Detection and Quantification of Acetaminophen in Aquatic Samples From Cádiz, Spain

Hannah R. Desmarais
DePaul University, hdesmara@depaul.edu

Michelle N. Mikos
DePaul University, mmikos1@depaul.edu

Follow this and additional works at: https://via.library.depaul.edu/depaul-disc

Part of the Chemistry Commons, Fresh Water Studies Commons, Life Sciences Commons, Medicine and Health Sciences Commons, and the Social and Behavioral Sciences Commons

Recommended Citation

Detection and Quantification of Acetaminophen in Aquatic Samples From Cádiz, Spain

Acknowledgements

Detection and Quantification of Acetaminophen in Aquatic Samples from Cádiz, Spain

Hannah R. Desmarais* and Michelle N. Mikos**

Department of Chemistry and Biochemistry

Kyle R. Grice, PhD[1]; Jason Bystriansky, PhD[2]; Faculty Advisors

Department of Chemistry and Biochemistry[1]; Department of Biological Sciences[2]

ABSTRACT Pharmaceutical contaminants in water are becoming increasingly prevalent. This could have potentially harmful effects on human health and aquatic environments, even at low concentrations. Acetaminophen, the active ingredient of Tylenol ®, was the target compound for this study, and the aim was to determine its presence and concentration in various aquatic samples in and around the city of Cádiz, Spain. It was predicted that acetaminophen would be detected in low concentrations in river water, drain outflow, University of Cádiz seawater flow, and lagoon water, but undetectable in the University of Cádiz sink water and fountain water. The presence and concentration of acetaminophen in various water samples was determined through Ultra-Performance Liquid Chromatography (UP-LC) and Mass Spectroscopy (MS). Acetaminophen was detected at a concentration between 0.01 to 0.5 mg L^{-1} in each sample analyzed. This suggests that filtration mechanisms are not effective enough to remove pharmaceutical contaminants from water. The concentration was highest in the seawater flow at the University of Cádiz and was at the lowest concentration in the public fountain water. These results suggest that pharmaceuticals may be present in aquatic samples at higher concentrations than originally believed, and even sources of filtered water may contain pharmaceuticals.

INTRODUCTION

The contamination of water in the environment by pharmaceuticals is a growing threat worldwide. Pharmaceuticals are introduced to the environment via multiple paths, with the following three paths being most significant: human excretion, improper disposal, or industrial waste.[10] This may ultimately result in the presence of pharmaceuticals in potable water or food, which can have adverse effects on human health, even at low concentrations.[3] Figure 1 displays potential pathways of medical compounds to

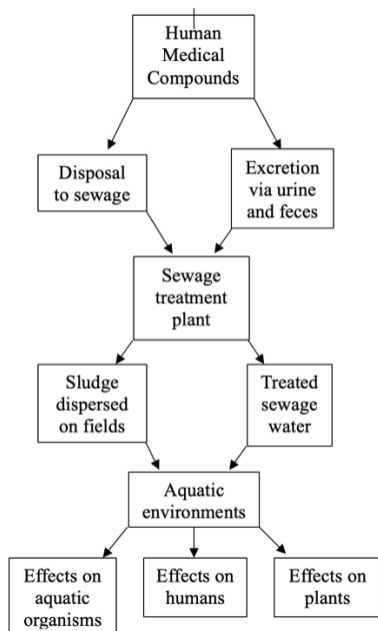reaching aquatic environments and the effects as a result.

Figure 1. Potential pathways of pharmaceuticals to reach aquatic environments.

