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Simultaneous extraction and determination of 34 multiclass endocrine disrupting compounds in river water using solid-phase extraction coupled with three liquid chromatography–mass spectrometry methods

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ABSTRACT

Endocrine-disrupting compounds (EDCs) encompass a diverse range of substances found in river water, and can have significant impacts on aquatic organisms and human health. In this study, a multiresidue analytical method was developed for determining 34 endocrine-disrupting compounds (EDCs), including pharmaceuticals, plasticizers, and hormones by utilizing a dual-cartridge solid-phase extraction (SPE) approach (Phenomenex® Strata-X and Oasis WAX) coupled with liquid chromatography–tandem mass spectrometry (LC–MS/MS). The optimized method achieved >70 % recovery for all analytes, demonstrating robust extraction efficiency. The reliability and robustness of the optimized method were ensured through meticulous validation procedures encompassing linearity, precision, recovery, limit of detection (LOD), and limit of quantification (LOQ). The method demonstrated satisfactory performance overall, meeting established precision levels and exhibiting LOD and LOQ values ranging from 0.1 ng/L to – 50 ng/L and 0.3 ng/L–200 ng/L, respectively. The linearity of the compounds indicated strong regression, with a goodness of fit (r) exceeding 0.99 for all targeted compounds. Satisfactory precision was achieved with a relative standard deviation (RSD) less than 18 %. However, two compounds showed lower precision during LC–MS/MS analysis, notably atenolol (21.97 %) and diltiazem (34.28 %). The validated method was used for the quantitative EDCs analysis of river water samples collected from five locations within the Langat River, Malaysia. Application of this method to real water samples from the Langat River revealed the presence of various EDCs, even upstream, underscoring the pervasive nature of EDC contamination in freshwater environments. This study contributes to the advancement of analytical chemistry methodologies for the comprehensive assessment of EDC occurrence in environmental waters, thereby facilitating informed decision-making processes for pollution control and public health protection.

1. Introduction

Endocrine disrupting compounds (EDCs) are chemicals that disrupt hormone function and potentially cause biological effects in organisms [1]. With a staggering 87,000 chemicals identified as EDCs, the World Health Organization (WHO) is concerned about their impact on both aquatic ecosystems and human health, even at miniscule concentrations (μg/L and ng/L) [2]. Rapid urbanization, followed by discharge from water treatment plants (WTP) and wastewater treatment plants (WWTPs), is the primary source of EDCs in surface water. This situation deteriorates because of the obsolete and ineffective wastewater treatment infrastructure. Ultimately, inadequately treated or untreated EDCs are discharged into water bodies during effluent release, leading to their widespread distribution and leaching into landfill and waste disposal

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sites. Consequently, EDCs disperse and accumulate in receiving water bodies, predominantly rivers, via effluent discharges. They can be carried by water currents or undergo various transformations such as absorption, settling, or percolation, eventually extending their reach to other environmental zones [3].

The world's largest chemical testing program, the European Regulation for Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), classifies EDCs as substances of very high concern (SVHC). Accordingly, EDCs are focal areas in ecotoxicology, and are a priority for identification and regulation. Identifying the sources of EDC contamination in river water is essential for implementing targeted pollution control measures [4–6]. EDCs have consistently been detected in riverine ecosystems across several countries, including the United States[7], Canada [8], Spain [9], England [10], Italy [11], Brazil [12], Malaysia [13], China [14], Japan [15], and Singapore [16]. Several countries have regulations and guidelines to limit the concentration of EDCs in water bodies [17]. Monitoring EDC levels ensures compliance with these regulations and facilitates appropriate remedial actions if the levels exceed permissible limits. Understanding the concentration of EDCs in river water enables risk assessments to evaluate their potential effects on aquatic organisms and human health. This information informs the decision-making processes aimed at mitigating risks and protecting ecosystems and public health.

The current trajectory in analytical chemistry research emphasizes the advancement of liquid chromatography–mass spectrometry (LC–MS)-based techniques that can concurrently identify multiple EDCs in a single analytical run. While gas chromatography-mass spectrometry (GC–MS) has traditionally been used for volatile and semi-volatile EDCs (e.g., certain plasticizers), its applicability is limited for polar, non-volatile, or thermally labile compounds (e.g., pharmaceuticals, hormones), which require derivatization and time-consuming step that introduces analytical variability [18]. Similarly, immunoassays offer high specificity for targeted compounds (e.g., hormones) but lack the universality needed for multiclass EDC analysis, often yielding false positives/negatives in complex environmental matrices. In contrast, LC–MS/MS obviates derivatization and enables simultaneous detection of structurally diverse EDCs, reducing analysis time and costs. However, analyzing contaminants of emerging concern (CECs) remains challenging due to their physicochemical diversity and matrix complexity. Techniques such as stir-bar-sorptive extraction (SBSE) and solid-phase microextraction (SPME) often struggle with low analyte recovery in environmental waters, while liquid–liquid extraction (LLE) requires large solvent volumes and lacks selectivity for trace-level analytes [19,20]. To address these limitations, this study obviates the need for reinjection of samples in tandem mass spectrometry (MS/MS) mode, thereby reducing both the analysis duration and costs. This study optimized a dual-cartridge solid phase extraction (SPE) approach combining reversed-phase and weak anion-exchange cartridge, which the reversed phase C18 sorbent efficiently retains hydrophobic pharmaceuticals, while the weak anion-exchange cartridge targets polar and ionizable compounds through anion-exchange mechanisms. By integrating SPE with LC–MS/MS, current method achieves high sensitivity and minimizes matrix effects, addressing critical gaps in existing workflows for environmental monitoring. It is recommended to utilize SPE extraction techniques because of streamlined resource utilization and enhanced efficiency in removing particulate matter from water samples [21].

