Parabens as endocrine disrupting compounds (EDCs): Analytical method for determination in environmental matrices

Rohaya Abd. Wahab[a]; Tuan Fauzan Tuan Omar[a,b] ; Mohd Yusoff Nurulnadia[a,b]
[a]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Mengabang Telipot, 21030 Kuala Nerus, Terengganu*
[b]*Ocean Pollution and Ecotoxicology Research Group, Faculty of Science and Marine Environment, Mengabang Telipot, 21030 Kuala Nerus, Terengganu*
**Corresponding author: tuan.fauzan@umt.edu.my*

Abstracts
Endocrine disrupting compounds (EDCs) discharged into the wastewater are persistent due to their continuous introduction into the environment. Recently, environmental pollution and the impacts of parabens have generated increased concern. Due to their antimicrobial and antifungal properties, parabens are a class of EDCs commonly used as preservatives. in cosmetic, personal care, food, and pharmaceutical applications. The widespread uses of parabens in a variety of applications have resulted in environmental contamination. As a result, the determination of parabens using an optimized solid phase extraction (SPE) clean up together with high-performance liquid chromatography (HPLC) instruments to analyze the concentrations of parabens in environmental matrices samples was successfully documented in this paper.
Keywords: endocrine disrupting compounds (EDCs); parabens; environmental matrices; solid phase extraction (SPE); high performance liquid chromatography (HPLC)

1. Introduction

There is increasing evidence that parabens are identified as endocrine-disrupting compounds by their capacity to modulate the endocrine system (EDCs) (Wei et al., 2021). EDCs are a diverse group of chemicals that cause abnormal development by altering organism's hormonal and homeostatic systems introduced into the environment due to anthropogenic activities (Tiwari et al., 2016). EDCs can mimic or antagonize the effects of natural hormones and bind to cell receptors to block their natural functions. There are two potential mechanisms through which EDCs can interfere with hormone action. First, a chemical interacts directly with a hormone receptor; second, a hormone-receptor protein complex is directly affected (Kasonga et al., 2021). Furthermore, EDCs can potentially interfere with endogenous hormones and affect the reproductive capacity of aquatic organisms and humans; finally, can cause serious harm to the

reproductive, nervous, and immune systems of aquatic organisms (Wang et al., 2016; Deng et al., 2019).

Parabens are endocrine-disrupting compounds (EDCs) to be preservatives in personal care products, cosmetics, pharmaceuticals, and food (Bledzka et al., 2014). Parabens effectively prevent fungi, bacteria, and yeast from spoiling products and, at the same time, do not alter cosmetics or foods' fragrance, flavor, or other properties (Barabasz et al., 2019).

Methylparaben (MeP), ethylparaben (EtP), and propylparaben (PrP) are the three most frequently utilized parabens, and their usage has grown substantially throughout the years (Vale et al., 2022). Additionally, combining two or more parabens can increase their antibacterial action without exceeding their respective legal maximum concentrations (Ma et al., 2018; Alampanos & Samanidou, 2021; Wei et al., 2021). Most of the time, a mix of parabens with different solubility properties is used so that their synergistic effects can make the system more effective against contamination (Wei et al., 2021)**.**

2. Physicochemical characteristics of parabens

Parabens are the 4-hydroxybenzoic acid alkyl esters. Based on the position of the ester groups at the C-4 position, parabens are typically divided into seven distinct types: methyl-, ethyl-, propyl-, isopropyl-, butyl-, and isobutyl-parabens. Methylparaben (MeP) and ethylparaben (EtP) are short-chain parabens, while propylparaben (PrP), isopropylparaben (i-PrP), butylparaben (BuP), isobutylparaben (i-BuP), and benzylparaben are long-chain parabens. (BzP) (Haman et al., 2015). With the alkyl chain increase, parabens' water solubility diminishes while their stability, antibacterial activity, and cell penetrability rise. Because most microbial growth happens in water, parabens with a greater alkyl chain length are less effective (Alampanos & Samanidou, 2021; Wei et al., 2021).

These compounds possess unique characteristics, including low volatility, very stable that is resistant to hydrolyze, and good water solubility (Jalilian et al., 2019). Physically, parabens are white crystals or crystalline powders at room temperature; chemically, they are readily soluble in alcohol, ether, and acetone (Fransway et al., 2019). Most parabens can retain in acidic conditions. On the other hand, parabens break down into p-hydroxybenzoic acid and the corresponding alcohol in alkaline solutions (Lin et al., 2011; Błedzka et al., 2014; Li et al., 2016).