Pharmaceuticals contaminating water can have various adverse effects on human health if present even at low levels. The potential adverse effects include acute and chronic damage, accumulation in tissues, reproductive damage, inhibition of cell generation, and behavioral changes.[9] Pharmaceutical compounds may also bioaccumulate in aquatic organisms and seafood, which then may be consumed by humans or other organisms. If bioaccumulation occurs in adult females of aquatic species, these compounds may be passed down to offspring during pregnancy.[12] Contaminated waters may also be used for irrigation of plants grown for consumption by humans and animals, which may cause damage to plants or pose hazards to their consumers.[6] Pharmaceutical contamination of water may also have toxic effects on the aquatic environments for organisms living in those environments. Some of the effects in fish include endocrine and hormonal disruption, reduced rates of food consumption, and oxygen depletion.[6] Acetaminophen caused hepatic oxidative stress and increased carboxylesterase activity, and acetylcholinesterase was depressed in the gills in adult *Mytilus galloprovincialis*, a species of bivalve, a marine mollusc.[11] Pharmaceuticals may

also inhibit the growth of aquatic plants, such as *Pseudokirchneriella subcapitata*, a species of algae.[5] Therefore, it is important to monitor the presence of phamaceuticals in the environment.

Acetaminophen, or paracetamol, is the most commonly consumed pain-relieving pharmaceutical worldwide. It has the trade name *Tylenol ®*, and it is classified as a non-steroidal anti-inflammatory drug (NSAID). It is most often taken as a pain-reliever but is also taken for its fever-reducing effects. Acetaminophen may also be combined with other medications to treat cough and cold symptoms, such as in the medication *Dayquil ®*.[2] If taken in excess of the recommended amount, liver damage or overdose may occur.[9] Pure acetaminophen is an odorless white solid with a molecular weight of 151.16 g/mol. It has a pKa of 6 and is soluble in water and methanol, as well as other solvents.[2] Figure 2 displays the chemical structure of acetaminophen.
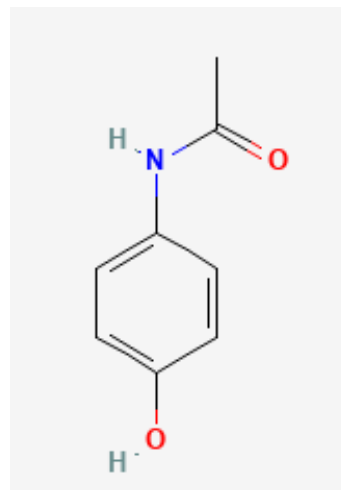
Figure 2. Chemical structure of acetaminophen.[8]

Acetaminophen is typically found at concentrations in the ng L^{-1} to μg L^{-1} range in aquatic environments.[8] It can be introduced to the environment by human excretion in urine. Less than 5% is excreted as unconjugated acetaminophen, and at least 90% of the dose is excreted within 24 hours of ingestion.[2] Since it is a very commonly used pain reliever beginning at age 2 for humans, its abundance around the globe is high. Due to the improper disposal that often occurs, a large contamination of water can result.

The aim of this study was to determine the presence and concentration of acetaminophen in various water sources in Cádiz, Spain. This can contribute to knowledge of the current levels in the environment, and the effectiveness of water treatment methods, providing insight on ways to improve them. This study also aims to increase awareness of the contamination of water that humans are constantly exposed to. This can promote safer disposal of pharmaceutical compounds, protecting aquatic environment and the organisms in them, as well as the health of humans.

Water samples were collected from 6 locations chosen, based on adjacence to the University of Cádiz, and filtered using vacuum filtration techniques to remove any large particles such as grass or dirt from the sample. The pH of each water sample was adjusted to ensure that acetaminophen itself would not be degraded during sample storage after collection. Solid phase extraction techniques (SPE) were also used to isolate the compound of focus, acetaminophen. SPE functions by retaining the analyte and sample compounds on the SPE material to later be eluted using a wash solvent. This results in a solution containing mostly the analyte for analysis purposes. Since the amounts of acetaminophen are low in comparison to the sample as an entirety, it is important to isolate it for purification. All samples were kept at room temperature. UP-LC, in a positive ion phase, was conducted to determine the presence and concentration of acetaminophen using a C18 1.7 µm (2.1x 50mm column) column. Based on background information, low concentrations of acetaminophen were expected in aquatic environment samples. It was expected that acetaminophen would be detected in river water, drain outflow, and lagoon water, but undetectable in the University of Cádiz sink water and seawater from the tap, and fountain water.

