Application Note

EMPOWERING RESULTS

Elemental Analysis | GC Mass Spectrometry | Metallography

Instrument: Pegasus® HRT$^+$ 4D

Characterization of PFAS and Other Pollutants in Household Dust Using GCxGC-HRTOFMS and Spectral Analysis Tools (SAT)

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Key Words: PFAS, GCxGC, High-Resolution Time-of-Flight Mass Spectrometry, Mass Defect Plots

Introduction

Currently, there is an increasing interest in environmental screening for harmful substances in both outdoor and indoor settings. Clean air, water, and soil are critical for the sustainability of life, and frequent exposure to hazardous materials via inhalation or ingestion during work, rest, or play can be detrimental to health. Therefore, it is important to regularly screen for known and unknown toxic materials in the environment. Unfortunately, monitoring hazardous chemicals, such as persistent organic pollutants (POPs), is challenging due to the enormous number of compounds, their large concentration ranges, and different physiochemical properties. An example of a complex environmental sample is household dust. Dust is a repository of a wide array of substances and is therefore an excellent indicator of chemical exposure.[1] It has been investigated by numerous researchers using different analytical techniques.[2]

The goal of this work was to develop a methodology for the untargeted analysis of a dust standard reference material (NIST 2585) as well as dust samples collected from household and office settings for both legacy and emerging pollutants. The method uses two-dimensional gas chromatography (GCxGC) and high-resolution mass spectrometry (HRTOFMS), is comprehensive, and covers several classes of pollutants in the samples, such as Perfluorinated alkyl substances (PFAS), Polychlorinated Biphenyls (PCBs), and pesticides. This differs from other common targeted methods, which only focus on monitoring specific pollutants. Additionally, emerging pollutants, such as many classes of volatile PFAS, are often absent from current targeted screening methods and databases. These are particularly challenging to analyze but were also covered with this method and were annotated through complementary EI- and CI-GCxGC-HRTOFMS data and advanced analysis software, like Spectral Analysis Tools (SAT). With these tools, a GCxGC contour plot of high-resolution negative chemical ionization data for a complex dust sample with thousands of peak markers can be simplified to display only analytes with specific elements, such as fluorine in the case of PFAS or chlorine in the case of PCBs and Chlordanes. (Figure 1). This method facilitates the untargeted screening of these complex samples and the focused investigation of specific classes of hazardous materials.

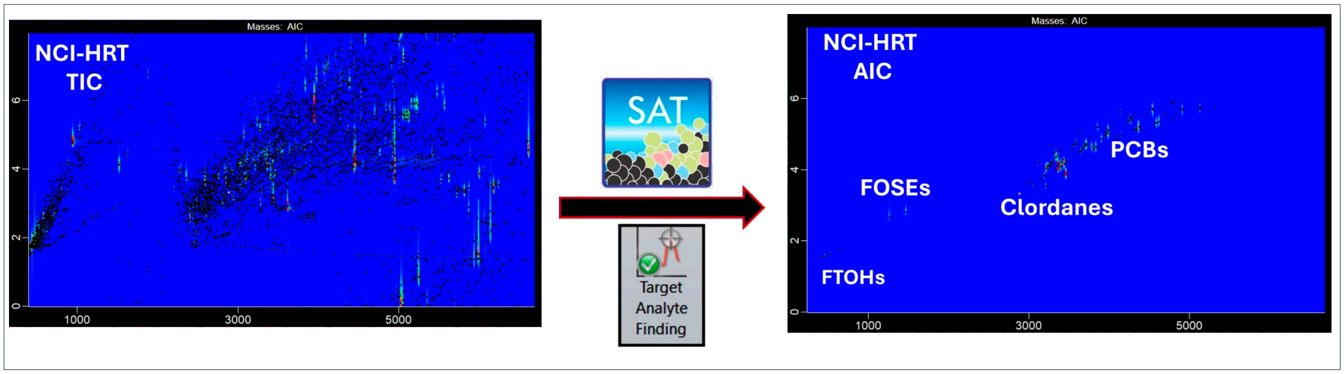


Figure 1. Negative Chemical Ionization–HRT Contour Plot (TIC) displaying thousands of peak markers after Peak Find (Untargeted) Data Processing (Left) for the reference dust standard. The utilization of SAT and Target Analyte Finding (TAF) options in the software resulted in characterization of trace Perfluorooctane Sulfanamide Ethanols (FOSEs), Fluorotelomer Alcohols (FTOHs), Polychlorinated Biphenyls (PCBs), and Chlordanes (Right). This versatile method allows for untargeted screening and focused review of these samples for potential hazardous materials.