The ratio of a compound's concentration in n-octanol and water under equilibrium conditions at a particular temperature is known as the water partition coefficient (Kow). Simultaneously, as the length of the alkyl chain rises, the value of the octanol-water partition coefficient (K_{ow}) increases, resulting in a decrease in water solubility. Compounds that have an increased

molecular weight readily adsorbed to sediments. Table 1 shows the physicochemical characteristics of targeted parabens.

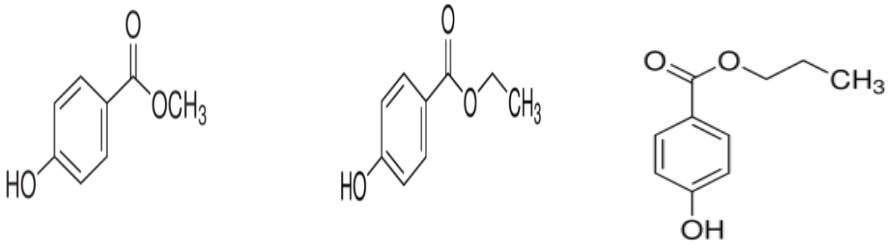
Table 1 The physicochemical characteristics of targeted parabens

Chemical	CAS Number[a]	Molecular formular[a]	Molecular weight[a]	Water solubility (mg /L)[b]	pK_a [b]	LogK_{OW} [b]
Methylparaben	99-76-3	$C_8H_8O_3$	152.14	2 500	8.31	2.00
Ethylparaben	120-47-8	$C_9H_{10}O_3$	166.17	885	8.50	2.49
Propylparaben	94-13-3	$C_{10}H_{12}O_3$	180.20	500	8.23	2.98

pK_a: Acid dissociation constant; K_{OW}: Octanol-water partition coefficient.

Source: [a]Wei et al., (2021) , [b]Lu et al., (2021)

Based on the information in Table 1, all the chemical structure of targeted parabens is shown in Figure 1.



Methylparaben Ethylparaben Propylparaben

Figure 1 Chemical structure of methylparaben, ethylparaben and propylparaben

3. Sources and fate of parabens in the ecosystem

EDCs are introduced directly and inadvertently into the aquatic environment through point-source pollution from sources like industrial discharges, urban wastewater effluents, and a variety of nonpoint or diffuse sources, such as surface runoff (Noutsopoulos et al., 2019;

Kasonga et al., 2021). The primary sources include wastewater from industrial, hospital, domestic, and agricultural activities (K'oreje et al., 2020). Consequently, this could also affect drinking water sources, potentially hazardous to human health. Additionally, numerous studies have shown that personal care product residues in aquatic products impact human health (Lulijwa et al., 2020; Chen et al., 2021).

It has been demonstrated that these endocrine-mediated mechanisms have endocrine effects on the organism by interfering with multiple biological systems via distinct pathways and mechanisms (Ismail et al., 2020). Fan et al. (2021) reported that EDCs had attracted global concern in recent decades due to their significant ecological and human health risks, like deteriorating quality and quantity of human sperm, adult obesity, neurotoxicity, diabetes, and hormone-related cancers.

Most personal care products have one or two antimicrobial parabens, which are potential entry points for parabens into the ecosystem of rivers. The second possibility is that the parabens originated in the municipal sewage system. As ingredients of personal care products, pharmaceuticals, and cosmetics, parabens end up in the sewage system. These chemicals will accumulate and cause irreversible environmental changes due to their difficulty in biodegrading (Barabasz et al., 2019). Figure 2 illustrates the origins and pathways of paraben exposure in the ecosystem.