METHODS

The methods outlined were developed from those employed in *Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya.*[7]

Standards Preparation

One liter of water was collected from the following geographic coordinates in Cádiz, Spain: University of Cádiz tap water, collected from a bathroom sink; University of Cádiz seawater, collected from a laboratory tap; River of Saint Peter (36.531685, -6.215187); drain outflow into River of Saint Peter (36.531963, 6.214791); Lagoon of the Algaida (36.532993, -6.210134); and water flowing through the fountain in the Plaza of Flowers. The samples were filtered through 0.45 µm Whatman filter paper to remove particulates, and 250 mL of each sample were acidified with 33% acetic acid solution to a pH between 6.8 and 7.1. To increase extraction efficiency, approximately 1 g L^{-1} $Na_2EDTA \cdot 2\ H_2O$ was added to each sample. Oasis HLB SPE cartridges were activated with 6 mL methanol and 6 mL distilled water, and 100 mL of each sample were filtered onto each cartridge, which should bind any acetaminophen in the water. The cartridges were then rinsed 4 times with 6 mL of distilled water. Each sample was eluted with 6 mL methanol, which acted as the wash solvent. The 6 mL of methanol containing the sample was then collected and distributed into LC-MS vials which were filled to 1.5 mL of methanol each. These samples were later diluted 9:1 H_2O to methanol in order for improved data collection using the LC-MS instrumentation.

Samples Collection and Preparation

One liter of water was collected from the following sites in Cádiz, Spain: University of Cádiz tap water, University of Cádiz seawater, River of Saint Peter, drain outflow flowing into the River of Saint Peter, Lagoon of the Algaida, and the fountain in the Plaza of Flowers. The samples were filtered through filter paper to remove particulates, and 250 mL of each sample were acidified with 33% acetic acid solution to a pH between 6.8 and 7.1. To increase extraction efficiency, approximately 1 g L^{-1} $Na_2EDTA \cdot 2$ H_2O was added to each sample. The Oasis HLB SPE cartridges were activated with 6 mL methanol and 6 mL distilled water, and 100 mL of each sample were filtered onto each cartridge, which should bind any acetaminophen in the

water. The cartridges were then rinsed 4 times with 6 mL of distilled water. Each sample was eluted with 6 mL methanol, which acted as the wash solvent. The 6 mL of methanol containing the sample was then collected and distributed into LC-MS vials which were filled to 1.5 mL of methanol each. These samples were later diluted 9:1 H_2O to methanol in order for improved data collection using the LC-MS instrumentation.

HPLC Analysis

The samples and standards previously made were analyzed using HPLC/UPLC. The LC-MS instrument was a Waters Alliance HPLCE E2695 separation module. It was equipped with a Waters UV/Vis detector model 2489 and a Waters mass spectrometer model SQ Detector 2. The column used was a Waters Acquity UPLC BEH C18 column with 50 mm length, 2.1 mm inner diameter, and 1.7 μm particles. (Product 186002350). Solvent A of the mobile phase was 0.1% formic acid in water. Solvent B of the mobile phase was 0.1% formic acid in acetonitrile. A gradient method with a gas flow rate of 900, capillary voltage of 1.50, cone voltage of 0, and fluidics set to waste was set. Solvent concentration varied from 0-100% acetonitrile. An inlet method with a flow rate of 0.4 mL/min was maintained for sample analysis. An MS method of 2.4 minutes run time was used with an injection volume of 10 μL. Mass spectra data was acquired from 50-400 m/z and UV data at 274 nm. Samples were run for one replicate, then the machine was programmed to shut down. For analysis, ions were extracted at 152 m/z. The chromatographs for MS data were then smoothed to reduce noisy signals and exported. UV data was not smoothed but was also extracted at 152 m/z. The retention time was determined to be approximately 1.23 minutes for the samples.

