19th International Conference on Environmental Science and Technology

Kos, Greece, 3 to 6 September 2025

Comprehensive Target, Suspect, and Non-Target Screening Using LC-HRMS of Pharmaceuticals and Their Transformation Products in Biological Treatment Systems

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Abstract This study employed LC-HRMS-based target, suspect, and non-target screening to investigate the occurrence, behavior, and environmental risk of pharmaceuticals (PCs) and their transformation products (TPs) in a biological treatment system (BTS). A total of 82 compounds (43 confirmed, 39 tentatively identified) were detected. PCA and HCA revealed temporal variations in concentrations and removal efficiencies, with some compounds exhibiting persistent or increasing trends. Fold change analysis enabled classification of TPs as human, microbial, or dual-type metabolites, highlighting active transformation processes in the BTS. PMT/PBT assessments showed that many TPs (e.g., CBZ-EP, CFN-PT, VST-A) exhibited high persistence, mobility, or potential toxicity. Structural modifications, such as ether, ketone, or hydroxylation groups, often contributed to these properties. Compounds like VST-A and PFN-M300 showed increased mobility and resistance to degradation, even after treatment. These findings underscore the importance of including diverse PCs and TPs in environmental assessments. The study demonstrates the applicability of comprehensive screening and statistical methods for identifying substances of environmental relevance and transformation behavior in the BTS. These findings indicate the necessity of comprehensive monitoring and may contribute to future regulatory development.

Keywords: Emerging pollutants, Wastewater treatment, LC-HRMS, Biotransformation, Non-target screening

1. Introduction

With the increasing development and use of emerging chemicals, the possibility of emerging pollutants entering aquatic environments is growing. South Korea, ranked 11th in pharmaceutical consumption among OECD countries in 2022, faces challenges in managing PCs. These compounds often enter the environment via wastewater treatment plants (WWTPs), where they are not fully removed, posing ecological and human health risks due to endocrine disruption, genotoxicity, and carcinogenicity. Instead, they can transform parent compounds into TPs, or deconjugate human metabolites back to parent compounds. These TPs including metabolites often lack structural and toxicological data, making monitoring difficult. Liquid chromatography and high-resolution mass spectrometry (LC-HRMS) enables accurate separation and structural characterization of unknown compounds owing to its high sensitivity and selectivity. LC-HRMS analysis generates thousands to tens of thousands of peaks. However, only a small portion of these can be quantified through target screening. Suspect and non-target screening approaches allow for the identification of a broader range of substances, including those not previously confirmed. Therefore, in complex matrices of WWTPs where various metabolites and TPs are present, suspect and non-target approaches are particularly essential. This study investigates PCs and TPs in a BTS using LC-HRMS, applies PCA and HCA for statistical analysis, and evaluates persistence, mobility, and toxicity via PMT/PBT assessment.

2. Material and method

A total of 69 pharmaceutical reference standards and 36 internal standards were analyzed using LC-HRMS in influent (INF) and effluent (EFF) samples collected from the BTS. Three sampling campaigns (May–July 2023) collected 18 INF and 18 EFF samples, which were filtered, spiked with ISTDs, and extracted using SPE. LC-HRMS analysis used HPLC-Orbitrap MS under positive and negative modes. Target screening was conducted using internal standard calibration; suspect list was established based on predicted TPs from databases and literature; non-target screening used Compound Discoverer with biotransformation logic. TPs were identified based on MS/MS data and confidence levels. Removal efficiency was assessed using calculated removal rates and classified by removal behavior. PMT/PBT assessment used QSAR tools (BIOWIN3, KOCWIN/KOWWIN/ECOSAR) for persistence, mobility, and toxicity. Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and FC analysis were applied to interpret patterns of compound behavior pre- and post-treatment.

3. Results and discussion

3.1. Target screening result

To evaluate the behavior of PCs and their TPs in the BTS, target compounds were quantified in INF and EFF samples across three sampling campaigns (total 43 subs.). Caffeine, and metformin were the most abundant compounds in INF, while carbamazepine, diclofenac and valsartan acid dominated in EFF. PCA separated INF and EFF samples, revealing high removal efficiencies for many compounds, but poor or negative

removal for others like carbamazepine, tramadol N-desmethyl, and valsartan acid, indicating persistence or biotransformation. HCA further categorized compounds into groups based on removal behavior, including elimination, consistent presence, and formation. Some compounds exhibited variable removal across campaigns, suggesting time-dependent or operational influences. The combined PCA and HCA results highlighted temporal shifts in compound concentrations and removal efficiency, confirming that influent levels and compound characteristics play critical roles in their fate during biological treatment.

