IMPROVING NON-TARGET ANALYSIS BY HPLC-ESI/HRMS IN THE CONTEXT OF THE EPA COLLABORATIVE TRIAL PROJECT (ENTACT)

Brian Ng[1,2], Natalia Quinete[3], and Piero Gardinali[1,2,3]

[1]Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8th Street, Miami, Florida 33199
[2]CREST Center for Aquatic Chemistry and Environment, Biscayne Bay Campus, 3000 Northeast 151st Street, Miami, FL 33181
[3]Southeast Environmental Research Center, OE-148, Florida International University, 11200 SW 8th Street, Miami, Florida 33199 E-mail: bng002@fiu.edu

FIU Center for Aquatic Chemistry and Environment
NSF Center of Research Excellence in Science and Technology

FIU FLORIDA INTERNATIONAL UNIVERSITY

ABSTRACT

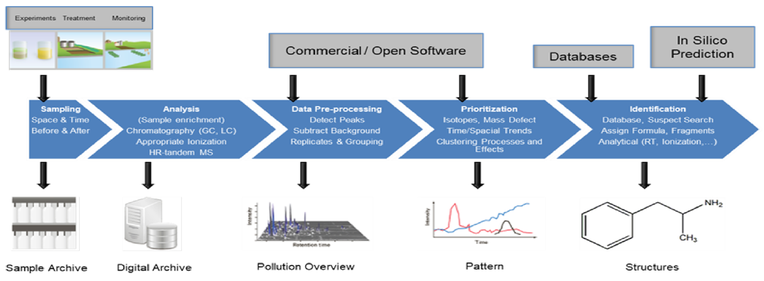
Advancements in analytical chemistry, especially mass spectrometry, have redefined the field of contaminant detection.[1] Majority of approaches for screening of environmental contaminants target individual chemical compounds or classes of chemical compounds using highly specific analytical methods. Despite their ability for low level detection and quantification, novel contaminants or transformation products which may still pose a risk to humans and wildlife are often overlooked by these methods. Non-targeted analysis requires no prior knowledge of compounds and has become very popular in the last couple years for the determination of new and emerging contaminants, or transformation products. The ENTACT samples were analyzed using a generic but robust HPLC-ESI/HRMS method. High resolution mass spectra (HRMS) were obtained using a Q-Exactive Orbitrap mass spectrometer operated at 140,000 resolution, in both positive and negative ionization mode. The obtained spectra were processed for the detection and identification of the "unknown" compounds in the standard mixtures using the unique small molecule structure identification software, Compound Discoverer to obtain a list of tentative identified compounds. This generated a massive amount of data, which was further post-processed by observation. The analyses of the ENTACT samples were done blinded and unblinded and the true positive % detection approximately doubled for the latter. The use of quality control samples was used to assess method performance during this study.

OBJECTIVES

- Establish a non-targeted screening workflow for the tentative identification of unknown compounds based on HPLC-ESI/HRMS and Compound Discoverer.
- Evaluation of the developed workflow for the detection of "unknowns" in the ENTACT samples (10 liquid mixtures and 3 types of samples: house dust, human serum and silicone wristbands).

MATERIALS AND METHODS

Non-target Analysis Workflow for environmental analysis adopted from Hollender *et. al.*[2]



UHPLC-High Resolution Mass Spectrometry:

Figure 1. Thermo Q-Exactive Orbitrap.

- Thermo Q-Exactive Orbitrap
- ESI sources
- FS:100-800 m/z 140,000 resolution
- MS^2 for confirmation: NCE 30
- Positive and Negative modes
- 4 runs per sample (MS^1, MS^2)
- Total time per run: 15 min
- Quality control samples
- Mass tolerance: <5 ppm
- Spray Voltage (V) 5000
- Capillary Temperature (˚C) 350
- Sheath Gas (a.u) 30
- Aux Gas (a.u) 2
- S-Lens RF Level 50

RESULTS

Data Processing Workflow using Compound Discovery v. 3.0

1) Peak picking
2) Background subtraction
3) Assign chemical formula
4) Apply filters
5) Search databases

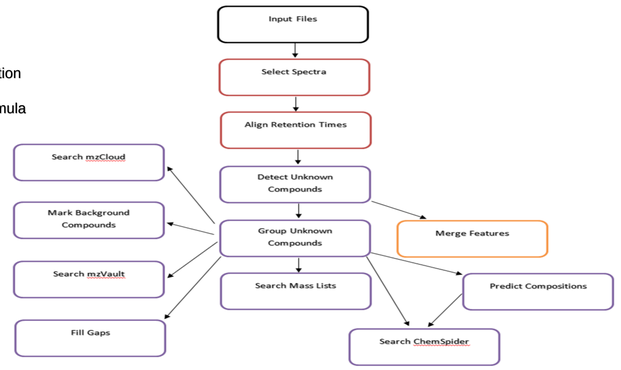


Figure 2. Compound Discoverer non-target screening workflow.