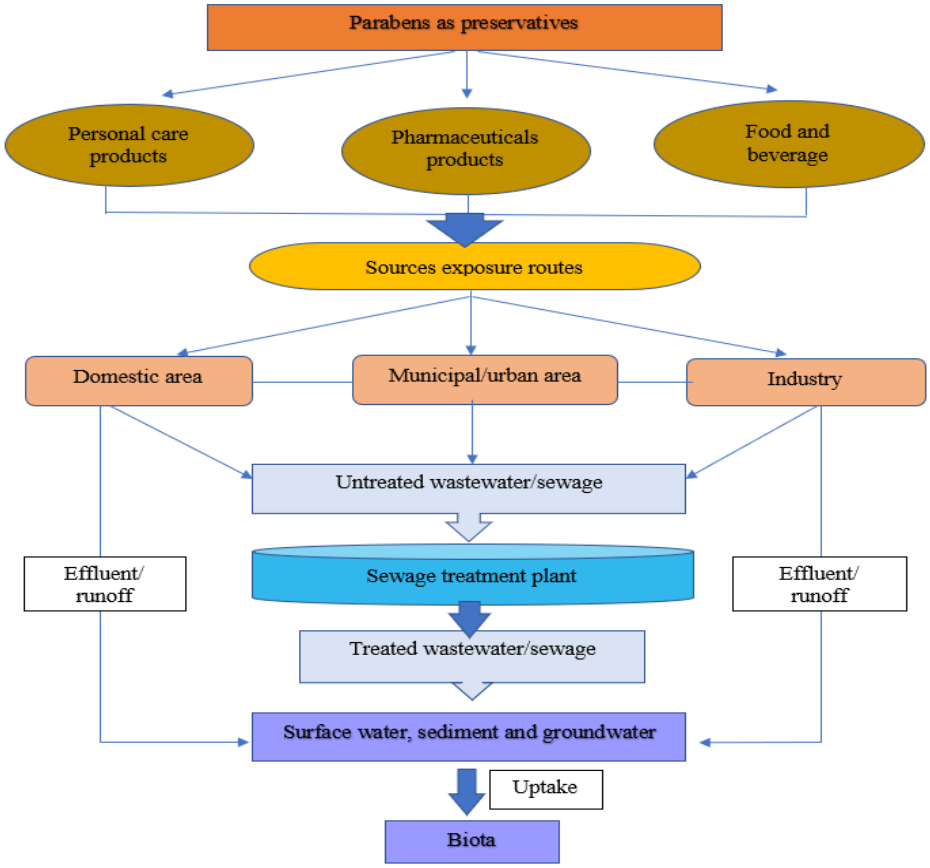


Figure 2 The origins and pathways of paraben exposure in the ecosystem.

4. Determination of parabens in environmental matrices

Prior to any analytical determination, sample preparation is required. This is because analytical instruments are not sensitive enough to find trace analytes directly in complex matrices. Target analytes were isolated and/or enriched throughout this step to prepare the samples for further analysis. The clean-up process will ensure the final extracts are clean, crucial for instrument analysis and detecting analytes at low levels. The extraction procedure is optimized to get the required accuracy and sensitivity. The aim is to minimize interferences as much as possible and eliminate co-extracted compounds.

Many techniques have been developed for effectively extracting the analyte because it is identified at low levels in aquatic environments. These techniques include liquid-liquid extraction, steam distillation extraction, and solid-phase extraction. The main advantages of the SPE method are that it has a high recovery percentage, is easy to automate, has analysis time by reducing the number of steps, works with chromatographic analysis, and uses less organic solvent. Moreover, SPE is a method of sample preparation applied in a wide variety of fields because it has many advantages over more conventional methods (Ötles & Kartal, 2016).

SPE focuses on partitioning solutes between a liquid phase (the sample matrix) and a solid phase. After extraction, solid sorbent pre-concentrates and purifies analytes from the sample. (Zwir-Ferenc & Biziuk, 2006). Since SPE requires less time and solvent, it has become the preferred method for extracting the analyte of interest and clean-up interferences from water samples (Baharom et al., 2020).

SPE is one of the most prevalent techniques for extracting pollutants from aqueous samples due to its simplest use and efficiency (Márquez-Sillero et al., 2010; Marta-Sanchez et al., 2018) It has been extensively reported for paraben extraction (Renz et al., 2013; Carmona et al., 2014). In addition, these approaches provide excellent selectivity and sensitivity while reducing or eliminating the amount of organic solvent used (Marta-Sanchez et al., 2018; Omar et al., 2021). Furthermore, SPE is able to be used in conjunction with a variety of other analysis techniques when employed to sample data. The four crucial main steps of SPE are outlined in Figure 3.