RESULTS

To quantify presence of acetaminophen in aquatic samples, mass spectrometry can be used as the detection method, which allows for identifying and quantifying the presence of acetaminophen based on the appearance of the molecular ion in the MS. Water samples from various locations were analyzed using liquid chromatography-mass spectrometry, LC-MS, leading to the isolation and determination of acetaminophen present in the sample. This was done through MS detector data in the form of acetaminophen present in each sample was calculated. MS was used as the quantitative method as it allows for the desired component, in this case acetaminophen, to be extracted at a specific value of 152 m/z, reducing peak overlap or interference from other analytes.

A stock solution of 1 mg/L of acetaminophen was made and diluted to make multiple standard solutions with concentrations ranging from 1 mg/L to 1 μg/L. Water samples were also collected, and acetaminophen was eluted from them using Waters SPE cartridges. The series of standard solutions, in addition to the water samples, were injected into the LC-MS machine. This data can be seen in Figure 3.
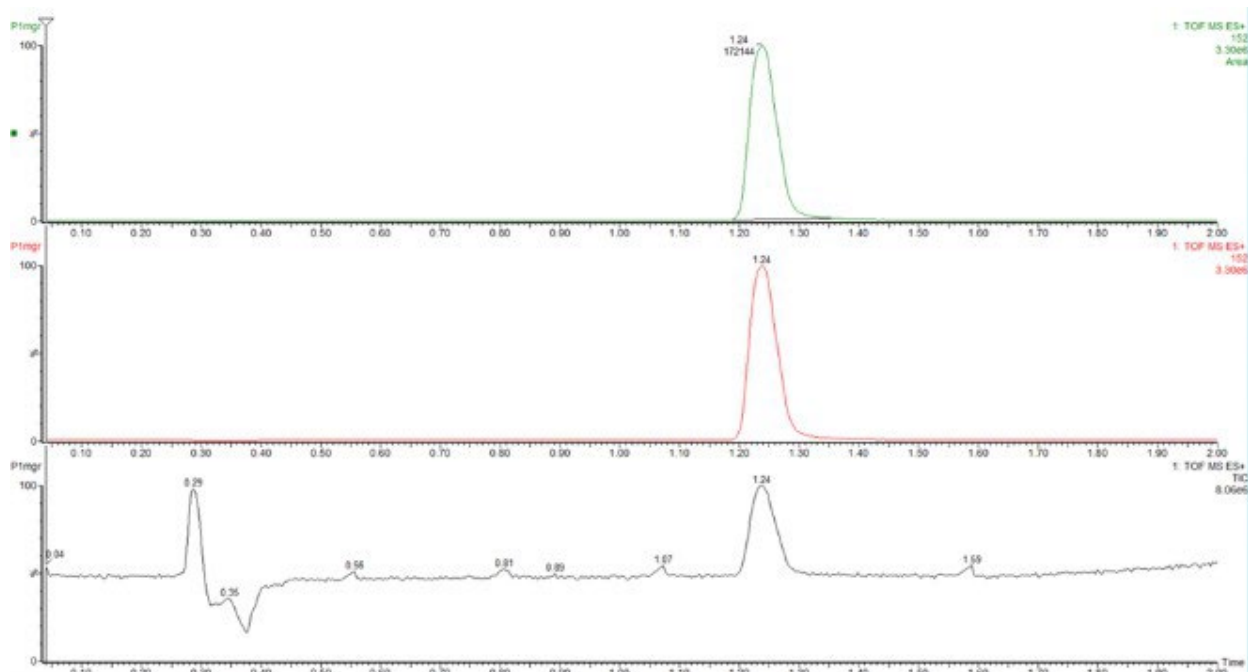
Figure 3. Chromatogram for 1 mg/L standard solution, each representing one run of the solution.

