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High-Sensitivity Drug and Microbial Metabolite Detection in Environmental Study Samples with Untargeted Fragment Ion and CCS Screening

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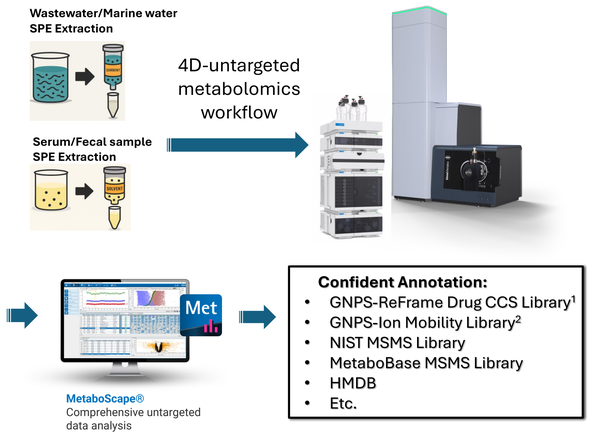
Introduction

Exposomics research seeks to characterize xenobiotics that are often present at trace levels in highly complex biological and environmental matrices. Challenges include low-abundance pharmaceuticals, environmental contaminants, and microbially derived metabolites requiring analytical sensitivity and dynamic range; relevant compounds occur at concentrations insufficient to reliably trigger MS/MS fragmentation, limiting confident spectral-based annotation; ion mobility-derived collision cross section (CCS) values provide a powerful orthogonal dimension for compound identification, while a substantial proportion of environmentally relevant small molecules fall below 200 Da requiring expanded mobility coverage. This study applies LC-TIMS-MS/MS with trapped ion mobility spectrometry (TIMS) and advanced untargeted data acquisition methods for the characterization of trace-level bioactive molecules across a wide mass range in complex environmental and biological samples.



[1]GNPS-ReFrame Drug CCS Library
Contains MS/MS spectra collected from mixtures of drug compounds from the Reframe drug library (https://reframedb.org/). The library contains collision cross section (CCS) values and MSMS spectra in positive ESI mode at 20eV and 40eV CE.

[2]GNPS-Ion Mobility Library
Contains MS/MS spectra collected from multiplexed mixtures including combinatorial synthesis reaction mixtures or crude mixtures of drugs, bile acids, and other small molecules. The compound name includes the experimental collision cross section (CCS) values.

Methods

Four representative exposomics sample types were selected for analysis: SPE-extracted wastewater, marine water, and solvent-extracted human plasma and human feces. Samples were analyzed using a Bruker Bio-AQ column (2 µm, 150 × 2.1 mm) at 45°C, with a 19-minute gradient (5-95% B) and 0.4 mL/min flow rate. Mobile phases consisted of A: 0.1% formic acid in water and B: acetonitrile with 0.1% formic acid.

DDA data was acquired on a timsMetabo with Mobility Range Enhancement (MoRE) in positive mode at mass range of 20-1300. At the beginning of each sample run within the dead volume, a small plug of sodium formate and Agilent L-TuneMix were injected as mass and mobility calibrants, respectively. Data were analyzed with MetaboScape 2026 to identify and characterize drugs and metabolites using GNPS-ReFrame Drug CCS library, GNPS-Ion Mobility library, and other metabolite and natural product libraries.

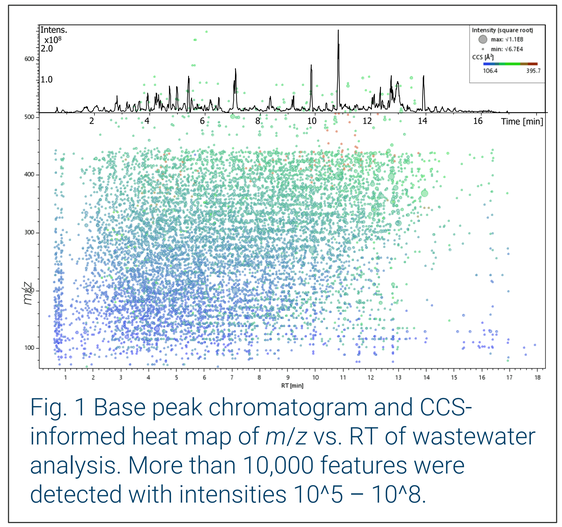


Fig. 1 Base peak chromatogram and CCS-informed heat map of *m/z* vs. RT of wastewater analysis. More than 10,000 features were detected with intensities 10^5 – 10^8.

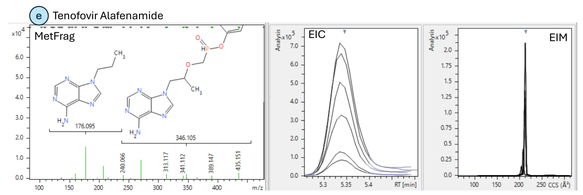


Fig. 3. (e) An HIV-drug, tenofovir alafenamide, was spiked to fecal samples and annotated with Target List. MetFrag with explanation of fragment masses embedded in MetaboScape MSMS spectrum view when InChI or SMILES is provided.

