SHIMADZU

WP 290

LC-MS/MS QTOF analysis of river water identifies contaminants of environmental concern by non-targeted profiling

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Overview

- Non-targeted analysis (NTA) software workflows coupled to high-resolution LC/MS were applied to river water samples to identify contaminants of environmental concern (CECs), enabling broad chemical space coverage beyond conventional target lists and supporting hypothesis-free detection of emerging and previously unreported compounds.
- The LC/QTOF analysis provided insight into the distribution of pharmaceuticals, personal-care product ingredients, industrial chemicals, and select agricultural inputs, which can provide an indirect assessment of upstream wastewater treatment performance and diffuse environmental inputs.

1. Introduction

Identifying contaminants of environmental concern in water-based epidemiological investigations has provided mechanistic insight into wastewater treatment performance and transformation processes. Both targeted and non-targeted approaches have been employed to detect emerging contaminants potentially originating from treated or untreated wastewater discharges, particularly under storm-driven overflow conditions events. In this study, river water grab samples were collected across tidal and freshwater environments systems. High-resolution LC/QTOF with DIA-MS/MS was applied using a direct injection reversed-phase workflow method. Data analysis leveraged novel non-targeted profiling software to identify differences in the distribution of detected components between and within defined sample groups.

2. Materials and Methods

River water samples were taken from twelve sites on two river systems in London, England. The River Thames represents a complex, highly impacted urban river system that integrates treated effluent, storm-driven sewer overflows, and diffuse catchment inputs, making it a critical sentinel site for monitoring CECs. It is also tidal therefore samples were taken during high-to-low tide. The River Hogsmill is a small, highly urbanized tributary that functions as a sensitive sentinel sub-catchment, reflecting localized wastewater inputs, surface runoff, and episodic discharges. Aliquots from each sample were taken to create a pooled QC to run throughout the analysis. Samples were measured by direct injection (40 µL) and separated using a generic reversed phase forensic toxicology gradient.

Sample preparation

- Water samples were collected from two urban water ways; 500 µL samples were prepared using Millipore centrifugal filters (Ultrafree - MC – GV, Dispose - PVDF 0.22um) for 4 min at 12,000 g.

Reversed phase LC Separation: Nexera X2

- Column: Shim-pack Velox™ Biphenyl (100 x 2.1 mm, 2.7 µm); column temp. 40 ºC, flow rate: 0.3 mL/min, 17 min total analysis time.
- Binary gradient; methanol:water gradient with 2 mM ammonium formate and 0.002% formic acid

High resolution QTOF analysis: LCMS-9050

- MS scan m/z 100-1000, 100 msec scan time.
- MS/MS DIA 35 consecutive scans (m/z 100-500 20 Da precursor isolation width, m/z 500-1025 35 Da precursor isolation width), 25 msec scan time (0.925 sec total cycle time).

2.1 Non-Targeted Analysis

Insight Profiler non-targeted analysis software was applied to LC/QTOF data acquired with samples from both river systems to identify CECs.

Feature Detection and Alignment
Detects and aligns all ion signals that behave as a peak. Default settings applied; threshold set to low.

Statistics and filters
Applied to find ion signals of significance and to remove ions of high variance.

Compound identification
Using large scale screening lists and multiple libraries to identify ion signals of interest.

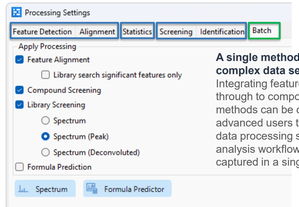

Figure 1. The Insight Profiler processing application method editor, designed to create a singular workflow for feature detection of unknowns to compound identification.

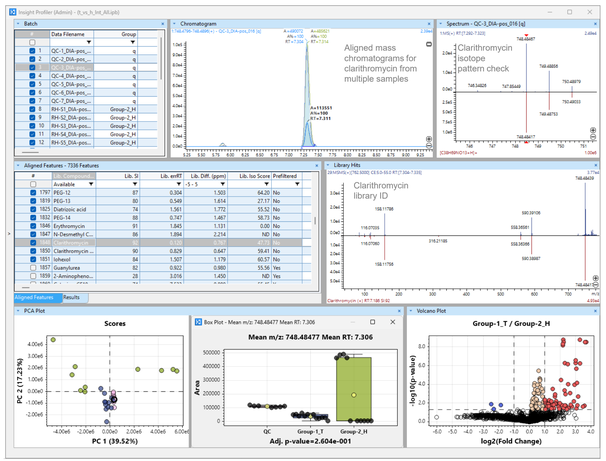

Figure 2. Data analysis of a small, highly urbanized tributary (green) compared to a complex, highly impacted urban river system (blue); pooled QC samples (pink).
The antibiotic clarithromycin is highlighted in the data review software with four sites in Group-2_H showing far higher concentrations at sample points in the small, highly urbanized tributary (green).