After analyzing the samples as shown in Figure 3, a standard curve, as shown in Figure 4, was constructed using the concentrations of the standards and the peak areas according to the MS data.
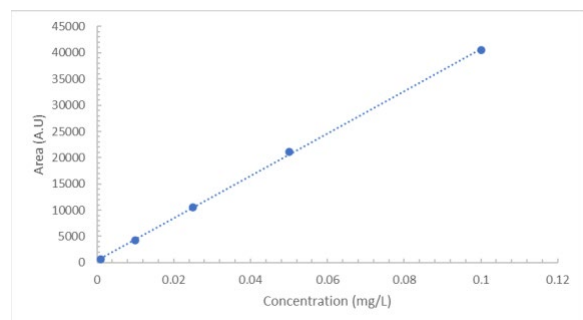

Figure 4. Standard Curve for the area of the acetaminophen peaks in standard solutions using MS data from a chromatogram. At 274 nm, the concentration of each standard was plotted in relation to its peak area detected, measured in absorbance units (AU). The line of best fit for the data was determined to have an equation of y = [403204 (±4606) (A.U./(mg/L))] x + [397.62 (±237) (A.U.)] (R^2=0.99961).

Using the data collected from MS data, Table 1 was created relating the concentration of the standard solutions and the peak area they were recorded to have.

Standard Concentration (mg/L)	Mean Peak Area (A.U)
0.10	40458
0.050	21073
0.025	10568
0.010	4244
0.0010	641

Table 1. Mean peak areas for acetaminophen standards.

Using the observed peak areas for the data set, calculations were completed to determine the concentration of acetaminophen present in the water samples. This was done with data retrieved from the line of best fit of the standard plot. Using simple calculations, the average concentration for the undiluted concentrations of acetaminophen was determined. This data is shown in Table

Sample	Mean Peak Area (A.U.)	Concentration (mg/L)
Fountain in the Plaza of Flowers	5323 (± 2604)	0.012 (± 0.006)
Drain Outflow into River of Saint Peter	8730 (± 111)	0.0207 (± 0.0003)
River of Saint Peter	21128 (± 11954)	0.05 (± 0.03)
Lagoon of the Algaida	21417 (± 594)	0.052 (± 0.001)
University of Cádiz Tap Water	45411 (± 2556)	0.112 (± 0.006)
University of Cádiz Seawater	211578 (± 91790)	0.5 (± 0.2)

Table 2. Mean peak areas and concentrations for water samples.