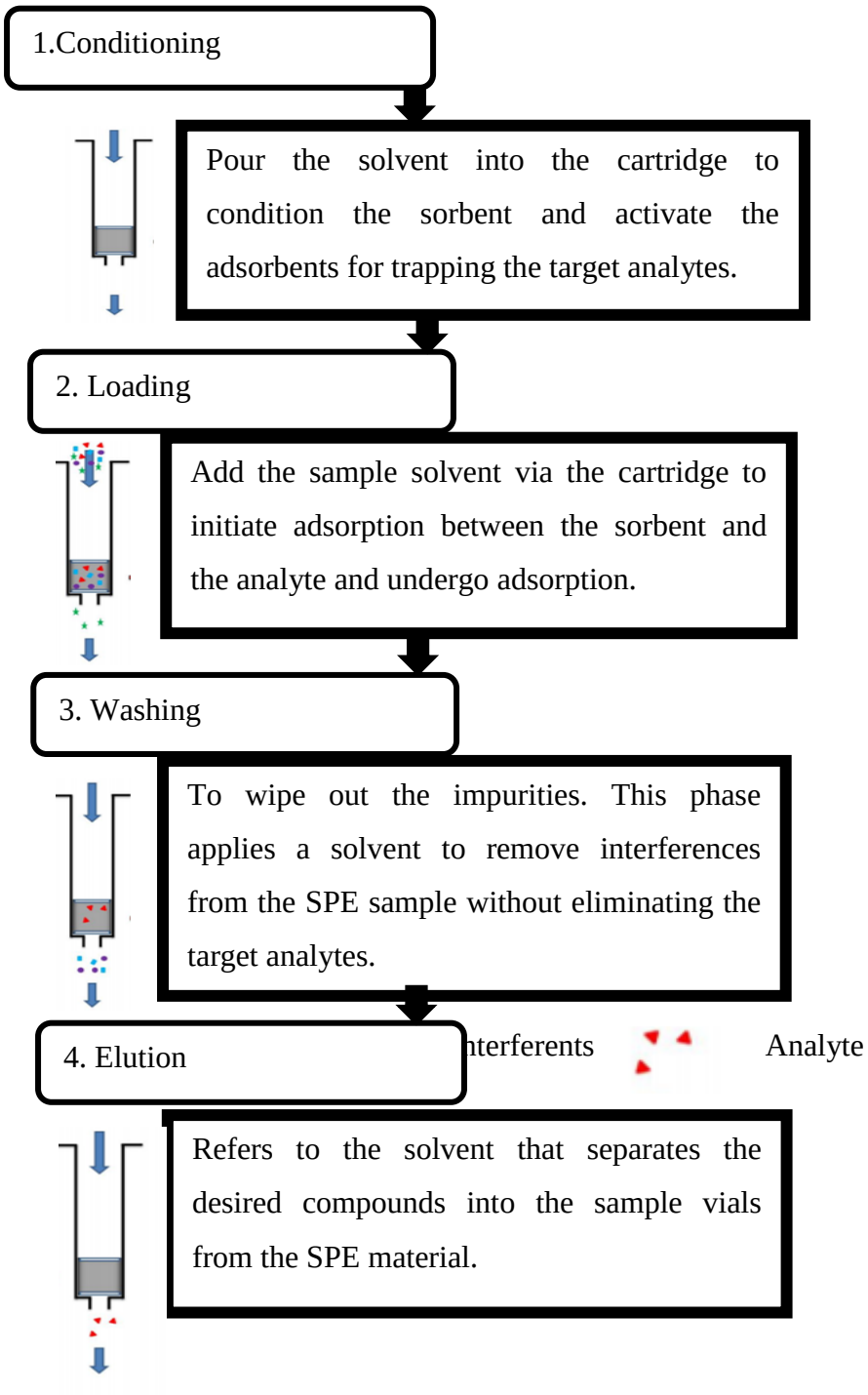

1.Conditioning
Pour the solvent into the cartridge to condition the sorbent and activate the adsorbents for trapping the target analytes.
2. Loading
Add the sample solvent via the cartridge to initiate adsorption between the sorbent and the analyte and undergo adsorption.
3. Washing
To wipe out the impurities. This phase applies a solvent to remove interferences from the SPE sample without eliminating the target analytes.
4. Elution
nterferents
Analyte
Refers to the solvent that separates the desired compounds into the sample vials from the SPE material.

Figure 3 The four crucial main steps of SPE.

High performance liquid chromatography (HPLC) is the remarkably versatile and commonly utilized elution chromatography. This chromatographic technique was developed in the early 1970s as an alternative to GC and utilized liquid as the mobile phase (Deng et al., 2019). This technique is applied in analytical chemistry to separate, identify, and quantify each component in various organic, inorganic, and biological materials.

HPLC procedures were developed by trial and error using analyst expertise, knowledge, and judgment. Since the compounds need to be derivatized for gas chromatographic separation to proceed, HPLC techniques are more widely applied for parabens determination (Márquez-Sillero et al., 2010). In any instance, sample preparation procedures must be followed before injecting analytes into the chromatographic system.

Typical components of an HPLC instrument are a degasser, sampler, pumps, and detector. The sampler injects a sample mixture into the flow of the mobile phase. The flow of the mobile phase then moves the sample mixture to the column. Pumps ensure the mobile phase moves via the column quickly and easily. The signal produced by the detector corresponds to the sample component concentration. A digital microprocessor controls an HPLC instrument and also does data analysis. Due to its greater adaptability, HPLC has become increasingly popular for application in analyzing compounds in recent years (Locatelli et al., 2016). Figure 4 describes four basic principal parts of HPLC and an important parameter that should be considered during clean up and HPLC analysis is summarized in Table 2

