What is Identification? Comprehensive Characterization of Exposome Samples via GC×GC High Resolution TOFMS

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Introduction

Historically, targeted analysis has been used to evaluate complex environmental samples. This constrained testing often misses emerging or unexpected contaminants. Recent improvements in chromatographic separation, detection, and processing allow for evaluation of these samples using non-targeted techniques. Further, the EPA is conducting a multiple system evaluation for non-targeted analysis methods using samples designed to mimic the exposome. The project contains two phases; first a blinded study of 10 standard mixtures was performed. Subsequently in phase two, the ability to successfully detect and identify these mixtures spiked into matrix samples was evaluated. Each mixture contained ~100 to 400 compounds with potential impurities, degradants, and reaction products. This presentation describes the logic used for identification of unknowns, the results, and the lessons learned from the process.

LECO Pegasus® GC-HRT+ 4D

- GCxGC dramatically improves chromatographic resolution and peak detection
- Industry leading deconvolution and non-target detection
- Multiplexing mass analyzer increases sensitivity 10X
- High Resolution Accurate Mass (HRAM) data allows for molecular and fragment ions formula calculations and verification
- ChromaTOF® brand software – A single software platform for hardware control and data processing

Data Collection Conditions

Each of the 10 standards and 3 matrix spike samples were analyzed in EI and CI modes for both GC and GCxGC separations using the settings described below. The CI data were utilized to confirm the identification for EI spectra with either a low abundance or non-existent molecular ion.

Mass Spectrometer	**LECO Pegasus GC-HRT+ 4D**
Ion Source Temperature	250 °C (EI) 200 °C (CI)
Acquisition Mode	High Resolution, ≥ 25K @ m/z 219 (FWHH)
Ionization Mode	EI and or CI (Reagent Gas CH_4 + 5% NH_3)
Mass Range (m/z)	29-1000 (EI); 60-1000 (CI)
Acquisition Rate	200 spectra/s (GCxGC); 6 spectra/s (GC)
Gas Chromatograph	**LECO GCxGC**
Injection	1 µL (diluted 10:1 in DCM) Split 10:1, Inlet Temp 250 °C (Splitless for CI)
Carrier Gas	He at 1.4 mL/min, Constant Flow
Columns	Primary 30 m x 0.25 mm x 0.25 µm Rxi-5MS (Restek, Bellefonte PA) Secondary 0.6 m x 0.25 mm x 0.25 µm Rxi-17Sil MS (Restek, Bellefonte PA)
Oven Program	Primary Oven 40 °C (1 min), 10 °C /min to 330 hold 30 min Secondary Oven +15 °C Offset
Modulation Period (GCxGC)	4 seconds

GCxGC Resolution Improves Peak Identification

1D Separation

GCxGC Separation & Deconvolution

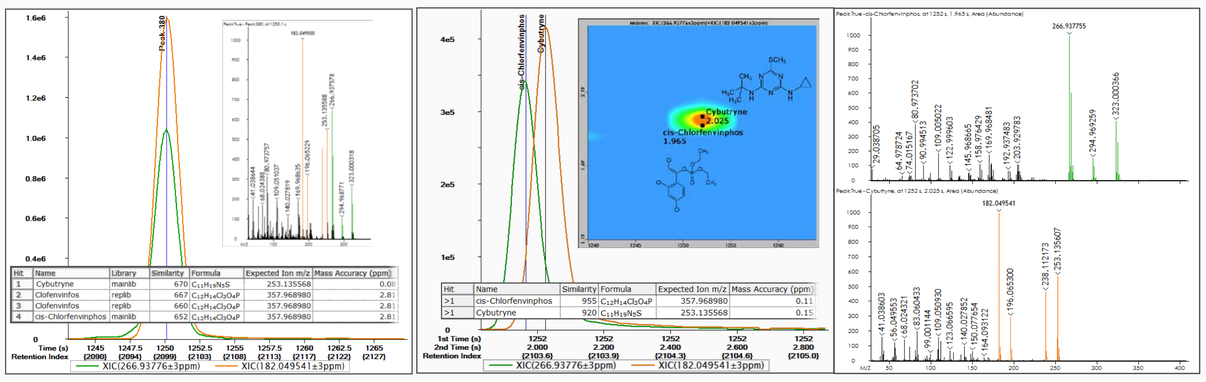

Figure 1. Comparison of traditional 1D and GCxGC separations.

