GC-MS/MS Analysis of Persistent Organic Pollutants in Small Volumes of Human Plasma

Technical Overview

Authors

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Introduction

Technological advancements in the fields of analytical chemistry and biochemistry have allowed for characterization of a broad range of chemical exposures that may be collectively involved with the onset of chronic illnesses. New developments in mass spectrometry (MS) have yielded measurements of thousands of chemicals in a single human specimen [1]. The comprehensive measurement and characterization of all chemical exposures in the human internal environment is a main objective of the exposome.

Within the exposome paradigm, the internal environment is composed of all bio-active chemicals circulating in the body regardless of their origin, for example, genetically derived or exposure derived [2]. Examples include dietary chemicals, drugs, pollution, bio-transformation products (metabolites), foreign DNA, and other exogenous and endogenous chemicals and sources of exposure [3]. Mass spectrometric strategies can be evoked to detect these chemical analytes using small volumes of human samples from population studies to determine risk factors for chronic illnesses.

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Exposomics

Exposomics is the application of tools such as NMR, mass spectrometry, and bioinformatics to characterize and measure the exposome. Exposomics focuses primarily on complex chronic illnesses, where a single exposure or genetic trait cannot fully explain disease risk in a population. Using well-designed case-control studies, differences in exposure profiles can be determined between healthy and diseased populations. If these case-control studies are nested within larger prospective cohorts, causal associations can be validated between the measured analytes and the outcome in patients prior to disease onset.

It is important to note that exposomics requires both targeted and untargeted methods of analysis to measure both high and low concentrations of chemicals in human samples. Many external environmental pollutants exist in the human system at 1,000-times lower concentrations than those stemming from foods, drug products, or metabolites (Figure 1), and are often below the detection limits of untargeted methods in the laboratory setting [4]. To measure these low-abundance molecules, one can use MS/MS methods in the liquid phase, gas phase, or both. Metals and complexed organometallic species can be measured through ICP-MS. It is further important to note, that while most analyses are conducted in blood, this does not preclude the analyses of other matrices such as lymph, saliva, urine, or breast milk.

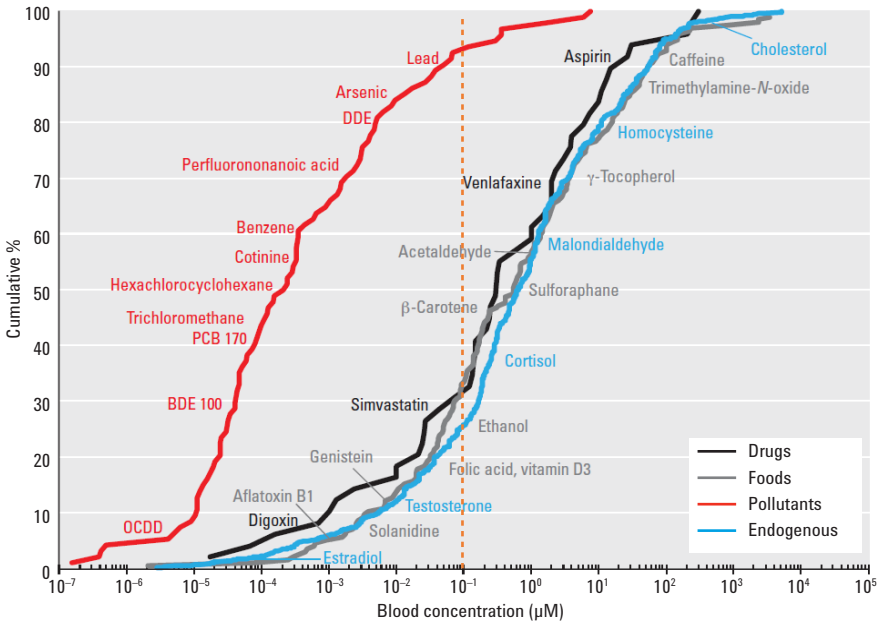

Figure 1. Blood concentrations of common exposure compounds. Chemicals on the right of the vertical dotted line are typically detected in untargeted (discovery-based) LC-TOF methods. Many on the left of the vertical line are not detected and require targeted MS/MS methodologies. This figure has been reproduced from Environmental Health Perspectives (Rappaport, et al. 2014)