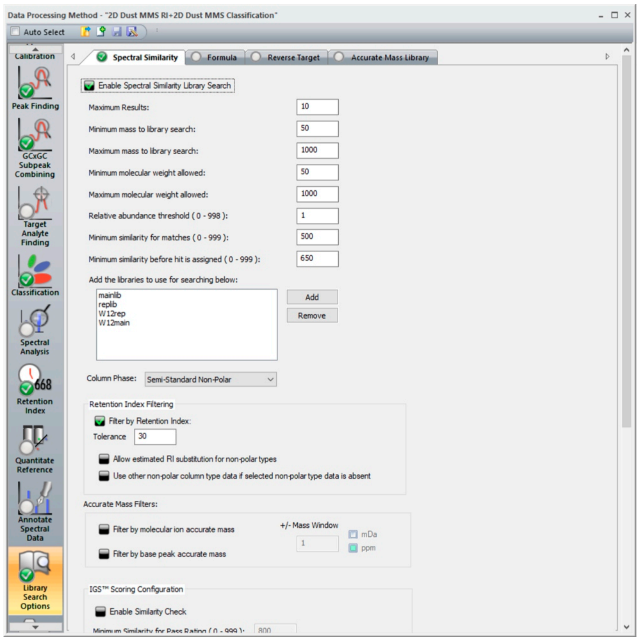
Experimental

The results presented in this application note are for NIST household dust SRM 2585. A sample of the dust was weighed (0.14 ±0.01 g) and transferred to a 10 mL glass centrifuge tube, then 3 mL of extraction solvent (3:1) dichloromethane/hexane was added, and the tube was capped. The heterogeneous mixture was vortexed for 10 s and then extracted for 20 min via sonication. The supernatant was removed, and the residue was extracted again with an additional 3 mL of solvent. The combined extracts were centrifuged (10 min, 3500 rpm) and filtered using a syringe filter to remove any remaining dust particles. The solution was then placed under N_2 gas to evaporate the extraction solvents. The resulting residue was reconstituted in 400 µL of dichloromethane and transferred to 2 mL GC vials for analysis. The instrument used for data collection was a LECO *Pegasus* HRT$^+$ 4D equipped with a novel Multi-Mode Source (MMS), which can acquire Electron Ionization (EI), Positive Chemical Ionization (PCI), and Negative Chemical Ionization (NCI) with the same hardware. The data was collected using the parameters listed in Table 1.

Table 1. Instruments Parameters

Gas Chromatograph	**Agilent 8890 with LECO Dual Stage QuadJet™ Modulator**
Injection	2 µL liquid injection, Splitless, 70 °C to 300 °C at 500 °C/min (Agilent MMI)
Carrier Gas	He @ 1.0 mL/min, Corrected Constant Flow
Primary Column	HP-5MS UI, 30 m x 0.25 mm i.d. x 0.25 µm
Secondary Column	BPX-50, 0.60 m x 0.10 mm x 0.10 µm
Temperature Program	80 °C (1 min) ramp 20 °C/min to 140 °C (Hold 30 min), then ramp to 200 °C at 10 °C/min, and ramp 1.5 °C/min to 300 °C (Hold 5 min). Secondary oven maintained +5 °C relative to primary oven
Modulation Period	8.0 s; modulator maintained +15 °C relative to secondary oven
Transfer Line	300 °C
Mass Spectrometer	**LECO *Pegasus* HRT$^+$ 4D**
Source Temperature	EI, 250°C; PCI, NCI, 165 °C
Acquisition Mode	High Resolution, R ≥ 25,000 for m/z 219, Mass Accuracy ≤1 ppm
Ionization	EI, PCI/NCI (Reagent Gas = CH_4)
Mass Range (m/z)	EI 50-1000; PCI 60-1000; NCI, 30-1000
Acquisition Rate	125 sps

The GCxGC-EI-HRTOFMS data were utilized to characterize compounds through automated Peak Find (untargeted) processing, which consisted of spectral similarity searches of large spectral databases, retention index filtering, and formula determinations using high-resolution accurate mass ions (Figure 2). Positive and negative CI spectra provided complementary information to increase confidence in compound annotation.



☑ Enable Spectral Similarity Library Search

Maximum Results: 10

Minimum mass to library search: 50

Maximum mass to library search: 1000

Minimum molecular weight allowed: 50

Maximum molecular weight allowed: 1000

Relative abundance threshold (0 - 998): 1

Minimum similarity for matches (0 - 999): 500

Minimum similarity before hit is assigned (0 - 999): 650

Add the libraries to use for searching below:

mainlib
replib
W12rep
W12main

Column Phase: Semi-Standard Non-Polar

Retention Index Filtering

☑ Filter by Retention Index:

Tolerance 30

☐ Allow estimated RI substitution for non-polar types

☐ Use other non-polar column type data if selected non-polar type data is absent

Accurate Mass Filters:

☐ Filter by molecular ion accurate mass

☐ Filter by base peak accurate mass

+/- Mass Window 1 ☐ mDa ☑ ppm

IGS™ Scoring Configuration

☐ Enable Similarity Check

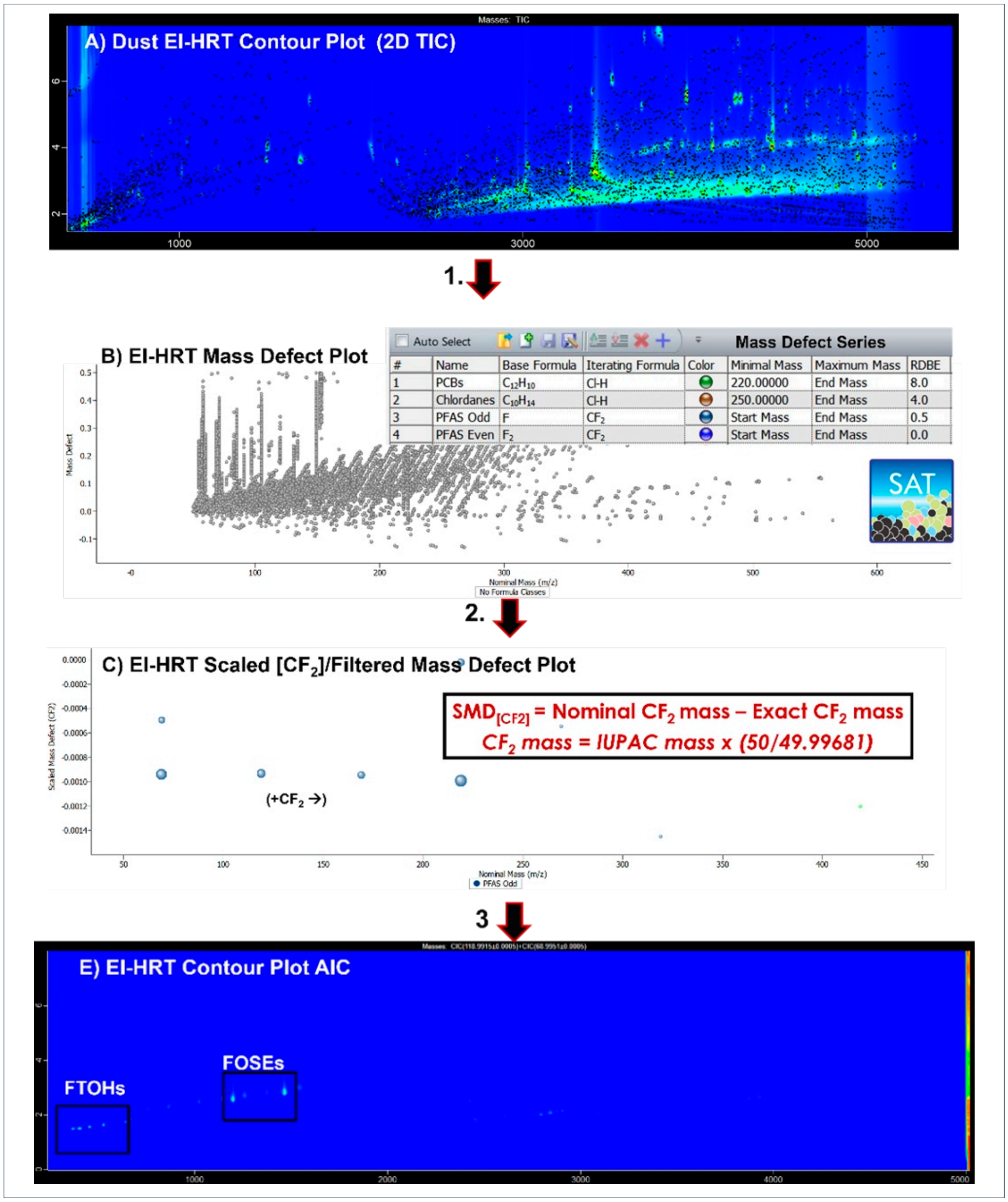
Minimum Similarity for Pass Rating (0 - 999): 800

- ✓ Peak Find (Deconvolution)
- ✓ Group Classification
- ✓ Database Searches
  - NIST
  - Wiley
  - ChemSpider
  - EPA ChemTox
- ✓ Retention Index Filtering
- ✓ Formula Determinations
  - Molecular Ions
  - Fragment Ions
  - Adducts

Figure 2. Peak Find data processing method.

Results and Discussion

This workflow and standard peak finding software allow for thorough untargeted screening of complex samples. The analytical methodology resulted in the confident annotation of numerous compounds, including hydrocarbons, acids, amines, alcohols, aldehydes, ketones, phenols, terpenes, fatty acids, steroids, phosphates, illicit drugs, over-the-counter drugs, and the more concentrated POPs in dust.[3] The additional software tools also helped screen the rich comprehensive data for various low-level analytes of interest. For example, the data were then processed using advanced SAT and TAF to search for trace PFAS and POPs in the complex dust matrix (Figure 3). These pollutants were characterized by using a combination of EI, PCI, and NCI high-resolution accurate mass data. In addition, the scaled mass defect plot feature from SAT was used to locate the PFAS and significantly simplify data processing. For example, Fluorotelomer Alcohols (FTOHs) and Perfluorooctane Sulfanamide Ethanols (FOSEs) were annotated. The processing workflow (Figure 3) includes 1) summation of all ions in the EI-HRT contour plot (TIC, peak markers displayed), 2) the use of the SAT mass defect feature to scale (CF2) and filter the mass defect plot to display the selected POP classes (e.g., PFAS odd for fragment ions), and 3) display the classes on the simplified contour plot (XIC).



Mass Defect Series

#	Name	Base Formula	Iterating Formula	Color	Minimal Mass	Maximum Mass	RDBE
1	PCBs	$C_{12}H_{10}$	Cl-H	●	220.00000	End Mass	8.0
2	Chlordanes	$C_{10}H_{14}$	Cl-H	●	250.00000	End Mass	4.0
3	PFAS Odd	F	CF_2	●	Start Mass	End Mass	0.5
4	PFAS Even	F_2	CF_2	●	Start Mass	End Mass	0.0

$SMD_{[CF2]}$ = Nominal CF_2 mass – Exact CF_2 mass
CF_2 mass = IUPAC mass x (50/49.99681)

Figure 3. The utilization of SAT to quickly simplify and locate PFAS in complex samples using GCxGC-EI-HRT data.

After highlighting select compound classes (Figure 3), individual class analytes were characterized. For example, PFAS were annotated using a combination of Peak True (Deconvoluted) EI- and PCI-HRT data, as shown in Figure 4. The EI spectrum compared favorably with the library mass spectrum of N-MeFOSE (Similarity = 808/1000). There was no molecular ion present in either the observed or library spectra, but the complementary PCI-HRT data exhibited an intense protonated molecular ion $[MH]^+$ at m/z = 558.00217 (Mass accuracy = -0.81 PPM, Isotopic fidelity = 980/1000), supporting the annotation