Figure 1 demonstrates how the nearly identical chromatographic profiles and perfect 1D coelution makes deconvolution of these two peaks impossible, regardless of the MS resolving power, resulting in a combined spectrum with poor match scores. With GCxGC, the two peaks are separated by only 0.06 seconds, but that difference is enough to allow ChromaTOF to effectively deconvolve the two compounds, dramatically improving similarity scores, M^+ mass accuracy, and overall match confidence. In this work GCxGC played a critical role in providing clean mass spectra for processing and interpretation.

Data Analysis & Identification Confidence

As part of the sample evaluation, chromatographic peaks were assigned a confidence score using a HRAM GC-MS specific scoring system, which was developed by LECO and accepted by the EPA for this project. Each reported peak was assigned an identification confidence score (A, B, or X) based on the following criteria.

Tier A – All of the following are true:

- Forward spectral similarity score ≥700
- Molecular ion present and within 5 ppm of the expected m/z; may be confirmed with CI data
- Masses w/abundance ≥ 30% of base peak are within ±5 ppm based on their proposed molecular formula from the library spectrum
- RI value ±50 compared to NIST (semi-standard-non-polar)
- The reviewing analyst must be confident with the peak deconvolution and identification

Tier B – An "A" with some failing criteria; typically missing M^+ or too many isomers to make definitive ID

Tier X – ID was made/changed based on unblinded review.

All match filtering, similarity, and mass accuracy calculations were performed by ChromaTOF based on the selected library match formulas. Without automatic fragment mass accuracy calculations, that identification step would be tedious and time prohibitive. Identifications with confidence scores of A or B were reported to the EPA during the blinded phase. After the initial review, the EPA released the list of spiked components, and allowed for reevaluation of the sample data. Any identifications that were changed as a result of the target list were scored as "X". The match scoring system proved to be so useful that it was added to a subsequent version of ChromaTOF.

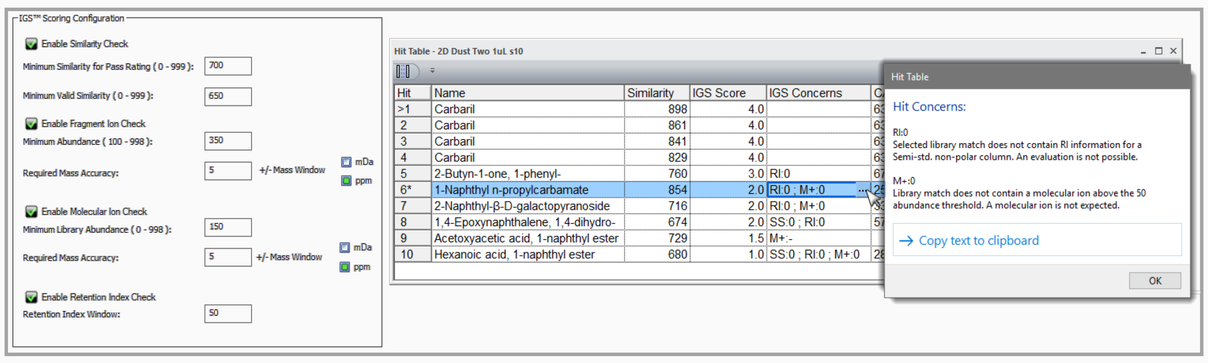

Figure 2. Image of the Identification Grading System (IGS) tool setup in the data processing method (left). Any of the evaluation criteria and limits may be enabled/edited at the user's discretion. Each criteria will award either +1 (pass), -1.5 (fail), or 0 (null result) points. The total score is used to rank the library matches in the Hit Table (right). If a library match does not receive a perfect score (4.0), the IGS Concerns column details why 0 or -1.5 points were awarded. In the image above, the highlighted match has a high similarity score, but because there was no RI information in the library and the library spectra did not include a molecular ion, these two criteria were awarded 0 points and the match was ranked lower in the Hit Table.

Phase I - Peak Detection Efficiency - Standards

Comparing the list of target compounds present in NIST 2017 against the number of found matches in each sample gives a good representation of how well the Pegasus HRT+ 4D performed in both the blinded and unblinded phases. In Phase 1 (blinded), the Pegasus HRT+ 4D and ChromaTOF found, on average, ~85% of the spiked compounds that have spectra in NIST 17. The success rate increased to ~92% once the target list was revealed. An additional point worth consideration is the likelihood that at least some of the spiked compounds reacted in solution and were therefore no longer present at the time of analysis.

