Comprehensive, Non-Target Characterization of Environmental Exposome Samples Using GC×GC and High Resolution Time-of-Flight Mass Spectrometry

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Purpose

Historically, targeted analysis has been used to evaluate complex environmental samples. This constrained testing often misses emerging or unexpected compounds. 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 in samples designed to mimic the exposome. The project contains two phases, first a blinded study of complex mixtures is evaluated. Subsequently in phase two, the individual standard component lists are revealed and results revised as necessary. Each standard mixture contains between 100-400 spiked analytes with potential contaminants, degradants, and reaction products. This presentation describes the logic used for identification of unknowns, the results, and the lessons learned from the process.

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

Data Collection Conditions

Each of the 10 samples was collected in EI and CI for both 1D and GC×GC separations using the settings described in Table 1. The GC×GC files were primarily used during data review with the CI utilized to confirm the identification for those spectra with either a low abundance or non-existent EI molecular ion. Examples of the most and least complex samples can be seen in Figure 2 (bottom center).

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/sec GC×GC (6 spectra/sec for 1D)
Gas Chromatograph	**Agilent 7890B w/ LECO 2nd Oven & Dual Stage, Quad Jet Thermal Modulator**
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-17 sil 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 (GC×GC)	4 seconds

LECO Pegasus GC-HRT+ 4D

GC×GC Resolution Improves Peak Identification

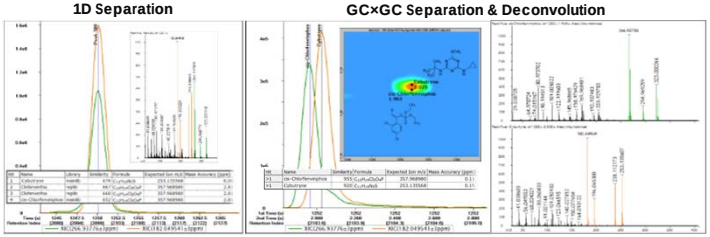

Figure 1. Comparison of traditional 1D and GC×GC separations.

The nearly identical chromatographic profiles and perfect 1D coelution makes deconvolution of these two peaks impossible regardless of the MS resolving power. The result is a combined spectrum with poor match scores. With GC×GC the two peaks are separated by only 0.06 seconds, but that difference is enough to allow ChromaTOF to efficaciously deconvolve the two compounds, dramatically improving similarity scores, M+ mass accuracy, and overall match confidence.

Data Analysis and Identification Scoring

As part of the sample evaluation chromatographic peak identifications are assigned a confidence score. A HRAM GC-MS specific scoring system developed by LECO and accepted by the EPA for this project. Each reported peak was assigned a 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 the molecular formula of the library spectrum
- RI value ±50 of the library spectrum (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 – Post initial findings submission; ID was made based on list being unblinded

All match filtering, similarity, and mass accuracy calculations were automatically preformed by ChromaTOF based on the selected library match formulas. Without the automatic fragment mass accuracy calculations, that identification step would be tedious and time prohibitive. The fragment m/z evaluation step was particularly helpful for selecting the correct identification among isobaric candidates. Identifications with confidence scores of A or B were reported to the EPA during the blinded phase (target list was unknown). After receipt of our 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. A typical example case of this type of update was from one isomer to another.

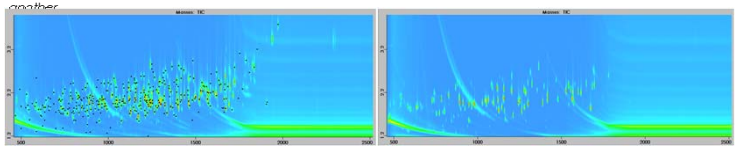

Figure 2. Example GC×GC separations.

NIST 17 Coverage of Spiked Compounds

To evaluate the success rate, we needed to understand which of the spiked compounds are likely to be detected by the system. As an initial evaluation, we determined how many of the spiked compounds are present in NIST 17 per sample. NIST 17 coverage varied significantly (72-91%) from sample-to-sample with the overall average coverage at approximately 81%

Table 2. Comparison of NIST 17 coverage for the spiked compounds in each sample.

Sample #	# of Spiked Compounds	# of Spikes in NIST17 (# absent in NIST)		% of Spikes w/ NIST 17 Spectrum
1	95	75	(20)	78.9
2	365	261	(104)	71.5
3	185	136	(49)	73.5
4	95	86	(9)	90.5
5	365	264	(101)	72.3
6	95	82	(12)	87.4
7	365	307	(58)	84.1
8	95	83	(12)	87.4
9	95	78	(17)	82.1
10	185	146	(39)	78.9
		Σ=1519	**(431)**	**$\bar{x}$=80.7**

A compound may not appear in NIST 17 because it may not be GC amenable. Also, some of the spiked compounds appear to be research analogue compounds, and a spectra may not have been submitted to NIST. As part of this project, the EPA provided single component samples of each of the target compounds. Future work with these samples should provide information for a HRAM GC-MS library that would improve detection and identification of these compounds in later studies of environmental samples.

Peak Detection Efficiency

Comparing the list of the target compounds present in NIST 2017 against the number of found matches in each sample gives a good indication of how well the system 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. The success rate increased to ~92% once the target list was released. An additional important consideration is the likelihood that at least some of the spiked compounds reacted in sample and were not actually present in the sample at the point of analysis.

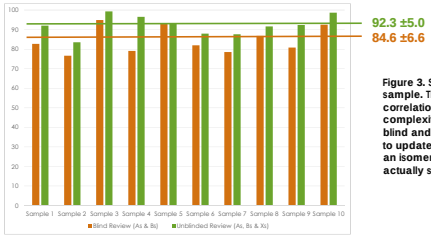

Figure 3. Summary of the success rate per sample. There does not appear to be a correlation between success rate and sample complexity. Most of the increases between the blind and unblinded review can be attributed to updates of initially identified compounds to an isomeric version of that compound that was actually spiked into the sample.

Conclusions

- GC×GC dramatically improved chromatographic peak resolution leading to superior deconvolution and identification for non-target analysis of complex samples
- LECO's industry-leading High Resolution Deconvolution® (HRD®) software feature provides clean mass spectra with unsurpassed spectral fidelity for library searching
- Increasing the coverage of existing GC-MS libraries would speed up non-target investigations and improve identification quality
- Evaluation of the individual compounds should help understand which compounds are not GC-MS amenable, and additionally aid users in future evaluations

For more information about this project please see the Chromatography Today online article at https://goo.gl/BV1Zy9