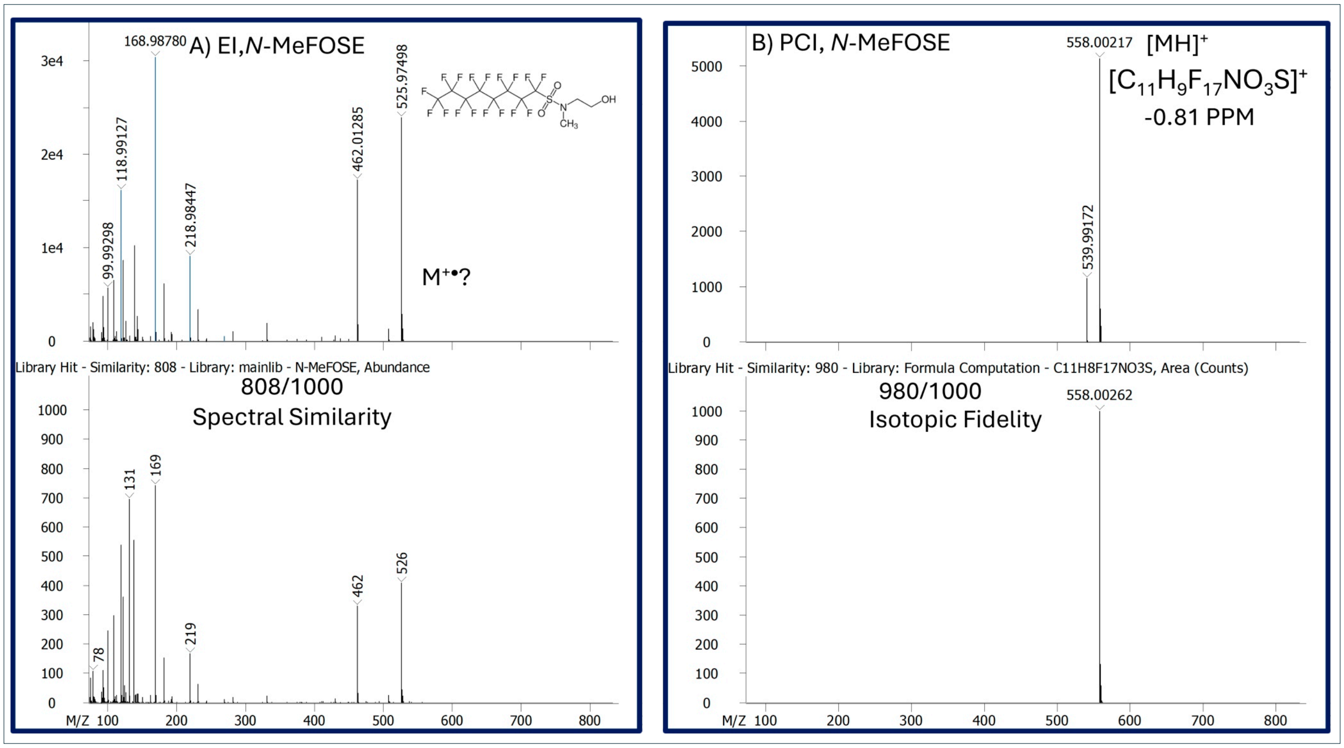
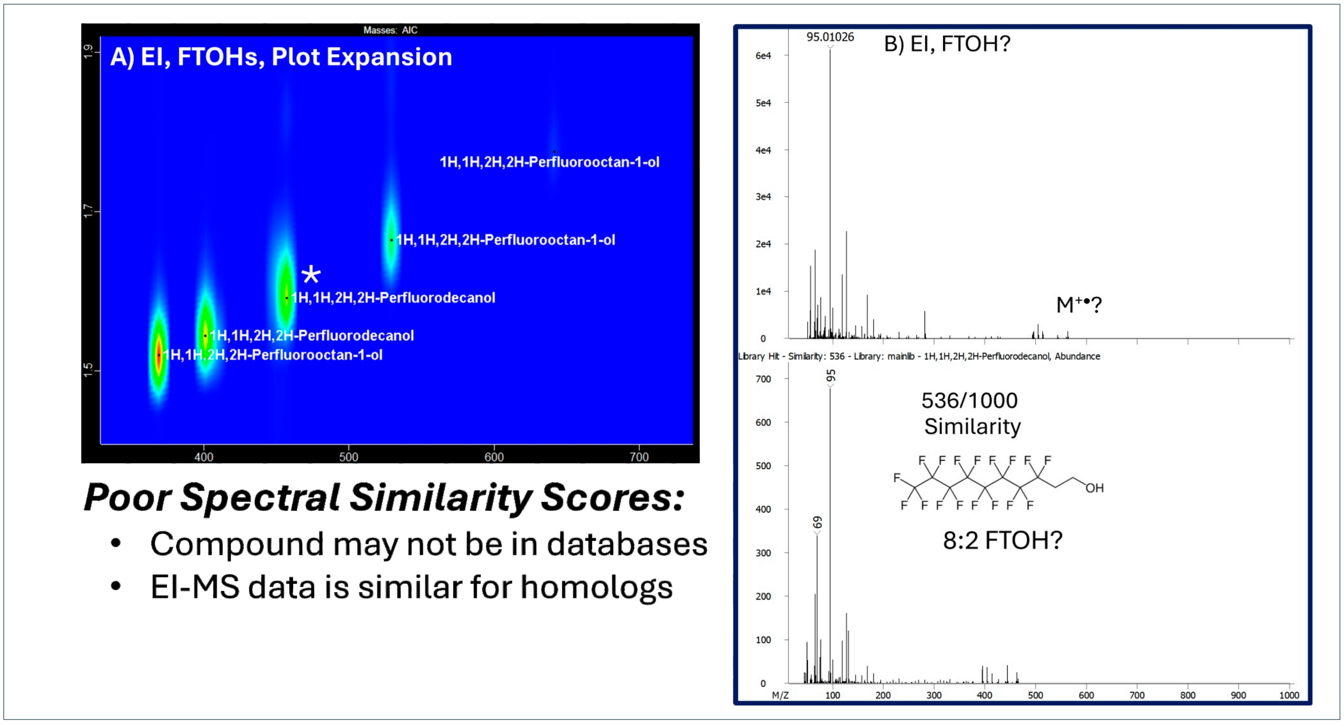


Figure 4. A) EI-HRT data (Top) and library mass spectra (Bottom) for N-MeFOSE. B) PCI-HRT data (Top) and theoretical isotopic fidelity comparison (Bottom) for N-MeFOSE.