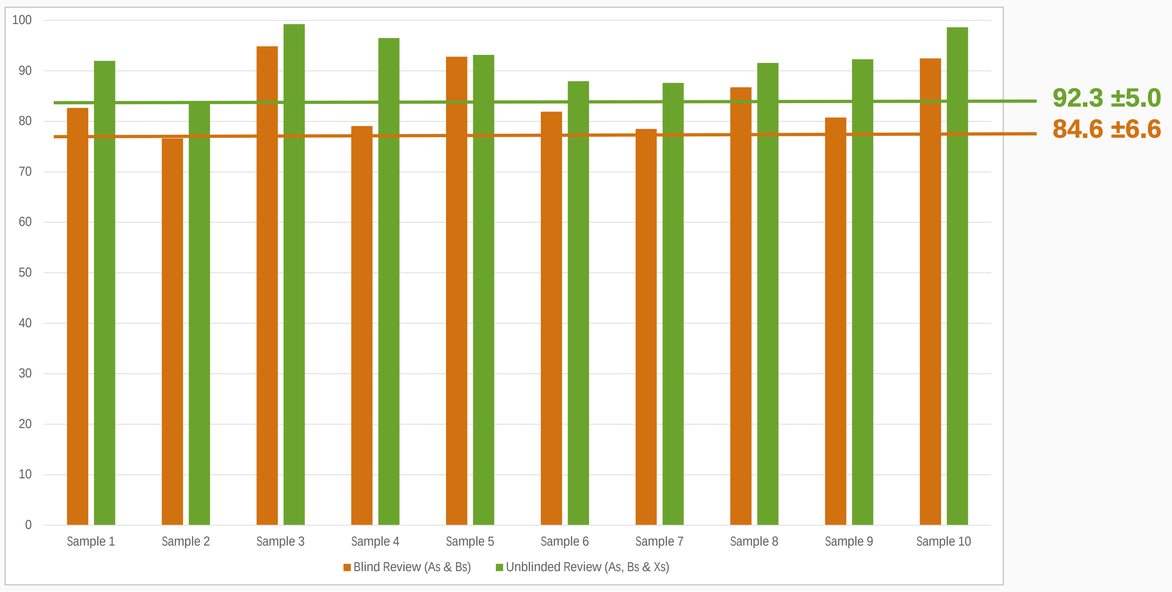

Figure 3. Summary of success rate per sample. There does not appear to be much, if any, correlation between success rate and sample complexity. Many of the increases between the blind and unblinded review can be attributed to updates of initially identified compounds to a related isomer.

Phase 2 - Peak Detection Efficiency - Samples

Unbeknownst to the testing groups, the spike solution was added to the serum, wristband, and dust matrix prior to extraction. Even if the extraction was 100% efficient, the data suggests that the concentration for the test analytes in the final extracts are orders of magnitude lower than the initial standards. For the serum and wristband samples the level of sample matrix was significantly lower than expected leading us to presume the extraction protocol was not particularly comprehensive. The combination of the much lower final concentration and potential extraction inefficiencies lead to a significant decrease in analyte detection in these samples with extraction loses as the most likely contributor.

