Suspect screening of environmental organic acids in human serum using high-resolution mass spectrometry (HRMS)

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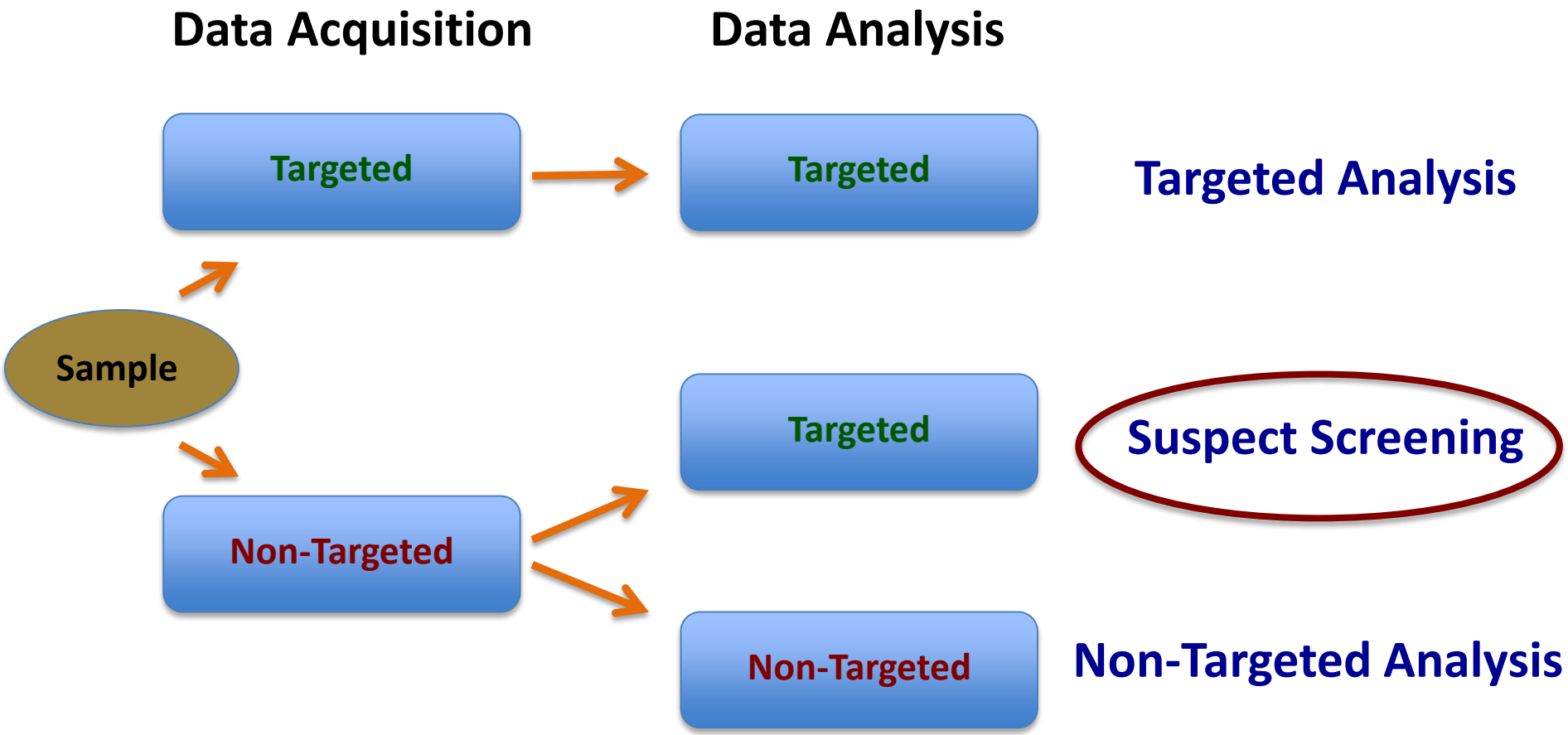
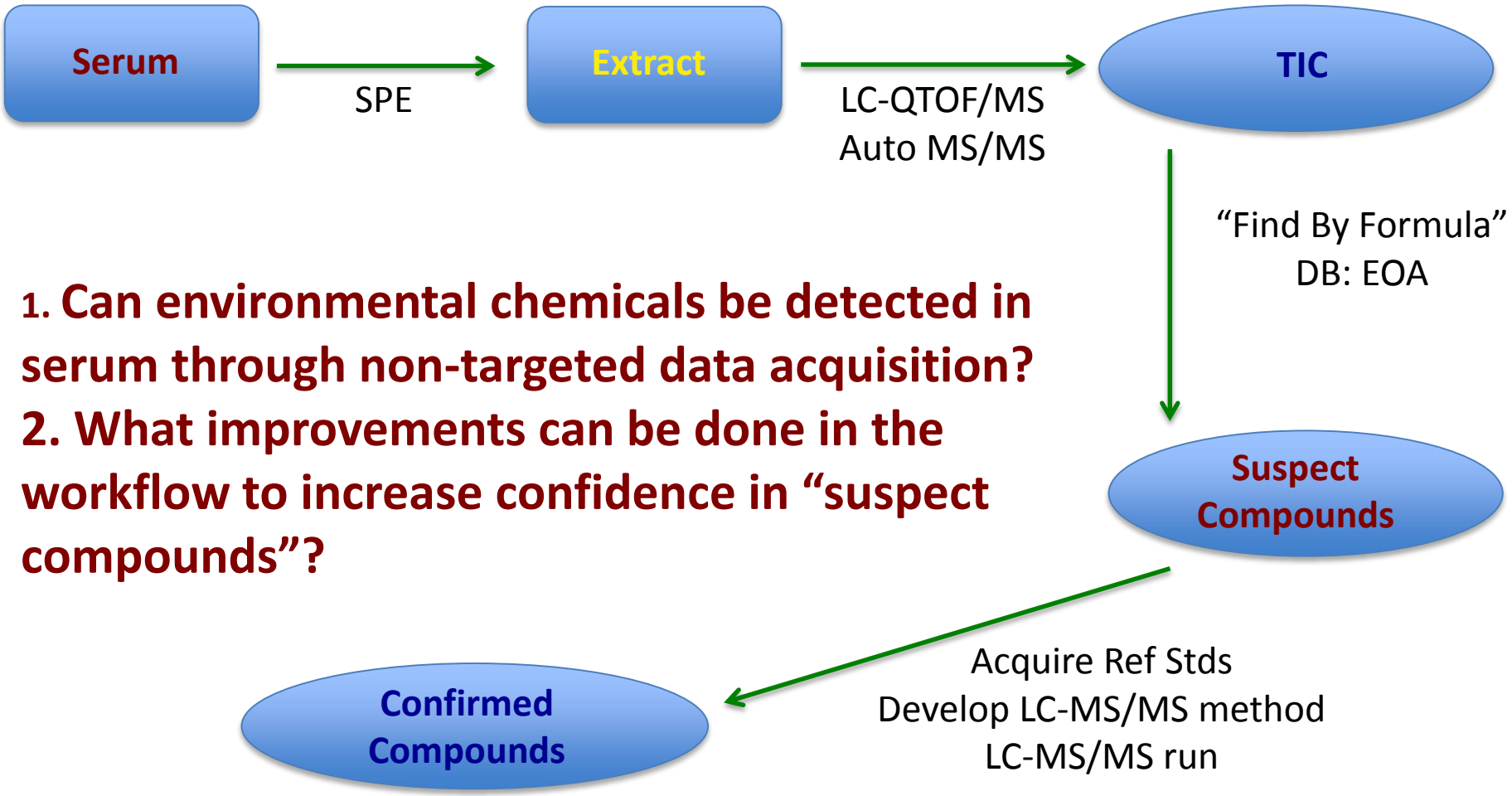
Types of Analyses Available Through HRMS