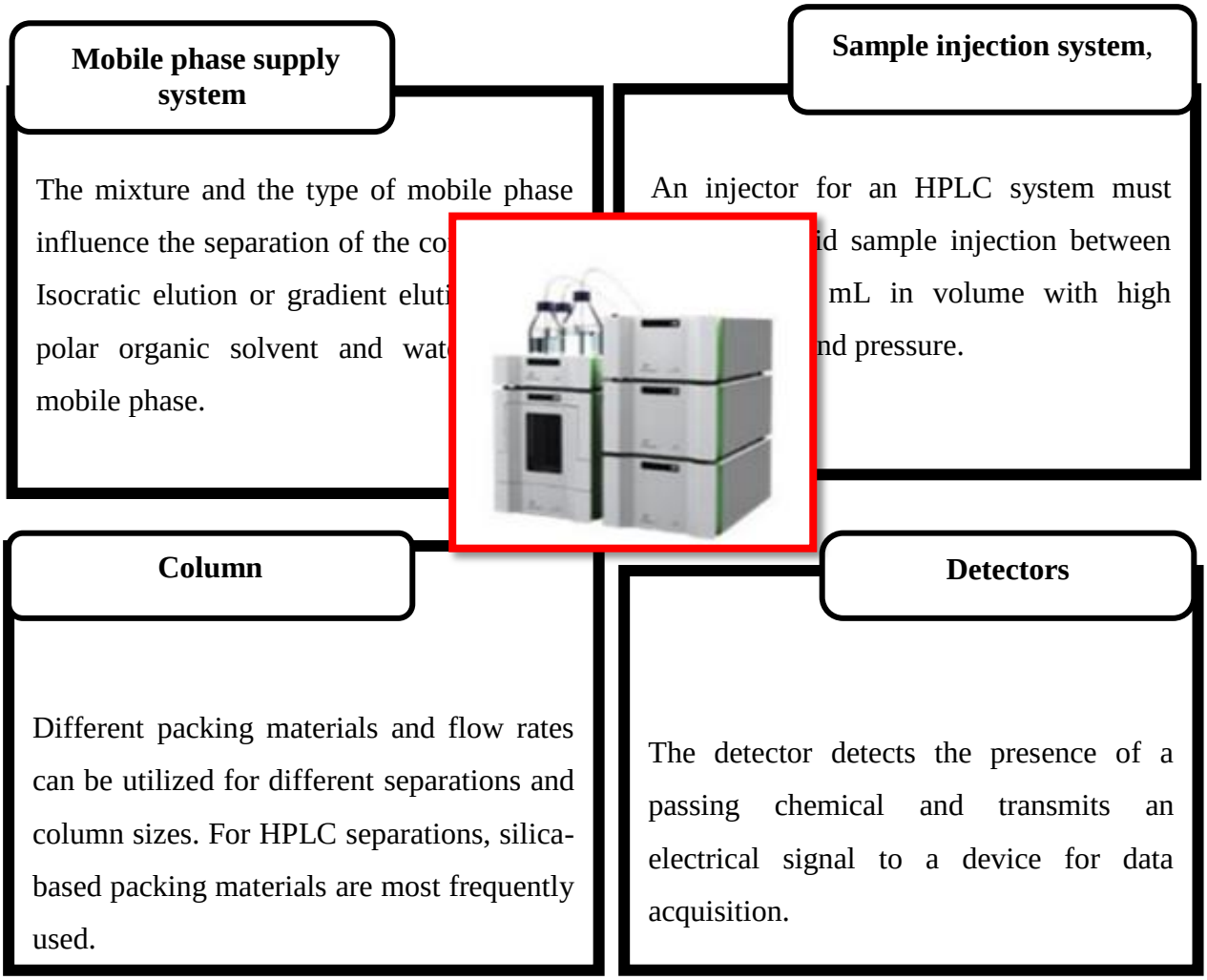


Figure 4 Four basic principle parts of HPLC

Table 2 Parameters for consideration during SPE clean up and HPLC analysis

Parameter	Function	Notes
Clean up sorbent	Reduction/elimination of matrix interferences	Choosing sorbents like polymeric C_{18} will help in the reduction and elimination of matrix interferences, allowing for achieving

		the lowest possible detection limit
Mobile phase	Separation of analyte	The peak separation of synthesized compounds can be improved by choosing an appropriate mobile phase composition.
Chromatographic column	Peak separation in HPLC	Selecting an appropriate chromatographic column will result in a good peak shape and the rapid elution of compounds.

Adapted from Omar et al., 2016

Figure 5 shows an overview methodology for the determination of parabens in environmental matrices. Essential steps include sample preparation and cleanup. To increase the selectivity, sensitivity, reliability, accuracy, and reproducibility of the analysis, the parabens are separated from the matrices and pre-concentrated in this step (Piao et al., 2014). The sample preparation method used largely depends on the type of matrix. Particles were removed from the water samples by filtration, and solid samples were usually homogenized, dried, and sieved before the subsequent extraction. Analytes are extracted from the sample matrix using organic solvents such as methanol, acetone, and acetonitrile for sediments and fish. In general, SPE is applied directly to liquid samples, while for solid samples, the supernatant of the analyte was extracted by ultrasonic extraction. HPLC makes the final determination as an instrument for quantifying parabens as one of the most commonly used method.