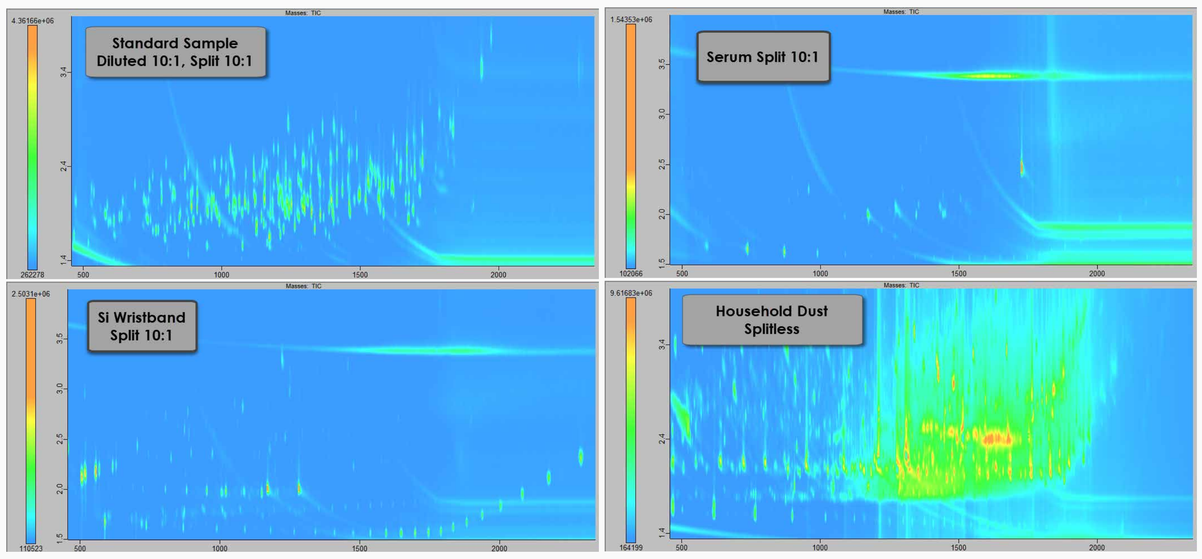

Figure 4. Comparisons of the contour plots for each of the sample classes to one of the standards from Phase 1. It appears that little of the sample matrix was extracted from the serum or wristband sample and this effect likely extends to the spiked compounds. The household dust sample is comparably matrix heavy though even the most concentrated matrix compound is ~200x less intense than the typical analyte signal in the phase 1 standard.

Table I. Success rates for detection of spiked compounds that are also present in NIST 17. Recent information from EPA indicates that if a compound was successfully extracted the concentration is likely several order of magnitude below the concentration likely to effect the most sensitive 5% of the population.

Sample	# of Spikes	Blinded Successes (of those in NIST)	Unblinded Successes (of those in NIST)
Serum (Split 10:1)	95	28.6%	38.6%
Wristband (Split 10:1)	185	39.7%	52.5%
Dust (Splitless)	365	38.3%	46.1%

Bonus Phase – Building a High Resolution Accurate Mass Library

After submitting Phase 1 results, the testing labs could request a series of 13, 384 well plates. Each well containing a single standard representing all compounds from Phase 1 and 2, plus ~3,500 additional compounds from the Toxcast database. Following the sample testing, we began collecting data for well plates and curating a high resolution, accurate mass GC-MS library.

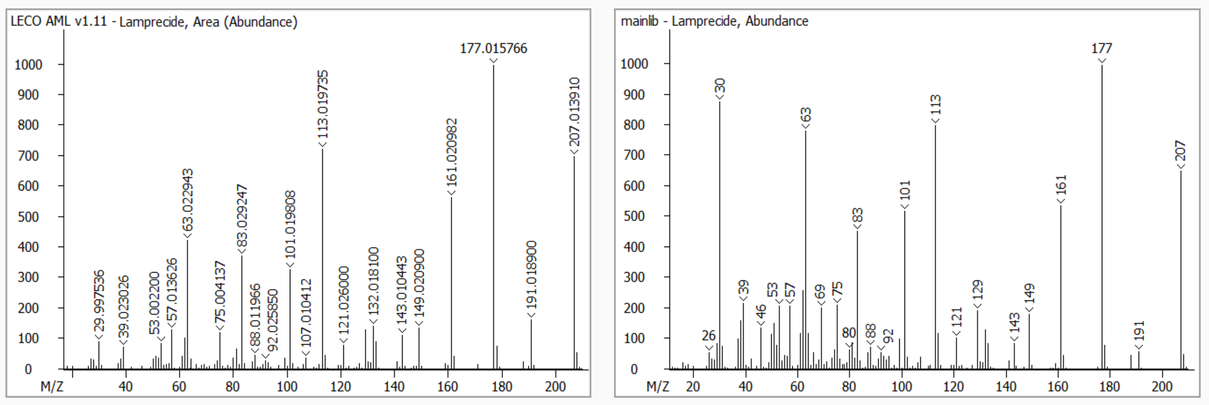

Figure 5. An example of the HRAM Lamprecide standard spectra collected on the HRT+ (left) compared to the NIST 17 "mainlib" spectra (right). The HRAM matches the established NIST spectra with similarity scores of 865/872 (forward/reverse). In the HRAM spectrum the molecular ion is within 0.65 ppm and all m/zs with an abundance >100 are within 2.3 ppm of the expected values.

Conclusions

- GCxGC dramatically improved chromatographic peak resolution leading to superior deconvolution and pure spectra for identification of non-target compounds in complex standard mixtures.
- LECO's industry-leading High Resolution Deconvolution® (HRD®) software feature provides clean mass spectra with unsurpassed spectral fidelity for library searching and spectral interpretation.
- Creation of an Identification Grading System added confidence to target and non-target investigations.
- Adding spectra from analysis Toxcast compounds from individual well plates will substantially increase the diversity of LECO's current accurate mass library.
- Inefficient extraction recoveries for compounds spiked in matrix is suspected to have played a critical role in this study's Phase 2 success rate.