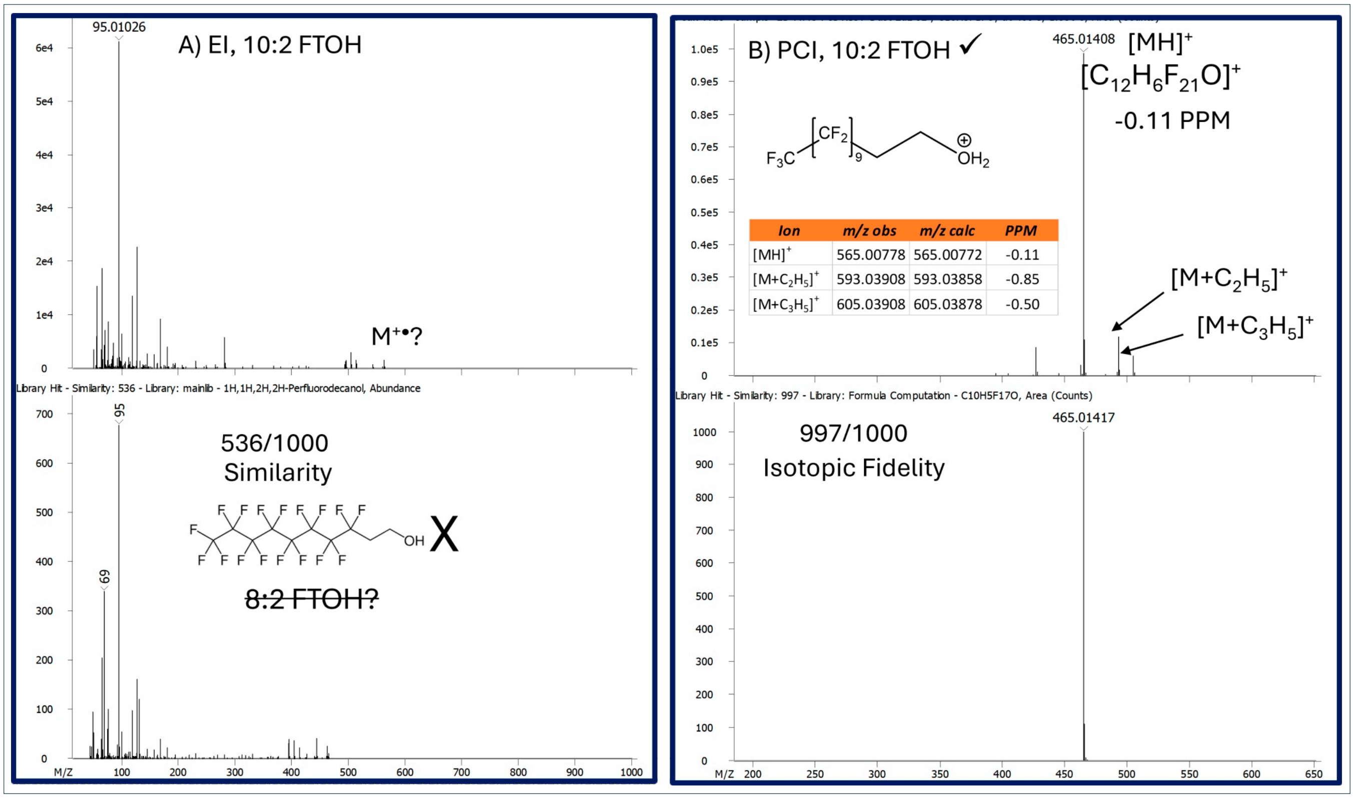
In other instances, the CI data improved compound annotations by correcting errors occasionally encountered during the comparison of an EI-HRT spectrum with databases that did not contain the compound of interest. For example, the EI spectra for a homologous series of FTOHs (highlighted in Figure 3) were very similar and did not display molecular ions, which resulted in incorrect initial annotations (Figure 5). However, the complementary PCI data provided molecular formula information (mass accuracy and isotopic fidelity) and facilitated the correct annotation of the FTOHs (Figure 6). The EI-HRT mass spectrum (Top) and the library match 8:2 FTOH (Bottom) do not exhibit molecular ions, and the similarity score for the database match is poor (536/1000) for 8:2 FTOH. The corresponding PCI spectrum includes an intense $[MH]^+$ with good Isotopic fidelity (990/1000) for the formula $C_{12}H_5F_{21}O$. The $[MH]^+$ together with the additional adducts $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ provide support for 10:2 FTOH.



Poor Spectral Similarity Scores:

- Compound may not be in databases
- EI-MS data is similar for homologs

Figure 5. A) EI-HRT Contour Plot expansion displaying a homologous series of FTOHs and B) library mass spectra for the incorrect hit 8:2 FTOH. Molecular ion information from PCI analysis (shown in Figure 6) corrected this annotation.



Ion	m/z obs	m/z calc	PPM
$[MH]^+$	565.00778	565.00772	-0.11
$[M+C_2H_5]^+$	593.03908	593.03858	-0.85
$[M+C_3H_5]^+$	605.03908	605.03878	-0.50

Figure 6. A) EI-HRT mass spectrum (Top) and incorrect library match (Bottom) for an unknown FTOH. B) Complementary PCI mass spectrum (Top, Right) and theoretical isotopic fidelity match (Bottom,) supported the annotation for 10:2 FTOH.

