014.06 .016.

Santos, L. H. M. L. M., Araújo, A. N., Fachini, A., Pena, A., Delerue-Matos, C., & Montenegro, M. C. B. S. M. (2013). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of Hazardous Materials*, 175, 1, 3, pp. 45–95. https://doi.org/10.1016/j.jhazmat.2 009.10.100.

Santos, L. H., Araújo, A. N., Fachini, A., Pena, A., Delerue-Matos, C., & Montenegro, M. C. B. S. M. (2013). Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of Hazardous Materials*, 175, 1, 3, pp. 45–95.

Tobiszewski, M. (2016). Metrics for green analytical chemistry. *Analytical Methods*, 8(, 15, pp. 2993–2999. https://doi.org/10. 1039/ C5AY03099E.

Verlicchi, P., Al Aukidy, M., & Zambello, E. (2012). Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Science of the Total Environment*, *429*,pp. 123–155. https://doi.org/10.1016/ j.scitotenv.2012.04.028.

World Health Organization (WHO). (2015). *Global Action Plan on Antimicrobial Resistance*. Geneva: WHO. https://www.who.int/publications/i/item/ 9789241509763