An example chromatogram of the data from one of the water samples containing acetaminophen can be seen in Figure 5, which can be compared to the standard in Figure 3.
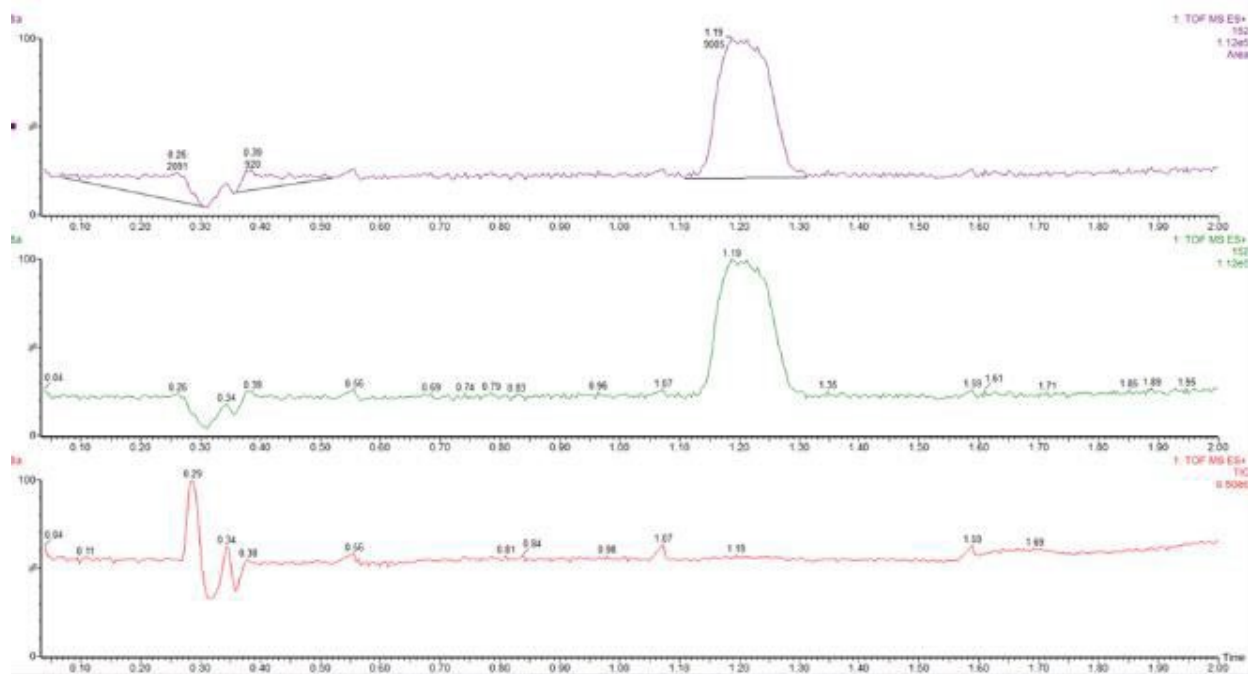
After analysis of Table 2, it can be determined that all samples contained detectable concentrations of acetaminophen. The curve created using standard samples, had a coefficient of determination with a value of approximately 0.9996, indicating a very good line of best fit between the data points that relate the concentrations of the standards to the peak areas. 3 replicates of each sample and standard were injected, with results being averaged only in the final step in creating Table 2. This led to more consistency and accuracy within the data.

Figure 5. Chromatogram for fountain water sample, runs 1-3. The peaks at about 1.19 minutes reflect the presence of acetaminophen.

DISCUSSION

Concentrations of acetaminophen were quantified using MS detection methods after the separation of the components done through LC. Using standard curves created from standard concentrations and peak areas, concentrations of acetaminophen present in various water samples were calculated. By doing so many replicates of each sample and standard, noise was factored out. The sample from the University of Cádiz seawater tap was determined to have the highest presence, with a detected concentration of 0.5238 mg L^{-1}. The sample with the lowest concentration was determined to be the sample obtained from the fountain in the Plaza of Flowers and was observed at 0.0122 mg/L.

According to the data, there was a presence of acetaminophen in all of the water samples tested. Experimental data collected displayed concentrations at the high end of typical values, though it has been detected at higher concentrations. As previously indicated, concentrations of acetaminophen are typically detected in the ng L^{-1} to µg L^{-1} range in aquatic

environments, and the concentrations detected in this experiment are at the higher end of this range. In other locations in Cádiz, Spain, acetaminophen has been detected at a concentration of 31,977-112,505 ng L^{-1} in wastewater influent, up to 41.5 ng L^{-1} in coastal water, and up to 2.8 ng L^{-1} in oceanic water.[12] The concentrations in coastal water are most relevant for comparison because the water collection sites in the environment were coastal. This suggests that the concentrations of acetaminophen in the samples collected in this study are much higher than those of coastal samples analyzed in previous studies. However, due to differing sources of water between the studies, it cannot be concluded that the concentrations were significantly different. In related studies, anti-inflammatory drugs such as acetaminophen are often detected in higher concentrations relative to other categories of drugs.[11] Because acetaminophen is a commonly used pharmaceutical, it is expected that it would be detected in relatively high concentrations in water samples.

Conclusion and Future Direction

Overall, it can be concluded that there were detectable traces of acetaminophen in each of the water samples tested. With a detection range of 0.01 to 0.5 mg L-1, the highest concentration was found to be in the seawater tap at the University of Cádiz. In the future, the analysis of presence of various other pharmaceuticals in water samples would be beneficial. The analysis of acetaminophen's presence in different water samples may also be further explored, as well as its degradation products. Finally, more long-term studies should be performed on plants and animals to see what, if any effects of long-term exposure to these levels of acetaminophen are. The effects on different species of algae, such as *Pseudokirchneriella subcapitata* may be analyzed, because these plants provide nutrition for many aquatic organisms. Various species of filter feeders, such as *Mytilus edulis*, may be studied to gain insight on the impact of acetaminophen on filtration .