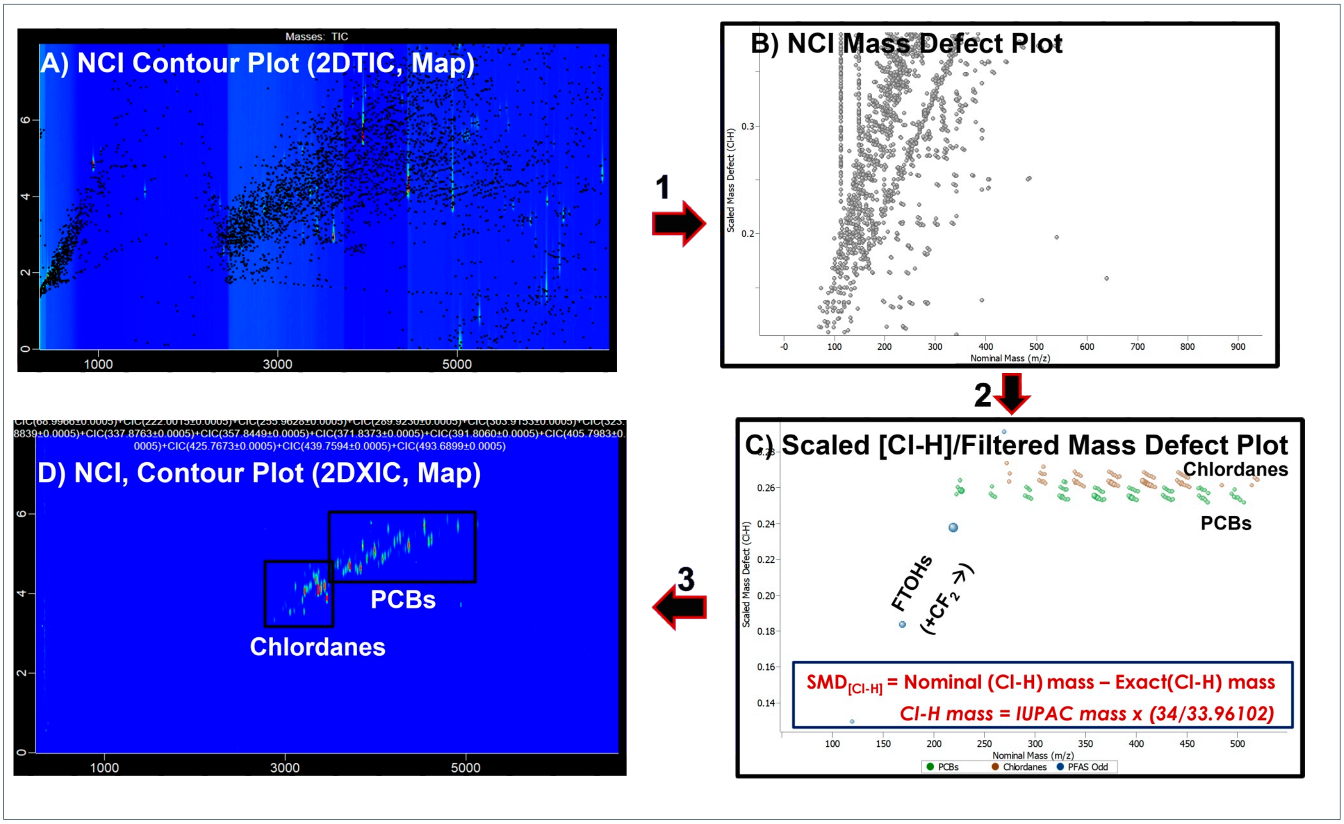
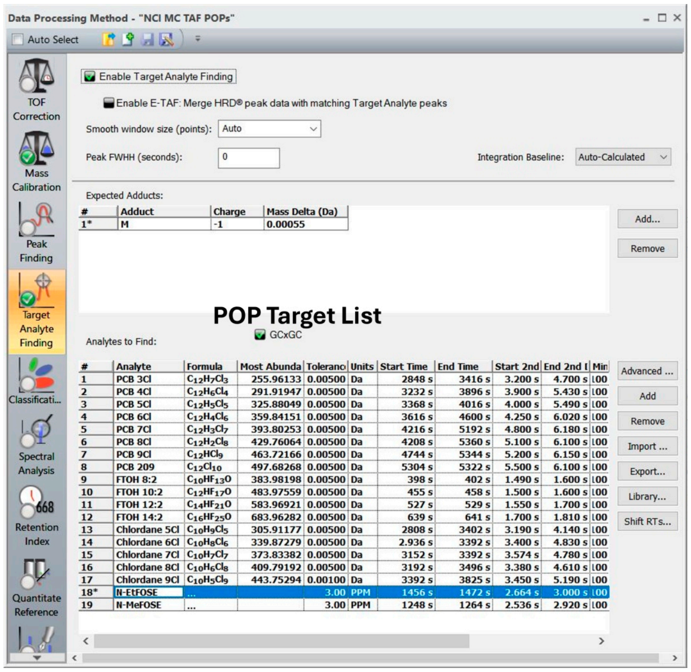


Figure 7. A) NCI contour plot with peak markers, B) Mass defect plot, C) Scaled and filtered mass defect plot, and D) NCI-HRT contour plot (XIC) displaying the location of chlordanes and PCBs in the NIST dust SRM.

The annotation strategy can be extended to other trace POPs in dust—such as PCBs and Chlordanes—by using NCI-HRT data acquisition. The modified processing workflow (Figure 7) includes 1) summation of all ions in the NCI-HRT contour plot (TIC, peak markers display), 2) the use of the SAT mass defect feature to scale and filter the mass defect plot (Cl-H) to display the selected POP classes (e.g., PCBs, Chlordanes), and 3) display the classes on the simplified contour plot (XIC). Once the location of the POPs is known, the data is processed using Target Analyte Finding (TAF). The TAF method includes a target POPs list with retention time windows and high-resolution accurate mass ions for each analyte (Figure 8), determined previously in the data review. TAF processing resulted in the annotation of FTOHs, FTOSEs, PCBs, and Chlordanes in the NIST dust SRM (Table 2).