Example of Targeted Exposomics Method

Herein, targeted exposomics studies are exemplified by measurement of persistent organic pollutants (POPs) detected in small volumes of plasma. As shown in Figure 1, the concentrations of POPs, such as DDE, PCBs, PBDEs, and dioxins, are among those that are below the limit of detection (LOD) for untargeted studies (0.1 µM). In fact, discovery-based analyses using liquid chromatography time-of-flight or Fourier transform mass spectrometry fail to detect approximately 70% of the exposome. Concomitant high sensitivity and specificity methodologies such as those offered by gas phase triple quadrupole mass spectrometry (GC-MS/MS) are necessary to achieve the LOD required to fully quantify and characterize the human exposome.

As bio-specimens from cohorts studies are precious, investigators are typically granted small volumes of biological fluid to run their analysis. However, most analytical procedures to measure POPs in blood require large volumes for extraction. For example, the Lab 28 methods associated with the National Health and Nutrition Examination Survey (NHANES) used to measure POPs in serum require as much as 10 mL in sample volume. Therefore, rigorous extractions and highly sensitive/specific mass spectrometry methods are crucial to ensure the analytes can be detected from sample volumes of 200 µL. Efforts are being made to reduce the sample volume to 100 µL or less.

Methods

Forty-nine human plasma samples and three pooled reference samples (200 µL each) were extracted. The procedure used is as follows:

1. Aliquot 200 µL of plasma.
2. Add 1 mL of 10 M urea.
3. Add 1 mL of 10% propanol/water, 1 mL MeOH, and 6 mL of petroleum ether.
4. Centrifuge and transfer the organic layer.
5. Filter through ~1 g of Florisil.
6. Elute with MTBE/pet ether.
7. Evaporate to dryness.
8. Reconstitute prior to injection.

An Agilent 7890B GC and an Agilent 7010A GC Triple Quadrupole Mass Spectrometer operated in EI MRM Mode was used. The column was an Agilent J&W HP-5ms Ultra Inert (30 m × 250 µm, 0.25 µm). The transfer line was set at 300 °C. The source temperature was 350 °C. The collision gas was nitrogen at 1.5 mL/min. Targeted MRM was performed for 67 POPs representing six POPs chemical classes. For each compound, one quantifying MRM and one qualifying MRM was defined. System performance and precision was monitored at three concentration levels. Post-extraction, $^{13}C_{12}$-DDT was added to the unknown samples and used to monitor parameters such as retention time precision and area counts of the technical replicates. Each sample was injected at 2 µL in triplicate.

Results and Discussion

The targeted POPs chosen for this study were based on those reported in the EPA water contaminant list. To that end, 16 polyaromatic hydrocarbons (PAHs), 12 dioxin-like polychlorinated biphenyls (DL-PCBs), 12 polybrominated diphenylethers (PBDEs), 17 organochlorine pesticides (OCPs), five dioxins, and five furans were measured in human plasma through targeted GC-MS/MS. Table 1 lists all the compounds in the analysis.

Table 1. Compound list.