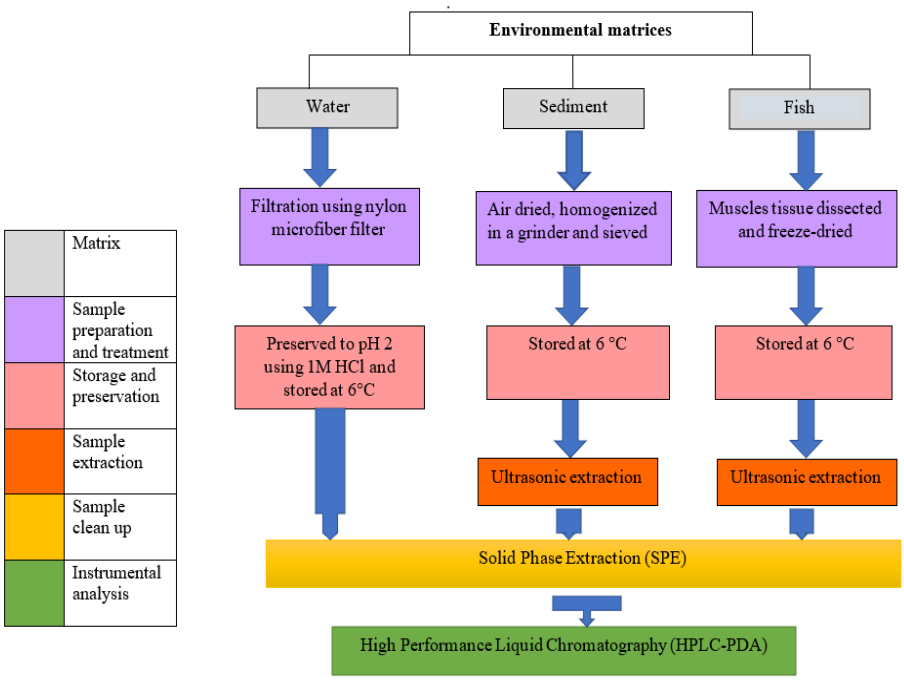


Figure 5 Overview methodology for detection of parabens in water, sediment and fish.

5. Conclusion

The quantity of EDCs that are entering the environment as a result of human activities is continuously growing. This is because of the rising demand for various products, such as pharmaceuticals and personal care items. Large quantities of parabens have entered aquatic systems via domestic and industrial wastewater due to their widespread use in consumer products. Aquatic ecosystems are particularly susceptible to anthropogenic contamination, primarily from household activities. Concerning the low concentration of parabens present in the sample matrices, it is necessary to complete the extraction and cleaning steps before the concentration is determined. Nevertheless, paraben detection helps authorities make informed decisions regarding the regulation, use, and disposal of parabens by monitoring their environmental release and impact on ecosystems.

Declaration of competing interests

The authors declare no competing financial interest.

Acknowledgments

Funding: This work was supported by Talent and Publication Enhancement-Research Grant (TAPE-RG/ 55237) provided by Universiti Malaysia Terengganu. The authors would like to thank staff of Faculty Science and Marine Environment, as well as Centre for Research and Field Services for their technical assistance.

REFERENCES

Alampanos, V., & Samanidou, V. (2021). An overview of sample preparation approaches prior to liquid chromatography methods for the determination of parabens in biological matrices. *Microchemical Journal*, *164*(January), 105995. https://doi.org/10.1016/j.microc.2021.105995

Baharom, M. Z., Yusoff, N. M., Khalik, W. M. A. W. M., Ariffin, M. M., Karim, J., & Sharifuddin, S. S. (2020). Extraction of 4-octylphenol and 4-nonylphenol in river water using solid-phase extraction and high-performance liquid chromatography. *Malaysian Journal of Analytical Sciences*, *24*(1), 146–158.