Data Processing Method - "NCI MC TAF POPs"

☑ Enable Target Analyte Finding

☐ Enable E-TAF: Merge HRD® peak data with matching Target Analyte peaks

Smooth window size (points): Auto

Peak FWHH (seconds): 0

Integration Baseline: Auto-Calculated

Expected Adducts:

#	Adduct	Charge	Mass Delta (Da)
1*	M	-1	0.00055

POP Target List

☑ GCxGC

Analytes to Find:

#	Analyte	Formula	Most Abunda	Toleranc	Units	Start Time	End Time	Start 2nd	End 2nd I	Min
1	PCB 3Cl	$C_{12}H_7Cl_3$	255.96133	0.00500	Da	2848 s	3416 s	3.200 s	4.700 s	100
2	PCB 4Cl	$C_{12}H_6Cl_4$	291.91947	0.00500	Da	3232 s	3896 s	3.900 s	5.430 s	100
3	PCB 5Cl	$C_{12}H_5Cl_5$	325.88049	0.00500	Da	3368 s	4016 s	4.000 s	5.490 s	100
4	PCB 6Cl	$C_{12}H_4Cl_6$	359.84151	0.00500	Da	3616 s	4600 s	4.250 s	6.020 s	100
5	PCB 7Cl	$C_{12}H_3Cl_7$	393.80253	0.00500	Da	4216 s	5192 s	4.800 s	6.180 s	100
6	PCB 8Cl	$C_{12}H_2Cl_8$	429.76064	0.00500	Da	4208 s	5360 s	5.100 s	6.100 s	100
7	PCB 9Cl	$C_{12}HCl_9$	463.72166	0.00500	Da	4744 s	5344 s	5.200 s	6.150 s	100
8	PCB 209	$C_{12}Cl_{10}$	497.68268	0.00500	Da	5304 s	5322 s	5.500 s	6.100 s	100
9	FTOH 8:2	$C_{10}HF_{13}O$	383.98198	0.00500	Da	398 s	402 s	1.490 s	1.600 s	100
10	FTOH 10:2	$C_{12}HF_{17}O$	483.97559	0.00500	Da	455 s	458 s	1.500 s	1.600 s	100
11	FTOH 12:2	$C_{14}HF_{21}O$	583.96921	0.00500	Da	527 s	529 s	1.550 s	1.700 s	100
12	FTOH 14:2	$C_{16}HF_{25}O$	683.96282	0.00500	Da	639 s	641 s	1.700 s	1.810 s	100
13	Chlordane 5Cl	$C_{10}H_9Cl_5$	305.91177	0.00500	Da	2808 s	3402 s	3.190 s	4.140 s	100
14	Chlordane 6Cl	$C_{10}H_8Cl_6$	339.87279	0.00500	Da	2.936 s	3392 s	3.400 s	4.830 s	100
15	Chlordane 7Cl	$C_{10}H_7Cl_7$	373.83382	0.00500	Da	3152 s	3392 s	3.574 s	4.780 s	100
16	Chlordane 8Cl	$C_{10}H_6Cl_8$	409.79192	0.00500	Da	3192 s	3496 s	3.380 s	4.610 s	100
17	Chlordane 9Cl	$C_{10}H_5Cl_9$	443.75294	0.00100	Da	3392 s	3825 s	3.450 s	5.190 s	100
18*	N-EtFOSE	...		3.00	PPM	1456 s	1472 s	2.664 s	3.000 s	100
19	N-MeFOSE	...		3.00	PPM	1248 s	1264 s	2.536 s	2.920 s	100

- ✓ Retrospective Analysis of Comprehensive Data
- ✓ Trace Analysis
- ✓ Quantitative Analysis
- ✓ Fast Processing

Figure 8. TAF processing method that includes a target list with retention windows and HRAM ions.

Table 2. TAF processing results, including analyte name, R.T., signal to noise, and peak area.

