

Open Characterization of 2.5 Micron, Particulate-Borne Semi-Volatile Organic Compounds by GC-TOFMS and UHPLC Orbitrap MS: Method Development, Performance and Applications

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Overview

The purpose of this work was to prove the concept that ambient fine particulate matter 2.5 microns in diameter (PM_{2.5}) collected by a Thermo Scientific™ TEOM (tapered element oscillating microbalance) filter can be used to monitor ambient semi-volatile organic compounds (SVOCs). A workflow including sample preparation, gas chromatography-time of flight mass spectrometry (GC-TOFMS), and ultra-high performance liquid chromatography (UHPLC) Thermo Scientific™ Orbitrap™ mass spectrometer (UHPLC Orbitrap MS) analysis of SVOCs is described. Applying this workflow, TEOM filters collected from two urban sampling stations were analyzed for targeted polycyclic aromatic compounds (PAHs) and non-targeted polar SVOCs such as pesticides and pharmaceutically active compounds.

Introduction

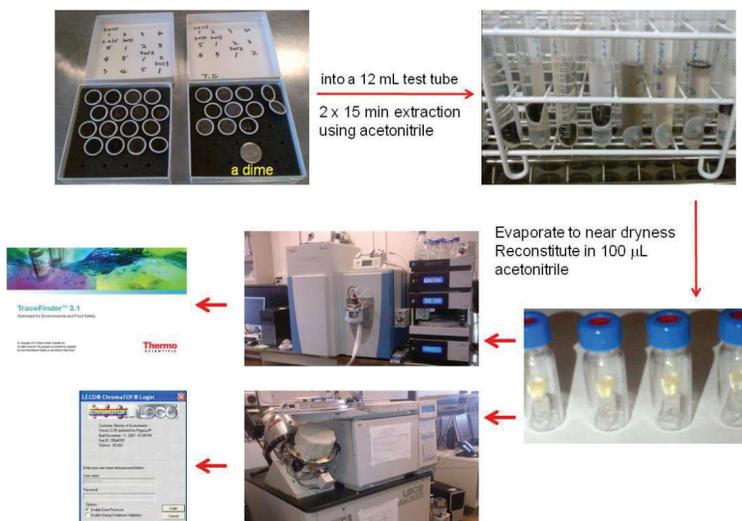
Airborne SVOCs, such as PAHs, are associated with fine particulate matter PM_{2.5}. PM_{2.5} also contains acidic components of airborne particulate and most of its mutagenic activity. The Ontario Ministry of the Environment (MOE) uses TEOM technologies to monitor PM_{2.5}. Information on individual SVOCs associated with PM_{2.5} can improve the knowledge gap related to the effects of PM_{2.5}. This work investigated the feasibility and analytical results obtained by using GC-TOFMS and UHPLC Orbitrap MS in the analysis of SVOCs in PM_{2.5} obtained from TEOM filters.

Methods

Chemicals and Sample Preparation

HPLC-grade acetonitrile (CH₃CN) was purchased from Thermo Fisher Scientific (Ottawa, ON, Canada). PAH stock solution consists of 21 individual PAHs that were purchased from Ultra Scientific Analytical Solutions (Brockville, ON, Canada). Pesticide analytical standards were courtesy of Dr. Jon Wong (United States Food and Drug Administration, College Park, MD). Neat standards of deuterium (D) labelled PAHs were purchased from CDN Isotope (Pointe-Claire, QC, Canada). Native and D-labelled intermediate standard solutions were prepared by mixing the corresponding stock solutions. Five levels of analytical standard solutions were prepared by diluting stock solution with toluene. A 60-day sampling period PM_{2.5} were collected using TEOM filters of 15 mm diameter at a flow rate of 3 L/min, resulting in a total sample volume of 259.2 M³. Five filters were randomly chosen from two urban sampling sites (A and B) with heavy industrial and shopping activities. Filters were ultrasonically extracted by 10 mL acetonitrile in 12-mL centrifuge tubes for 2 x 15 min period. Sample extracts were then filtered through a 0.2 micron syringe filter directly into another 12-mL centrifuge tube. The extract was evaporated to near dryness, reconstituted in 100 mL acetonitrile, transferred to an autosample vial with a 125-mL glass insert and stored in a refrigerator until ready for analysis (Figure 1).