While previous studies have employed SPE for multiclass EDC analysis, existing methods often focus on limited compound classes or smaller analyte panels. For instance, Wee et al. (2019) developed an SPE-LC–MS/MS method for 16 EDCs across pharmaceuticals and hormones, achieving recoveries of 55–115 % but omitting critical plasticizers and polar pharmaceuticals. Similarly, Li et al. (2021) utilized single-sorbent SPE (C18) for 25 EDCs, reporting challenges in recovering ionizable hormones and polar plasticizers due to restricted retention mechanisms. In contrast, this study introduces a dual-cartridge SPE strategy that enables simultaneous extraction of 34 multiclass EDCs spanning pharmaceuticals (28 compounds), plasticizers (3 compounds), and hormones (3 compounds). Furthermore, developed method integrate three optimized LC–MS/MS methods (M1–M3) to address chromatographic challenges posed by multiclass analytes, enabling precise quantification in a single workflow. The optimized method was validated for various parameters such as linearity, precision, recovery, limit of detection (LOD), and limit of quantification (LOQ) to ensure the reliability of the results. Current developed method advances environmental monitoring by offering unparalleled comprehensiveness with the LODs in range of 0.1–50 ng/L). Subsequently, the developed method was applied to the quantification of EDCs in river water collected from the Langat River for method validation using real environmental samples. This comprehensive analytical method aims to better inform on the environmental fate and occurrence of EDCs in environmental waters.

2. Materials and methods

2.1. Materials and chemicals

The 34 EDCs analyzed in this study were chosen based on their documented environmental occurrence, toxicological significance, and regulatory prioritization, ensuring a comprehensive assessment of high-risk compounds with recognized public health concerns [4]. Reference standards for 34 multiclass EDCs, including pharmaceuticals, plasticizers, and hormones, such as atenolol, caffeine, cefuroxime, chloramphenicol, dexamethasone, diclofenac, diltiazem, diphenhydramine, erythromycin, fluconazole, ibuprofen, ketoprofen, lidocaine, metformin, metronidazole, naproxen, nicotine, oseltamivir, paracetamol, primidone, ranitidine, salbutamol, sitagliptin, sulfadiazine, sulfamethoxazole, tramadol, triclosan, trimethoprim, testosterone, progesterone, estrone, bisphenol A, bisphenol F and bisphenol S above 97 % purity were purchased from Sigma-Aldrich (MA, USA) and Dr Ehrenstorfer GmbH (Augsburg, Germany). Internal standards were omitted due to the impracticality of employing 34 distinct standards for the diverse multiclass analytes, which could exacerbate matrix effects and complicate method optimization [22]. Internal standards must replicate the behavior of analytes and their responses to matrix effects. Otherwise, their effectiveness in ensuring an accurate analysis may be compromised [23]. Additionally, cost constraints and limited availability of suitable internal standards for certain analytes further justified this approach [24].

Individual stock solutions of each EDC were prepared at a concentration of 2500 mg/L in methanol. A mixed standard solution of 34 EDCs with a final concentration of 100 mg/L for each EDC was prepared by combining 4 mL of each individual stock solution in a 100 mL volumetric flask. The resulting mixture was then diluted to 100 mL with methanol and stored at –20 °C to prevent photochemical degradation. SPE cartridges (200 mg, 6 mL, Strata-X polymeric reversed phase C18) were obtained from Phenomenex (CA, USA), whereas SPE cartridges (225 mg, 60 μm, Oasis Plus short weak anion exchange (WAX) were purchased from Waters (MA, USA). Moreover, Gemini NX C18 columns (150 × 2.1 mm, 5 μm) and Gemini C6 Phenyl columns (150 × 2.00 mm, 5 μm) were obtained from Phenomenex (CA, USA). Comprehensive extraction of multiclass EDCs was ensured by the combination of C18 and WAX sorbents, thereby improving the robustness and sensitivity of the method across a wide array of analytes. Glass microfiber filters (0.2 μm, 47 mm) were obtained from Whatman (Buckinghamshire, UK). All solvents used during water extraction and instrument analysis, such as methanol, ascorbic acid, acetonitrile, ammonia solution, and ultrapure water, were of liquid chromatography (LC) grade and supplied by Fisher Scientific (NJ, USA).

2.2. Sample preparation

For the purpose of method development and validation, river water was collected from the Langat River (Fig. 1). The Langat River,