Barabasz, W., Pikulicka, A., Wzorek, Z., & Nowak, A. K. (2019). Ecotoxicological aspects of the use of parabens in the production of cosmetics. *Czasopismo Techniczne*, *12*, 99–124. https://doi.org/10.4467/2353737xct.19.126.11451

Błedzka, D., Gromadzińska, J., & Wasowicz, W. (2014). Parabens. From environmental studies to human health. *Environment International*, *67*, 27–42. https://doi.org/10.1016/j.envint.2014.02.007

Carmona, E., Andreu, V., & Picó, Y. (2014). Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: From waste to drinking water. *Science of the Total Environment*, *484*(1), 53–63. https://doi.org/10.1016/j.scitotenv.2014.02.085

Chen, H., Chen, W., Guo, H., Lin, H., & Zhang, Y. (2021). Pharmaceuticals and personal care products in the seawater around a typical subtropical tourist city of China and associated

ecological risk. *Environmental Science and Pollution Research*, *28*(18), 22716–22728. https://doi.org/10.1007/s11356-020-12335-1

Deng, Z. H., Li, N., Jiang, H. L., Lin, J. M., & Zhao, R. S. (2019). Pretreatment techniques and analytical methods for phenolic endocrine disrupting chemicals in food and environmental samples. *TrAC - Trends in Analytical Chemistry*, *119*. https://doi.org/10.1016/j.trac.2019.07.003

Fan, D., Yin, W., Gu, W., Liu, M., Liu, J., Wang, Z., & Shi, L. (2021). Occurrence, spatial distribution and risk assessment of high concern endocrine-disrupting chemicals in Jiangsu Province, China. *Chemosphere*, *285*(June), 131396. https://doi.org/10.1016/j.chemosphere.2021.131396

Fransway, A. F., Fransway, P. J., Belsito, D. V., Warshaw, E. M., Sasseville, D., Fowler, J. F., DeKoven, J. G., Pratt, M. D., Maibach, H. I., Taylor, J. S., Marks, J. G., Mathias, C. G. T., DeLeo, V. A., Zirwas, J. M., Zug, K. A., Atwater, A. R., Silverberg, J., & Reeder, M. J. (2019). Parabens. *Dermatitis*, *30*(1), 3–31. https://doi.org/10.1097/DER.0000000000000429

Haman, C., Dauchy, X., Rosin, C., & Munoz, J.-F. (2015). Occurrence, fate and behavior of parabens in aquatic environments: A review. *Water Research*, *68*, 1–11. https://doi.org/10.1016/j.watres.2014.09.030

Ismail, N. A. H., Wee, S. Y., Haron, D. E. M., Kamarulzaman, N. H., & Aris, A. Z. (2020). Occurrence of endocrine disrupting compounds in mariculture sediment of Pulau Kukup, Johor, Malaysia. *Marine Pollution Bulletin*, *150*(November 2018), 110735. https://doi.org/10.1016/j.marpolbul.2019.110735

Jalilian, N., Ebrahimzadeh, H., & Asgharinezhad, A. A. (2019). Preparation of magnetite/multiwalled carbon nanotubes/metal-organic framework composite for dispersive magnetic micro solid phase extraction of parabens and phthalate esters from water samples and various types of cream for their determination with liquid. *Journal of Chromatography A*, *1608*. https://doi.org/10.1016/j.chroma.2019.460426

K'oreje, K. O., Okoth, M., Van Langenhove, H., & Demeestere, K. (2020). Occurrence and treatment of contaminants of emerging concern in the African aquatic environment: Literature review and a look ahead. *Journal of Environmental Management*, *254*(November 2019), 109752. https://doi.org/10.1016/j.jenvman.2019.109752

Kasonga, T. K., Coetzee, M. A. A., Kamika, I., Ngole-Jeme, V. M., & Benteke Momba, M. N. (2021). Endocrine-disruptive chemicals as contaminants of emerging concern in wastewater and surface water: A review. *Journal of Environmental Management*, *277*(October 2020), 111485. https://doi.org/10.1016/j.jenvman.2020.111485

Li, W., Gao, L., Shi, Y., Wang, Y., Liu, J., & Cai, Y. (2016). Spatial distribution, temporal variation and risks of parabens and their chlorinated derivatives in urban surface water in Beijing, China. *Science of the Total Environment*, *539*, 262–270. https://doi.org/10.1016/j.scitotenv.2015.08.150

Lin, Y., Ferronato, C., Deng, N., & Chovelon, J. M. (2011). Study of benzylparaben

photocatalytic degradation by TiO2. *Applied Catalysis B: Environmental*, *104*(3–4), 353–360. https://doi.org/10.1016/j.apcatb.2011.03.006

Locatelli, M., Sciascia, F., Cifelli, R., Malatesta, L., Bruni, P., & Croce, F. (2016). Analytical methods for the endocrine disruptor compounds determination in environmental water samples. *Journal of Chromatography A*, *1434*, 1–18. https://doi.org/10.1016/j.chroma.2016.01.034

Lu, S., Lin, C., Lei, K., Xin, M., Wang, B., Ouyang, W., Liu, X., & He, M. (2021). Endocrine-disrupting chemicals in a typical urbanized bay of Yellow Sea, China: Distribution, risk assessment, and identification of priority pollutants. *Environmental Pollution*, *287*(February), 117588. https://doi.org/10.1016/j.envpol.2021.117588