REFERENCES

1. *Acetaminophen.* https://medlineplus.gov/druginfo/meds/a681004.html (accessed 2023-08-19).

2. *Acetaminophen.* https://pubchem.ncbi.nlm.nih.gov/compound/Acetaminophen (accessed 2023-08-19).

3. Baxfield, L.M.; Toccalino, P.L.; Belitz, K.; Foreman, W.T.; Furlong, E.T. Hormones and Pharmaceuticals in Groundwater Used as a Source of Drinking Water Across the United States. *Environ. Sci. Technol.* **2019**, *53* (6), 2950-2960. DOI: 10.1021/acs.est.8b05592

4. Biel-Maeso, M.; Baena-Nogueras, R.M.; Corada-Fernández, C.; Lara-Martín, P.A. 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.* **2017**, *612,* 649-659. DOI: 10.1016/j.scitotenv.2017.08.279

5. Gogoi, A.; Mazumder, P.; Tyagi, V.K.; Chaminda, G.G.T.; An, A.K.; Kuman, M. Occurrence and fate of emerging contaminants in water environment: A review. *Groundwater for Sustainable Development.* **2018**, *6*, 169-180. DOI: 10.1016/j.gsd.2017.12.009

6. Kayode-Afolayan, S.D.; Ahuekwe, E.F.; Nwinyi, O.C. Impacts of pharmaceutical effluents on aquatic ecosystems. *Scientific African.* **2022**, *17* (e01288). DOI: 10.1016/j.sciaf.2022.e01288

7. K'oreje, K.O.; Vergeynst, L.; Ombaka, D.; De Wispelaere, P.; Okoth, M.; Van Langenhove, H.; Demeestere, K. Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere.* **2016**, *149*, 238-244. DOI: 10.1016/j.chemosphere.2016.01.

8. Montaseri, H.; Forbes, P.B.C. Analytical techniques for the determination of acetaminophen: A review. *TrAC Trends in Analytical Chemistry*. **2018**, *108*, 122-134. DOI: 10.1016/j.trac.2018.08.023

9. Phillips, P.J.; Smith, S.G.; Kolpin, D.W.; Zaugg, S.D.; Buxton, H.T.; Furlong, E.T.; Esposito, K.; Stinson, B. Pharmaceutical Formulation Facilities as Sources of Opioids and Other Pharmaceuticals to Wastewater Treatmetn Plant Effluents. *Environ. Sci. Technol.* **2010**, *44* (13), 4910-4916. DOI: 10.1021/es100356f

10. Sebastine, I.M.; Wakeman, R.J. Consumption and Environmental Hazards of Pharmaceutical Substances in the UK. *Process Safety and Environmental Protection.* **2003**, *81* (4), 229-235. DOI: 10.1205/095758203322299743

11. Solé, M.; Shaw, J.P.; Frickers, P.E.; Readman, J.W.; Hutchinson, T.H. Effects on feeding rate and biomarker responses of marine mussels experimentally exposed to propranolol and acetaminophen. *Anal. Bioanal. Chem.* **2010**, *396*, 649–656. DOI: 10.1007/s00216-009-3182-1

12. Van de Merwe, J.P.; Chan, A.K.Y.; Lei, E.N.Y.; Yau, M.S.; Lam, M.H.W.; Wu, R.S.S. Bioaccumulation and maternal transfer of PBDE 47 in the marine medaka (*Oryzias melastigma*) following dietary exposure. *Aquatic Toxicology.* **2011**, *103* (3-4), 199-204.DOI: 10.1016/j.aquatox.2011.02.021