3. Results

3.1 Combined impact of environmental contaminants

Water samples were collected from multiple sites on two rivers to assess whether non-target analysis could differentiate samples based on location. Aliquots from each sample were combined to produce a pooled quality control (QC) sample. Insight Profiler was used to identify compounds with high confidence using the Shimadzu Forensic Toxicology database matching compounds within 5 ppm and retention time match within 1 minute. Results identified a high number of pharmaceutical compounds and metabolites, including venlafaxine, desmethylvenlafaxine, tramadol, O-desmethyltramadol, gabapentin, ketamine, clarithromycin, metronidazole, carbamazepine, and lidocaine. To illustrate the level of compounds measured from each site summed peak areas of positively identified compounds were plotted (Figure 3).

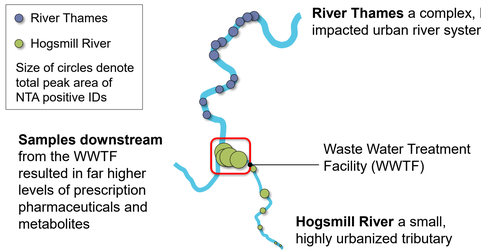

Figure 3. Samples taken downstream from a Waste Water Treatment Facility on small, highly urbanized tributary. Prescription pharmaceuticals and metabolites accounting for ~70% of all detected compounds.

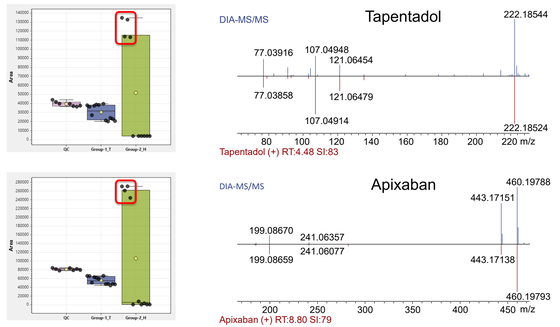

Figure 4. Tapentadol (a prescription opioid analgesic used for the treatment of moderate to severe pain) and Apixaban (a prescription anticoagulant used to prevent and treat blood clot) were both detected at far higher concentration in samples downstream from the WWTF on the small, highly urbanized tributary (indicated with the red box).

3.3 High confidence library identification

It was notable that acetaminophen (paracetamol) and caffeine, recognised as well established markers of wastewater influent, were detected at comparatively low levels in the small, highly urbanized tributary downstream of the WWTF indicating successful waster water treatment.

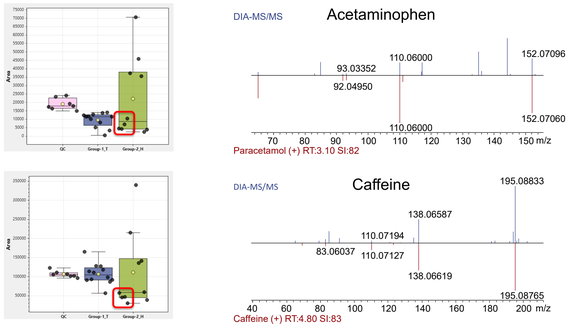

Figure 5. Acetaminophen (paracetamol) and caffeine, were consistently detected and used as indicators of anthropogenic wastewater inputs. Both were reduced post treatment.

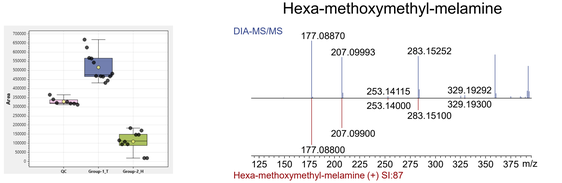

Figure 6. Insight Profiler enables large-scale suspect screening through support for multiple search lists and spectral libraries. Application of .msp libraries from MassBank resulted in the identification of hexa-methoxymethyl-melamine (HMMM), an additive widely used in coatings, plastics, and rubber and increasingly recognized as a road-derived emerging contaminant in urban aquatic environments. HMMM was found with the highest concentration at the first sampling location on the complex, highly impacted urban river system, consistent with proximal urban and transport-related inputs.

4. Conclusions

- Insight Profiler non-targeted analysis software was applied to the analysis of urban river water using a direct injection method.
- The results highlight the complex and dynamic changes occurring in urban river water in which a single source can have far reaching influences downstream however global trends can still identify contaminants such as HMMM with high confidence.