Streamlining HRMS data interpretation for wastewater impact on water quality

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Novel aspect:

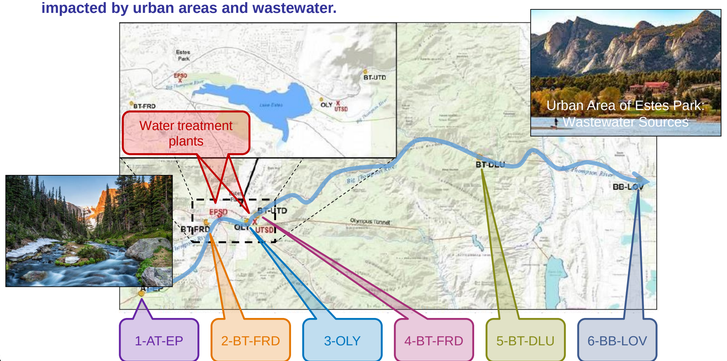
Iterative MS-MS and All Ions acquisition methods with statistical analysis techniques are used to identify unknown environmental contaminants in river water.

Introduction

- Pharmaceuticals, pesticides, personal care products, and industrial substances such as PFAS, are emerging contaminants, which are an environmental health concern when entering drinking water sources.
- Wastewater is a major source of these contaminants in rivers and streams. Furthermore, microbial degradation of wastewater contributes metabolites and degradation products, whose chemical structures are often unknown.
- High-resolution mass spectrometry is required to identify literally thousands of unknown compounds in a single data file.
- **The analytical challenge is an innovative approach to find and identify environmentally relevant contaminants using software tools for rapid analysis.**

Study Design

- Water samples were analyzed from 6 locations over a 5-year period along the Big Thompson River near Rocky Mountain National Park and Estes Park.
- **Sample sites varied from pristine mountain streams, used as controls, to downstream locations impacted by urban areas and wastewater.**



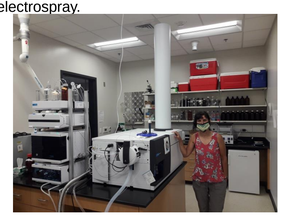
Sample Preparation

1. Collect 1-Liter of river water.
2. Transfer 125 mL to a bottle, add internal standard: carbamazepine d-10 (concentration 80ng/L).
3. Extract 100 mL on Oasis HLB cartridge (200mg) using Automated SPE system (Gilson GX-271 ASPEC)
4. Elute with 6 mL of MeOH.
5. Nitrogen dry to 0.5 mL final volume.
6. Inject 20 µL on LC/Q-TOF-MS.

Samples were concentrated 200x

Instrument conditions

- Reverse phase chromatography
- Data independent (All Ions MS/MS) and data dependent Iterative MS/MS acquisition were applied to all samples using positive ion electrospray.



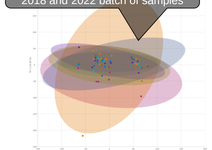
Agilent 1290 Infinity II LC coupled to a 6546 LC/Q-TOF

Data-Analysis Workflow

- **Novel software, Agilent MassHunter Explorer, was used for non-targeted data extraction and analysis.**
- LOESS normalized, extracted data was subjected to 1-way analysis of variance (ANOVA) to compare the pristine upstream location with all downstream locations. Further focus was provided by filtering significantly different compounds for those with a 10-fold change.
- Putative compound identifications with various confidence are reported

Setup	Find and Align Compounds	Normalize	Statistical Analysis	Compound Identification
• Non-targeted data acquired • Regular Pooled QC samples	• Non-targeted compound extracted	• LOESS	• 1-way ANOVA • Fold Change	• Accurate Mass • Isotope Pattern • Retention Time • NIST MS/MS • CSI:FingerID

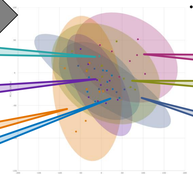
2020 batch separated from 2017, 2018 and 2022 batch of samples



PCA before normalization

After normalization, samples are grouped by locations

PooledQC's
1-AT-EP
2-BT-FRD
3-OLY

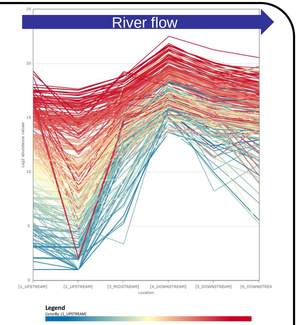


4-BT-UTD
5-BT-DLU
6-BB-LOV

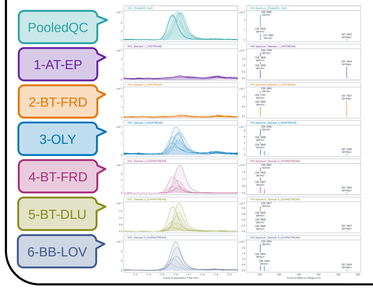
PCA after normalization

Results

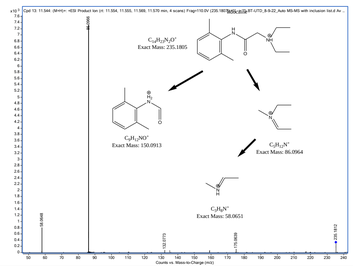
- **6745** compounds were extracted from the entire set of samples (N=58).
- **6036** compounds were observed in all Pooled QC's, allowing LOESS normalization
- **1705** compounds were found to be significantly different (p≤0.05) relative to the 1-AT-EP control pristine upstream water sample) by a 1-way ANOVA
- **275** compounds were 10x more abundant in downstream locations relative to the 1-AT-EP location… of which so far:
  - **29** compounds were known pharmaceuticals, pesticides and their metabolites identified with a RT and spectra from a chemical standard.
  - **8** compounds are putatively identified with matches to curated MS/MS spectra.
  - ~20% of the remaining 238 compounds have MS/MS spectra correlated to chemical structures, but further detailed investigation is required.



An Example of 1 of 8 Compounds Identified with a Putative ID: Lidocaine, a local anesthetic, appears after the wastewater treatment plant and decreases in intensity downstream



Fragmentation tree for the *m/z* 235.1807



Conclusions

- The combination of acquiring data with All Ions MS/MS and Iterative Auto MS/MS acquisition modes, aids rapid analysis and confidence in compound identification workflows.
- The use of the LOESS normalization technique allowed the use of multiple batches of samples (acquired at different times) to increase the power of the significance analyses.
- The data analysis workflow focuses attention on environmental contaminates of concern, of which Pharmaceuticals and metabolites were the majority of the putatively identified compounds along with lesser amounts of pesticides and industrial compounds.

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