Lulijwa, R., Rupia, E. J., & Alfaro, A. C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Reviews in Aquaculture*, *12*(2), 640–663. https://doi.org/10.1111/raq.12344

Ma, W. L., Zhao, X., Zhang, Z. F., Xu, T. F., Zhu, F. J., & Li, Y. F. (2018). Concentrations and fate of parabens and their metabolites in two typical wastewater treatment plants in northeastern China. *Science of the Total Environment*, *644*, 754–761. https://doi.org/10.1016/j.scitotenv.2018.06.358

Márquez-Sillero, I., Aguilera-Herrador, E., Cárdenas, S., & Valcárcel, M. (2010). Determination of parabens in cosmetic products using multi-walled carbon nanotubes as solid phase extraction sorbent and corona-charged aerosol detection system. *Journal of Chromatography A*, *1217*(1), 1–6. https://doi.org/10.1016/j.chroma.2009.11.005

Marta-Sanchez, A. V., Caldas, S. S., Schneider, A., Cardoso, S. M. V. S., & Primel, E. G. (2018). Trace analysis of parabens preservatives in drinking water treatment sludge, treated, and mineral water samples. *Environmental Science and Pollution Research*, *25*(15), 14460–14470. https://doi.org/10.1007/s11356-018-1583-4

Noutsopoulos, C., Koumaki, E., Sarantopoulos, V., & Mamais, D. (2019). Analytical and mathematical assessment of emerging pollutants fate in a river system. *Journal of Hazardous Materials*, *364*(October 2018), 48–58. https://doi.org/10.1016/j.jhazmat.2018.10.033

Omar, T. F. T., Ahmad, A., Aris, A. Z., & Yusoff, F. M. (2016). Endocrine disrupting compounds (EDCs) in environmental matrices: Review of analytical strategies for pharmaceuticals, estrogenic hormones, and alkylphenol compounds. *TrAC - Trends in Analytical Chemistry*, *85*, 241–259. https://doi.org/10.1016/j.trac.2016.08.004

Omar, T. F. T., Aris, A. Z., & Yusoff, F. M. (2021). Multiclass analysis of emerging organic contaminants in tropical marine biota using improved QuEChERS extraction followed by LC MS/MS. *Microchemical Journal*, *164*(February), 106063. https://doi.org/10.1016/j.microc.2021.106063

Ötles, S., & Kartal, C. (2016). Solid-Phase Extraction (SPE): Principles and applications in food samples. *Acta Scientiarum Polonorum, Technologia Alimentaria*, *15*(1), 5–15.

https://doi.org/10.17306/J.AFS.2016.1.1

Piao, C., Chen, L., & Wang, Y. (2014). A review of the extraction and chromatographic determination methods for the analysis of parabens. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *969*, 139–148. https://doi.org/10.1016/j.jchromb.2014.08.015

Renz, L., Volz, C., Michanowicz, D., Ferrar, K., Christian, C., Lenzner, D., & El-Hefnawy, T. (2013). A study of parabens and bisphenol A in surface water and fish brain tissue from the Greater Pittsburgh Area. *Ecotoxicology*, *22*(4), 632–641. https://doi.org/10.1007/s10646-013-1054-0

Tiwari, M., Sahu, S. K., & Pandit, G. G. (2016). Distribution and estrogenic potential of endocrine disrupting chemicals (EDCs) in estuarine sediments from Mumbai, India. *Environmental Science and Pollution Research*, *23*(18), 18789–18799. https://doi.org/10.1007/s11356-016-7070-x

Vale, F., Sousa, C. A., Sousa, H., Santos, L., & Simões, M. (2022). Parabens as emerging contaminants: Environmental persistence, current practices and treatment processes. *Journal of Cleaner Production*, *347*(December 2021). https://doi.org/10.1016/j.jclepro.2022.131244

Wang, B., Dong, F., Chen, S., Chen, M., Bai, Y., Tan, J., Li, F., & Wang, Q. (2016). Phenolic endocrine disrupting chemicals in an urban receiving river (Panlong river) of Yunnan-Guizhou plateau: Occurrence, bioaccumulation and sources. *Ecotoxicology and Environmental Safety*, *128*, 133–142. https://doi.org/10.1016/j.ecoenv.2016.02.018

Wei, F., Mortimer, M., Cheng, H., Sang, N., & Guo, L. H. (2021). Parabens as chemicals of emerging concern in the environment and humans: A review. *Science of the Total Environment*, *778*, 146150. https://doi.org/10.1016/j.scitotenv.2021.146150