Results

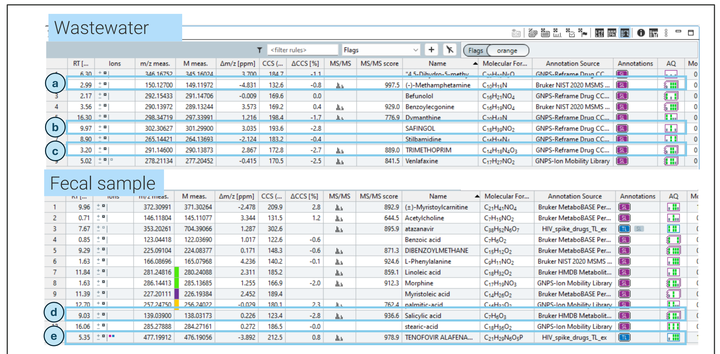


Fig. 2 MetaboScape feature tables with representative annotated from wastewater and fecal sample. See details of selected compounds in Fig. 3.

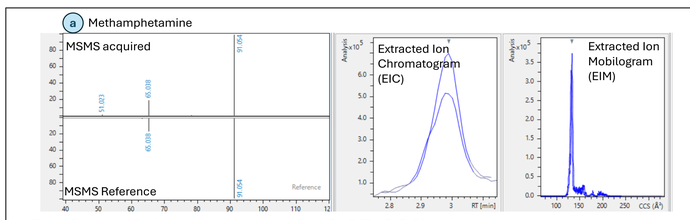


Fig. 3. (a) 4D feature (*m/z*, MSMS, RT, CCS) of methamphetamine annotated from wastewater with NIST 2020 library.

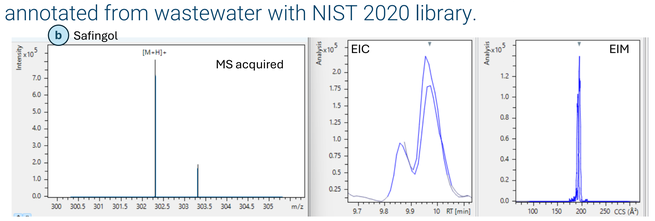


Fig. 3. (b) A lung cancer drug safingol annotated from wastewater with GNPS-ReFrame CCS library. When MSMS was absent, CCS value can increase annotation confidence.

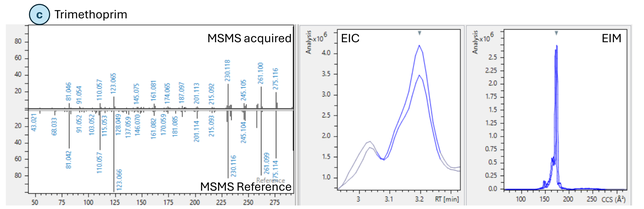


Fig. 3. (c) 4D feature of an antibiotic, trimethoprim, is annotated from wastewater with GNPS-ReFrame CCS library.

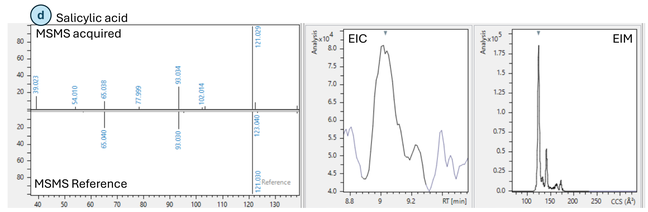
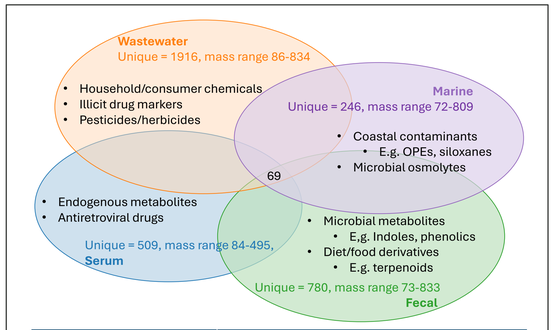


Fig. 3. (d) 4D feature of a microbial metabolite, salicylic acid, is annotated from fecal samples with HMDB spectral library.



Category	examples
Stimulants and markers	Caffeine, cotinine, natural products
Pharmaceuticals	CV drugs, NSAIDs, antidepressants, antiepileptics, antibiotics
Xenobiotics	Drugs, pesticides, plasticizer
Human Endogenous Metabolites	Amino acid, steroids, bile acids, eicosanoids, creatine,

Fig. 4. Venn diagram displaying unique chemical classes detected in each environmental matrices, with the common species summarized in the table

Summary

- Thousands of features are detected from environmental study samples in 19-min with high sensitivity and wide dynamic range (Fig 1.)
- Endogenous metabolites and xenobiotics are CCS-aware and annotated from those matrices, with many of them < 200 Da (Fig .2).
- Annotation comes from accurate mass, MSMS spectral match, and CCS. CCS errors of <3% increase annotation confidence when MSMS is not available (Fig 3).
- Pharmaceutical molecules, industrial chemicals, endogenous metabolites, and xenobiotics were detected from different environmental matrices (Fig.4)

Conclusion

- High sensitivity drug and microbial metabolite screening from environmental samples
- Automated annotation workflow using MetaboScape with CCS and Spectral libraries
- CCS measurement added level of confidence on annotation

Exposomics timsMetabo

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