Name	R.T. (s)	Peak S/N	Area
FTOH 8:2	400, 1.536	122	39745
FTOH 10:2	456, 1.584	182	45139
FTOH 12:2	528, 1.648	134	30844
FTOH 14:2	640, 1.760	59	8830
N-MeFOSE	1264, 2.688	126	135734
N-EtFOSE	1464, 2.832	220	136663
Chlordane 5Cl	2880, 3.344	28	2646
Chlordane 6Cl	2976, 3.632	34	3439
Chlordane 6Cl	3008, 3.688	25	2541
Chlordane 6Cl	3056, 3.536	126	15791
Chlordane 6Cl	3112, 4.184	151	16657
Chlordane 7Cl	3152, 3.744	23	2265
Chlordane 7Cl	3192, 3.840	240	26166
Chlordane 6Cl	3192, 3.848	63	7242
Chlordane 8Cl	3208, 3.552	354	33778
Chlordane 7Cl	3216, 4.056	601	97391
Chlordane 6Cl	3216, 4.064	558	85421
Chlordane 5Cl	3216, 4.064	84	10839
Chlordane 6Cl	3232, 4.128	88	17716
Chlordane 7Cl	3240, 4.112	94	22096
Chlordane 6Cl	3248, 4.128	34	3064
PCB 4Cl	3256, 4.088	22	3550
PCB 4Cl	3272, 4.192	133	23439
Chlordane 6Cl	3272, 4.688	59	8262
Chlordane 7Cl	3280, 4.240	25	2758
Chlordane 6Cl	3280, 4.248	21	2174
Chlordane 6Cl	3288, 4.136	25	2490
Chlordane 7Cl	3288, 4.168	35	3687
PCB 4Cl	3288, 4.224	54	9950
Chlordane 7Cl	3312, 4.472	119	21062
Chlordane 6Cl	3312, 4.504	39	7612
Chlordane 8Cl	3336, 4.000	51	4735
Chlordane 7Cl	3336, 4.360	614	111800
Chlordane 6Cl	3336, 4.360	85	15672
Chlordane 7Cl	3352, 4.408	92	16486
Chlordane 8Cl	3360, 4.112	3127	945592
Chlordane 7Cl	3360, 4.112	825	413840
Chlordane 6Cl	3360, 4.120	352	143938
Chlordane 5Cl	3360, 4.120	41	7889
PCB 5Cl	3368, 4.168	49	13475
PCB 4Cl	3368, 4.552	80	17205
Chlordane 8Cl	3392, 4.096	1115	145220
Chlordane 7Cl	3392, 4.104	22	2114
PCB 5Cl	3400, 4.224	249	54028
PCB 5Cl	3424, 4.296	37	6008
Chlordane 9Cl	3432, 3.848	54	5514
Chlordane 8Cl	3440, 4.176	1547	338027
Chlordane 9Cl	3464, 3.888	1733	336210
Chlordane 8Cl	3480, 4.232	85	9885
PCB 5Cl	3536, 4.616	65	19898
PCB 5Cl	3552, 4.720	52	11263
PCB 5Cl	3584, 4.720	227	44766
PCB 4Cl	3584, 4.784	88	23079
PCB 6Cl	3640, 4.536	130	23649
PCB 5Cl	3640, 5.080	23	4069
PCB 6Cl	3648, 4.520	128	23937
PCB 6Cl	3664, 4.632	80	14882
PCB 6Cl	3672, 4.576	60	11216
PCB 5Cl	3672, 4.592	67	14750
PCB 5Cl	3688, 4.664	130	23845
PCB 4Cl	3688, 4.672	25	4730
PCB 5Cl	3704, 4.688	32	5461
PCB 6Cl	3704, 4.736	257	54959
PCB 5Cl	3712, 4.712	902	307906
PCB 4Cl	3712, 4.720	97	29719
PCB 5Cl	3776, 4.912	24	3820
Chlordane 9Cl	3784, 4.656	365	56822
PCB 6Cl	3800, 4.576	208	44400
PCB 5Cl	3800, 4.584	23	3756
PCB 6Cl	3832, 4.640	957	285099
PCB 5Cl	3832, 4.656	99	18983
PCB 6Cl	3848, 5.216	22	7996
PCB 4Cl	3856, 5.200	46	9766
PCB 5Cl	3856, 5.208	329	74091
PCB 6Cl	3904, 4.872	339	85405
PCB 5Cl	3904, 4.888	31	5130
PCB 6Cl	3944, 4.968	45	13273
PCB 6Cl	3952, 5.064	46	8259
PCB 6Cl	3960, 5.024	41	8148
PCB 6Cl	3976, 5.024	107	21750
PCB 6Cl	3984, 5.096	714	280450
PCB 5Cl	3984, 5.120	138	34941
PCB 6Cl	4000, 5.016	198	39233
PCB 5Cl	4000, 5.016	24	4921
PCB 6Cl	4032, 5.288	40	11070
PCB 6Cl	4080, 4.872	51	14033
PCB 6Cl	4104, 4.960	46	10259
PCB 6Cl	4136, 5.632	127	39203
PCB 6Cl	4144, 4.976	85	27026
PCB 6Cl	4208, 5.312	34	6945
PCB 7Cl	4240, 5.376	160	38110
PCB 6Cl	4240, 5.384	22	4263
PCB 6Cl	4264, 5.456	58	10772
PCB 7Cl	4264, 5.496	80	15711
PCB 6Cl	4272, 5.392	302	70449
PCB 7Cl	4272, 5.432	23	4796
PCB 8Cl	4304, 5.128	36	7095
PCB 6Cl	4304, 5.504	52	9809
PCB 7Cl	4328, 5.120	109	26105
PCB 6Cl	4328, 5.136	26	4923
PCB 7Cl	4368, 5.200	995	397534
PCB 6Cl	4368, 5.216	283	78439
PCB 7Cl	4384, 5.128	42	8583
PCB 8Cl	4440, 5.528	50	10118
PCB 7Cl	4536, 5.720	370	86694
PCB 6Cl	4536, 5.728	129	27998
PCB 7Cl	4544, 5.672	277	74844
PCB 6Cl	4544, 5.680	88	22186
PCB 8Cl	4568, 5.216	21	4613
PCB 8Cl	4584, 5.312	318	119235
PCB 7Cl	4584, 5.320	91	32680
PCB 6Cl	4584, 5.336	20	3297
PCB 8Cl	4616, 5.360	427	152732
PCB 7Cl	4616, 5.376	103	38093
PCB 7Cl	4704, 5.552	31	6906
PCB 9Cl	4784, 5.416	70	16203
PCB 8Cl	4792, 5.864	73	27430
PCB 7Cl	4792, 5.912	22	5032
PCB 9Cl	4832, 5.560	26	5294
PCB 7Cl	4904, 5.728	22	5236
PCB 8Cl	4912, 5.672	308	97987
PCB 7Cl	4912, 5.680	116	26947
PCB 9Cl	5128, 5.720	150	51490
PCB 8Cl	5128, 5.760	51	10527
PCB 8Cl	5136, 5.688	34	7760
PCB 209	5312, 5.792	51	10229

Conclusion

LECO Corporation's *Pegasus* HRT$^+$ 4D is a powerful tool for the analysis of complex environmental samples, such as the NIST house dust SRM that was used to demonstrate this workflow. The instrument produces untargeted data that can be evaluated with various software tools. Automated Peak Find processing provides an extensive list of annotated sample components through spectral similarity searches and formula determinations using high-resolution, accurate mass ions, while the complementary use of Spectral Analysis Tools (SAT) and Target Analyte Finding (TAF) is a more effective method for the characterization of trace PFAS and other POPs in samples. Together, these analysis options provide thorough coverage of the rich data for these complex environmental samples.

References

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