FIGURE 1. Preparation and analysis of TEOM filter samples for GC-TOFMS and UHPLC Orbitrap MS analysis



GC-TOFMS and UHPLC-Orbitrap MS Analysis

Sample analysis of targeted PAHs was done by a LECO® Pegasus® III GC-TOFMS system using helium carrier gas and a DB®-5 30m 0.25mm 0.25m column. Details of the analysis is available on request (1). Polar SVOC analysis was achieved on a Thermo Scientific™ Dionex™ UltiMate™ 3000 UHPLC system, consisting of an HRG-3400RS binary pump, WPS-3000 autosampler, and a TCC-3400 column compartment. Separation was achieved by injecting 10 mL extracts into a Thermo Scientific™ BetaSil™ (positive mode) and an XB® C-18, 2.1 100 mm coreshell technology column for positive and negative mode Orbitrap MS analysis. Details of the UHPLC analysis is available on request (2). The UHPLC was interfaced to a Thermo Scientific™ Exactive™ Plus Orbitrap MS using a heated electrospray ionization (H-ESI II) interface. The Orbitrap MS system was tuned and calibrated in positive and negative modes by infusion of standard mixtures of MSCAL5 and MSCAL6. High purity nitrogen (>99%) was used in the H-ESI II source (35 L/min). Spray voltages used were (declustering potential) of 2,500 and 3,200 V for positive and negative modes. Mass spectrometric data was acquired at a resolving power of 140,000 (defined as full-width-at-half-maximum peak width at m/z 200, R_{FWHM}), resulting in a scanning rate of >1.5 scans/sec when using automatic gain control and a C-trap inject time of 50 msec.

Data Analysis

The GC-TOFMS analytical data collected were processed offline using LECO® ChromaTOF® deconvolution software and NIST mass spectral library. Thermo Scientific™ Xcalibur™ software was used for process mass spectral data for graphic presentation. Two databases were used with Thermo Scientific™ TraceFinder™ software to carry out targeted screening for 565 pesticides (courtesy of Dr. Jon Wong, U.S. FDA) and an in-house 382 emerging organic compounds (EOCs) including pharmaceutically active compounds, steroids, hormones, surfactants, and perfluorohydrocarbons. The pesticide database was used to screen compounds in positive mode while the EOC database was used in both positive and negative mode screenings. Adduct ions of $(M+H)^+$, $(M+NH_4)^+$, and $(M+Na)^+$ were used in the positive mode, while $(M-H)^-$ adduct ion was used in negative mode data. The TraceFinder software created an extracted ion chromatogram (XIC) using a mass extraction window (MEW) of 5 ppm. Analytes were identified using an XIC area threshold of 50,000 (approximately 25–50 pg/mL, ppt) depending on compound, a 2 ppm mass accuracy of the mono-isotopic mass (M) of the molecular ion, and an isotopic $(M+1)$ peak threshold of 90% with relative intensities variation of <10%. Typical screening time was about 65 sec/sample using the 565 pesticide database. Analytical results were interpreted manually for the top 10th percentile compounds and exported to Microsoft® Excel® with which analytical data were compiled for the presentation.

Results

Analytical Results Obtained From GC-TOFMS

Table 1 shows results obtained from targeted analysis of PAHs from the 10 PM_{2.5} filter samples. The occurrence of these detected PAHs in air samples have been known and reported since the late 1980s. Amberlite™ XAD™ resin (divinylbenzene polymer) was not used in the sampling media; therefore, PAHs with lower boiling point, that is, 2- or 3-ring PAHs such as naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene, and acenaphthene, are expected to break through during the 60-day sampling period and not be detected in all samples.

Identification of Non-targeted Compounds by UHPLC Orbitrap MS and TraceFinder Software

In the identification of non-targeted compounds, accurate mass of the mono-isotopic peak M , isotopic $(M+1)$ peaks, relative intensities of the $M/(M+1)$ peak, and retention time (RT) obtained from the XIC of UHPLC analysis (if analytical standard was available) could all be used to improve the confidence and credibility of analytical results. Figure 2 shows the true positive identification of bisphenol A, a known endocrine disrupting compound, in seven of the ten samples analyzed. The identification was achieved by a mass accuracy of <2 ppm of the M and $(M+1)$ between the simulated and measured mass spectral peaks (Figure 2C and 2D) and RT (Figure 2A and 2B) obtained from the XIC.

TABLE 1. Analytical results obtained from GC-TOFMS analysis of targeted PAHs.