3.2. Human metabolites and microbial metabolites in the biological treatment system.

A total of 39 TPs were identified in a BTS, classified as human, microbial, or dual-type based on FC and pathway analysis. FC analysis showed microbial TPs were mainly formed via hydroxylation or dealkylation, with VST-A and MFM-M300 as notable examples. PCA and HCA supported compound classification and transformation behavior. Biotransformation pathways of key TPs such as TRM-ND, DCF-M312, and CBZ derivatives were identified, indicating persistence or formation during treatment. Novel microbial TPs like FBF-M225 and dual-type metabolites such as VLF-OD and ASP-M342 suggest active transformation within BTS and the need for further monitoring.

3.3. PMT/PBT assessment

Compounds in the PM area (e.g., CFN-PT, CFN-M209) showed high persistence and mobility, with 38 out of 40 fulfilling PM criteria (BIOWIN3 ≤ 2.75, log KOC < 3.0). CBZ derivatives (CBZ-EP, CBZ-OX, CBZ-M267), FBF-M225, PFN-M300, and VLF-OD exhibited enhanced mobility due to structural changes like ether, ketone, or hydroxylation. VST-A and VST-M450, with low biodegradability and log K_{OC}, were notably persistent and mobile. TMST, IBST-M445, and LST-M421, shown in the PB area, also demand attention for poor biodegradation and possible bioaccumulation. Especially, CFN-PT, CFN-M209, IBST-M445, LST-M421, and TMST are not only classified as PM or PB substances but also exhibit high toxicity, which may lead to adverse environmental impacts.

4. Conclusion

Target and suspect/non-target screening confirmed 43 and tentatively identified 39 PCs and TPs in the BTS. PCA and HCA revealed concentration and removal trends across sampling periods. TPs were classified as human, microbial, or dual metabolites based on FC analysis. PMT/PBT assessments highlighted environmental risks, particularly associated with compounds such as CFN-PT, CFN-M209, IBST-M445, LST-M421, and TMST.

Therefore, given the presence of diverse PCs and TPs that may pose environmental risks, it is essential to comprehensively monitor not only well-known parent compounds but also newly formed compounds and metabolites in the WWTP.

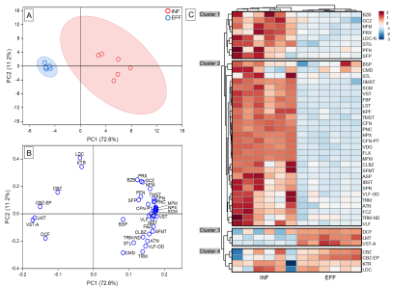

Figure 1. PCA and HCA of PCs and TPs quantified in the INF and EFF of the BTS from the first sampling campaign.

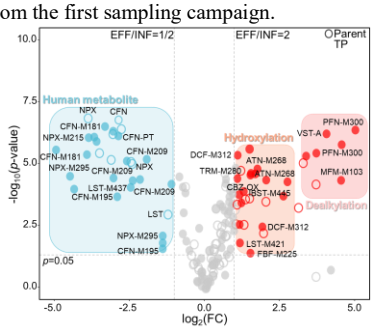

Figure 2. Classification of TPs detected in the BTS using FC analysis**.**

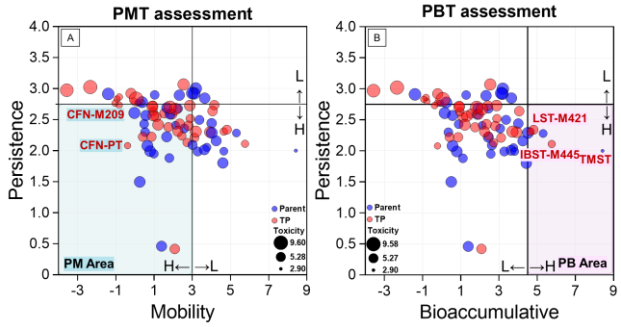

Figure 3. PMT/PBT assessments were conducted for PCs and TPs identified in the BTS.

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