Quality Control

Log Kow VS Retention Time

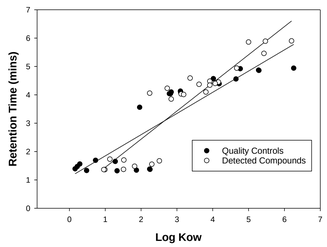


Figure 3. Correlation between Log K_{ow} and retention time in QC samples.

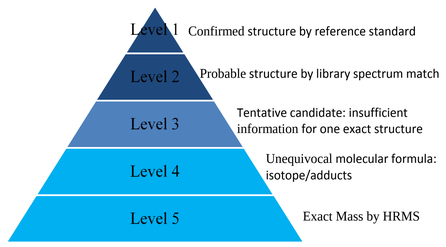


Figure 4. Schymanski level of confidence.[3]

ENTACT Samples

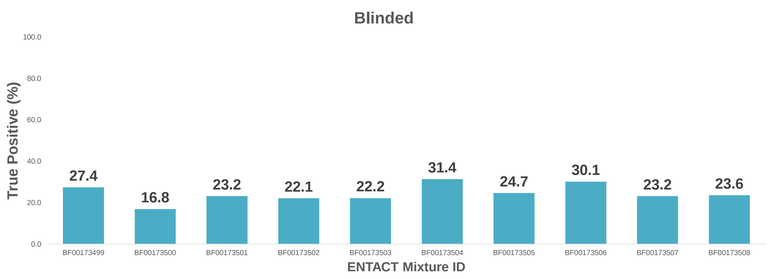


Table 1. Results of the 10 liquid mixtures (blinded).

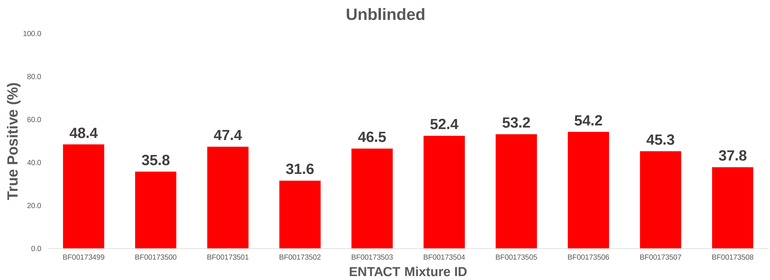


Table 2. Results of the 10 liquid mixtures (unblinded).

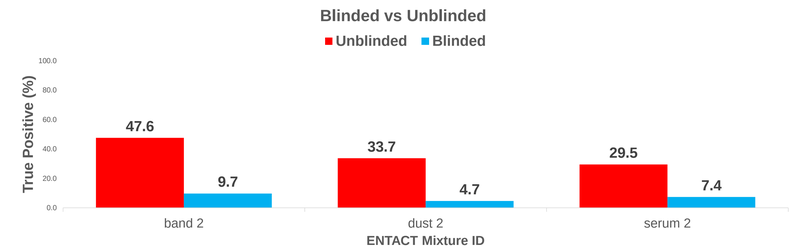


Table 3. Results of the 3 types of spiked samples (blinded vs unblinded).

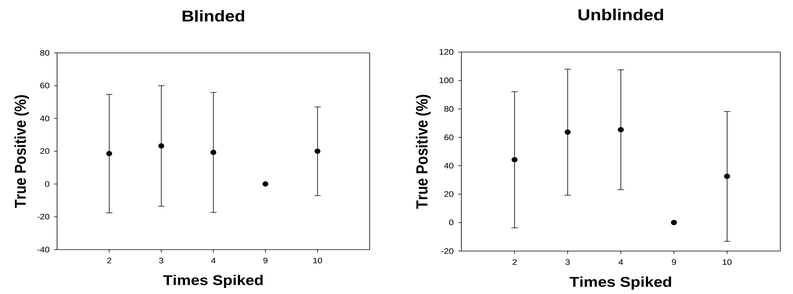


Table 4. Reproducibility of the method was assessed based on its ability to detect compounds spiked in multiple ENTACT mixtures but not necessarily at the same concentration level or matrix complexity.

CONCLUSIONS

- The use of QC samples with varying Log K_{ow} and their relationship with retention time helps restrict the massive amount of data generated as well as reduce false positives.
- The developed generic workflow detected an average of 24.5% of the ENTACT mixture compounds (blinded) and the detection rate increased to 45.3% after unblinding. Maybe some of the compounds that were not detect at all could have been GC amenable only or not ionizable with ESI.
- The study showed that by restricting the database (unblinded analysis), the ability to detect compounds increased. Maybe trying to use too many databases affected the ability to detect the correct compounds due to the way the algorithm determines the detected features.
- The capability to detect the same compound spiked in multiple samples could have been affected by the complexity of the matrix, due to the high amount of compounds as well as by differences in concentrations (16.2% blinded and 41.1% unblinded).
- Overall, the ENTACT samples contained a combined total of 1940 spiked compounds , and our developed method detected and identified a total of 911.

REFERENCES

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