Analyte	Analyte
4,4'-Dibromodiphenyl ether	1,2,3,7,8-Pentachlorodibenzofuran
2,4,4′-Tribromodiphenyl ether	1,2,3,4,7,8-Hexachlorodibenzofuran
2',3,4-Tribromodiphenyl ether	1,2,3,4,6,7,8-Heptachlorodibenzofuran
2,2′,4,4′-Tetrabromodiphenyl ether	Octachlorodibenzofuran
2,2′,4,5′-Tetrabromodiphenyl ether	3,3',4,4'-Tetrachlorobiphenyl
2,3′,4,4′-Tetrabromodiphenyl ether	3,4,4',5-Tetrachlorobiphenyl
2,4,4′,6-Tetrabromodiphenyl ether	2,3,3',4,4'-Pentachlorobiphenyl
2,2′,4,4′,5-Pentabromodiphenyl ether	2,3,4,4',5-Pentachlorobiphenyl
2,2′,4,4′,6-Pentabromodiphenyl ether	2,3',4,4',5-Pentachlorobiphenyl
2,2′,4,4′,5,5′-Hexabromodiphenyl ether	2',3,4,4',5-Pentachlorobiphenyl
2,2′,4,4′,5,6′-Hexabromodiphenyl ether	3,3',4,4',5-Pentachlorobiphenyl
2,2′,4,4′,6,6′-Hexabromodiphenyl ether	2,3,3',4,4',5-Hexachlorobiphenyl
Acenaphthene	2,3,3',4,4',5'-Hexachlorobiphenyl
Acenaphthylene	2,3',4,4',5,5'-Hexachlorobiphenyl
Anthracene	3,3',4,4',5,5'-Hexachlorobiphenyl
Benz(a)anthracene	2,3,3',4,4',5,5'-Heptachlorobiphenyl
Benzo(b)fluoranthene	a-BHC
Benzo(k)fluoranthene	b-BHC
Benzo(g,h,i)perylene	g-BHC
Benzo(a)pyrene	d-BHC
Chrysene	Heptachlor
Dibenz(a,h)anthracene	Aldrin
Fluoranthene	Heptachlor Epoxide (isomer B)
Fluorene	Endosulfane I
Indeno(1,2,3-cd)pyrene	DDE
Naphthalene	Dieldrin
Phenanthrene	Endrin
Pyrene	DDD (mitotane)
2,3,7,8-Tetrachlorodibenzo-p-dioxin	Endosulfane II
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	Endrin Aldehyde
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	DDT
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	Endosulfane sulphate
Octachlorodibenzo-p-dioxin	Methoxychlor
2,3,7,8-Tetrachlorodibenzofuran	

Technical replicate area precision for the $^{13}C_{12}$-DDT internal standard averaged 3% RSD (min = 0.38% RSD; max = 6.79% RSD; n = 153). Retention time precision was ±0.122 minutes (average retention time for ^{13}C-labeled DDT was 9.608 minutes). The analytical method was sensitive enough to detect analytes at concentrations as low as 5.0 pg/mL for some compounds using 200 µL extraction volumes. Post-acquisition data reduction revealed that 27 of the target compounds were detected in these population samples. Reported LODs were: 0.005–0.02 ng/mL for DL-PCBs, 0.05–0.15 ng/mL for OCPs, and 0.0075–0.075 ng/mL for PBDEs (Smith, *et al*. 2015). Recent work (unpublished) yielded minimum detection limits (MDL) for DL-PCBs ranging from 0.004–0.009 ng/mL; 0.005 ng/mL for α-HCH, β-HCH and γ-HCH; 0.001–0.006 ng/mL for *o,p′*-DDT and *p,p′*-DDT, and their transformation products excluding *p,p′*-DDD, for which an MDL of 0.028 ng/mL was determined.

Conclusions

The results demonstrated coverage of POP concentration ranges with detection limits as low as 4 pg/mL from the 200 µL plasma samples. The concentrations reported here were determined based on the wet sample volume without consideration of lipid profiles. The ability to measure POPs in human bio-fluids at very low concentration levels from small extraction volumes will vastly improve exposure assessment within the exposome paradigm. Targeted MS/MS analyses such as the one described herein can be used to determine qualitative and quantitative differences in exposure profiles in various populations.

References

1. J. Ivanisevic, *et al.* "Toward 'Omic Scale Metabolite Profiling: A Dual Separation–Mass Spectrometry Approach for Coverage of Lipid and Central Carbon Metabolism" *Anal. Chem*. **85(14)**, 6876–6884 (2013).
2. M. T. Smith, R. de la Rosa, S. I. Daniels. "Using exposomics to assess cumulative risks and promote health" *Environ. Mol. Mutagen*. **56(9)**, 715–723 (2015).
3. S. M. Rappaport, M. T. Smith. "Environment and disease risks" *Science* **330**, 460-461 (2010).
4. S. M. Rappaport, *et al*. "The Blood Exposome and Its Role in Discovering Causes of Disease" *Environ. Health Perspect.* **122(8)**, 769-774 (2014).

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