Compound Name	Site A					Site B					MDL
	#1	#2	#3	#4	#5	#1	#2	#3	#4	#5	
	Loading, Total ng										
Phenanthrene	19.9	16.1	21.1	27.4	15.5	16.0	15.6	20.9	9.3	14.3	4.1
Anthracene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	7.7
Fluoranthene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.6
Pyrene	7.5	13.1	10.6	19.8	9.7	15.9	<MDL	<MDL	<MDL	13.0	2.4
B(a)A	<MDL	<MDL	<MDL	<MDL	<MDL	5.5	<MDL	<MDL	<MDL	<MDL	3.5
Chrysene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.1
7,12-Dimethylbenz(a)Anthracene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	4.8
B(b)Fluorascence	<MDL	15.1	12.1	27.0	14.9	29.0	10.4	38.5	9.4	10.5	4.1
B(k)Fluorascence	<MDL	<MDL	<MDL	9.6	4.9	<MDL	3.7	<MDL	<MDL	<MDL	3.4
B(e)Pyrene	<MDL	7.5	6.6	14.5	7.0	14.9	5.9	19.5	4.5	5.3	3.6
B(a)Pyrene	<MDL	4.9	<MDL	9.6	<MDL	7.6	<MDL	8.6	<MDL	<MDL	4.2
Perylene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	5.2
Indeno(123-cd)Pyrene	<MDL	5.7	5.3	10.2	10.2	10.8	4.2	15.0	<MDL	<MDL	3.2
Dibenz(ah)Anthracene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	6.6
Benzo(ghi)Perylene	<MDL	8.3	8.0	14.8	8.1	15.6	7.1	21.3	4.8	5.1	4.4
Napthalene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	4.7
2-Methylnaphthalene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	6.7
1-Methylnaphthalene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	6.5
Acenaphthylene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	4.4
Acenaphthene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.6
Fluorene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.2

MDL: Method Detection Limit

FIGURE 2. True positive identification of bisphenol A.

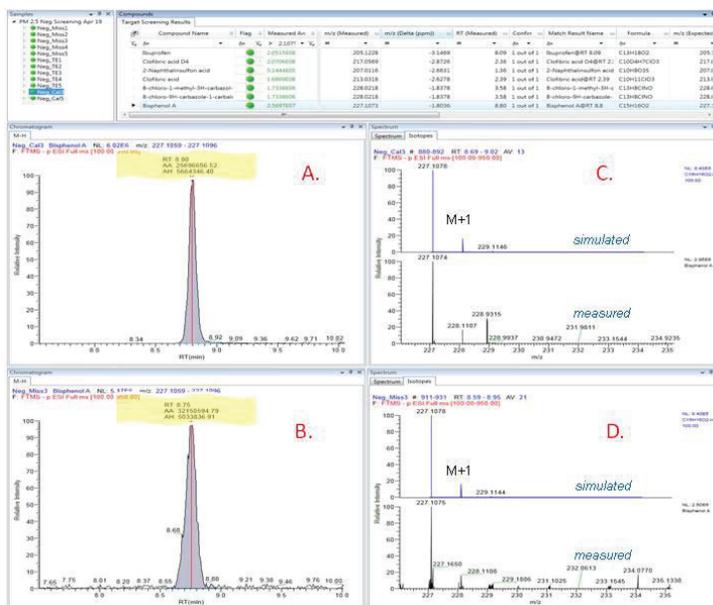


Figure 3 shows an example of the false positive identification of Gemfibrozil, a lipid lowering drug, in the analysis. From Figure 3C and 3D, one can observe that accurate mass of the simulated and measured mass of the M and (M+1) peaks meet the criteria given and would have been treated as positively identified. However, analytical standard of Gemfibrozil had an RT of 10.85 min instead of that 6.5 ± 0.1 min observed in the analysis (Figure 3A and 3B). A ChempSpider search showed there were 87 possible hits within the $M \pm 2$ ppm range of Gemfibrozil. Additional work will be required to positively identify this 6.5 ± 0.1 min compound. There were also situations in which compounds can be tentatively identified by the accurate mass of both M and (M+1) isotopic peaks (within 2 ppm) but need to be confirmed by an analytical standard.

Table 2 lists compounds found in these 10 TEOM samples along with their occurrence in the negative mode using the 385 ESOC database. Of the 26 compounds in the top 10th percentile of area counts, Bisphenol A was the only positively identified compound.

Table 3 lists compounds found in these 10 samples along with their occurrence in the positive mode.

FIGURE 3. False positive identification of Gemfibrozil.

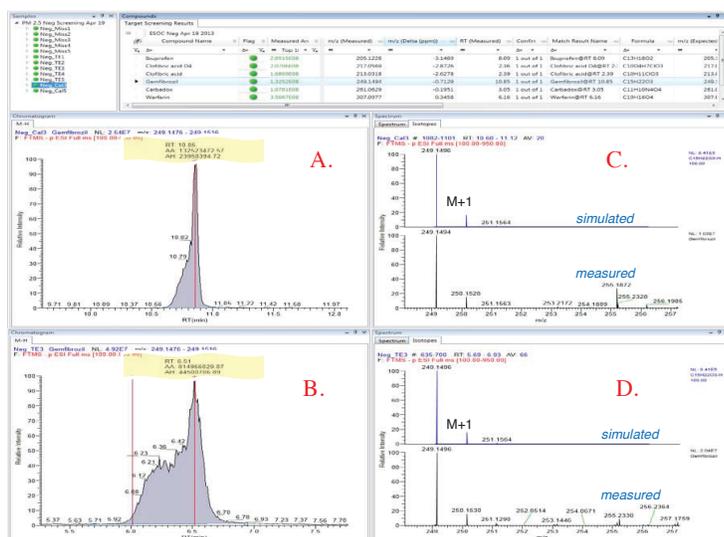


TABLE 2. Compounds with area counts in the top 10th percentile analyzed in the negative mode.

Compound Name	Occu.	Compound Name	Occu.
(1,4-dioxo-4,9-dihydro-1H-carbazol-8-yl) acetic acid	100%	2-(2-chloro-phenylamino)-benzaldehyde	80%
(8-hydroxyl-9H-carbazol-1-yl) acetic acid	100%	Chlortoluron	80%
1-chloro-8-methyl-9H-carbazole	100%	Perfluorooctane sulfonate	80%
Bisphenol A ¹	100%	19-Norethersterone ²	70%
DCL-Quinone	100%	2-Ethylhexyl-4-methoxycinnamate	70%
Dinoseb	100%	Monuron	70%
Gemfibrozil ²	100%	O-Desvenlafaxine	70%
Ibuprofen ²	100%	Oxolinic Acid	60%
Imidacloprid ²	100%	1-chloro-9H-carbazole	50%
Octylphenol	100%	Hydrocortisone ²	50%
2-chloro-N-phenylaniline	90%	Metolachlor-OXA	50%
3-Phenoxybenzoic acid	90%	Oxybenzone ²	40%
Climbazol	90%	2-(4,6-Diphenyl-1,3,5-triazin-2-yl)-5-[(hexyl)oxy]-phenol	10%

Occu.: Occurrence rate; 1: Confirmed by RT of an analytical standard;
2: Analytical standard has a different RT

TABLE 3. Compounds with area counts in the top 10th percentile analyzed in the positive mode

Compound Name	% Occu.	Compound Name	% Occu.
Atenolol*	100	Dodecyltrimethylammonium	90
Azoxystrobin	100	Sulfamethazine	90
Dexamethasone	100	Methyl-Benzotriazol	80
Estriol*	100	Metribuzin	70
Nonylphenol diethoxylate	100	Propanolol	50
Prometon	100	Simeton	50
Terbutometon	100	Terbutylazin-2-hydroxy	50
Benzyltrimethylammonium	90	Methylprednisolone	40

Occu.: Occurrence rate; *: Analytical standard has a different RT

Table 4 lists compounds found in these 10 TEOM samples along with their occurrence in the positive mode using the 565 pesticide database. Of the 16 compounds in the top 10th percentile of area counts, Flamprop-M-isopropyl, a herbicide used in wild oat control, was the only positively identified compound by accurate mass of M and isotopic (M+1) peaks and RT of an analytical standard. We cannot rationalize the source of this pesticide. Nicotine can be attributed to tobacco smoke and was found with high area counts (within 10th percentile of area counts) in nine of the ten samples investigated.

TABLE 4. Compounds with area counts in the top 10th percentile analyzed in the positive mode using the 565 pesticides.

Compound Name	% Occu.	Compound Name	% Occu.
Dodemorph	90%	Flamprop-M-isopropyl [†]	60%
Famphur-oxon	90%	Tebuthiuron	60%
Fenamiphos-deisopropyl	90%	Mepanipyrim	40%
Nicotine *	90%	Metolcarb	40%
Sedaxane	90%	Propamocarb	40%
Spiroxamine	90%	Trimethacarb, 2,3,5-	40%
Paclobutrazol	80%	XMC	40%
Siduron	80%	Xylycarb	40%

Occu.: Occurrence rate; †: Confirmed by RT of an analytical standard;

*: Analytical standard has a different RT

Conclusion

It is demonstrated that combining UHPLC Orbitrap MS and iterative data analysis by TraceFinder software using different compound databases in different retrospective steps can be a powerful tool in the analysis of airborne pollutants:

- True positive identification of targeted and non-targeted compounds including those traditionally known (that is, PAHs) and expected but have not been reported like nicotine and Bisphenol A.
- Tentative identification of ESOCs such as pharmaceuticals, personal care products, and pesticides (mostly fungicides) associated with airborne particulates.
- Identification by accurate mass of M and isotopic (M+1) peaks and their relative intensity of M/(M+1) can still result in false positive results. Confirmation by fragment ion(s), as suggested by SANCO (3), or library search can be useful to improve the confidence and credibility of results.
- UHPLC provides high peak capacity and RT for the identification of analytes eluted from the UHPLC column and is complimentary to Orbitrap MS.

References

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Acknowledgements

We would like to thank Ms. Marilyn Pitcher for analyzing the PAH samples and Dr. Jon Wong and Dr. Kai Zhang for providing the required database.

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