Types of Analyses Available Through HRMS

- Targeted Analysis
  - Reference standard available (RT, HRMS, MS/MS)
  - Acquisition: Targeted; Analysis: Targeted
- General Suspect Screening
  - Prior information available BUT no reference standard available
  - Acquisition: Non-Targeted; Analysis: Targeted
- Non-Targeted Analysis
  - NO prior information available
  - Acquisition: Non-Targeted; Analysis: Non-Targeted

Workflow



1. Can environmental chemicals be detected in serum through non-targeted data acquisition?

2. What improvements can be done in the workflow to increase confidence in "suspect compounds"?

Experimental Design

- Charcoal-stripped serum
  - Analyze for false positives using different criteria with varying degrees of stringency
- 70 Reference Standards in neat solvent
  - Acquire retention time and MS/MS spectra
- Charcoal-stripped serum spiked with reference standards (0.01- 500 ng/mL)
  - Establish LODs of EOAs in serum
- Pregnant women's serum
  - Run 10 samples to determine how doing GSS will compare with targeted screening

Analytes

- Environmental Phenols (27)
  - Bisphenol A, AF, AP, B, C, F, G, P, S, Z, Tetrabrombisphenol A, Tetrachlorobisphenol A; Benzophenone- 1, -3; 2-sec-butylphenol, 4-methylphenol, 4-tert-Butylphenol 4-tert-Octylphenol, 4-nonylphenol; Methyl, Ethyl, Propyl, Butyl Paraben; Triclosan; Eugenol, Methyl eugenol
- Phthalate metabolites (15)
  - MMP, MEP, MBP, MPP, MHxP, MOP, MBzP, MCHP, MEHP, MEHHP, MEOHP, MECPP, MiNP, MCOP, MCPP
- Perfluoroalkyl compounds (11)
  - PFHxA, PFHpA, PFOA, PFNA, PFDeA, PFUA, PFDoA, PFBuS, PFHxS, PFOS, PFOSA
- Pesticides and metabolites (12)
  - O-Phenylphenol, Pentachlorophenol, 2,4-Dichlorophenoxyacetic, 2,4,5-Trichlorophenoxyacetic acid, DMP, DEP, DMTP, DETP, DMDTP, DEDTP, Glyphosate, AMPA
- Phytoestrogen metabolites (5)
  - Enterodiol, Equol, Daidzein, Genistein, O-Desmethylangolensin

Sample Preparation and LC-QTOF/MS Run

Sample Preparation: Solid Phase Extraction (Waters Oasis HLB); 500 uL serum

Instrument: Agilent LC 1260- QTOF/MS 6550

Column: Agilent Poroshell 120 C-18 column (2.1X 100mm, 2.7um) at 50° C

Injection Volume: 50 uL **Flow Rate:** 0.5mL/min

HPLC: Gradient elution (A= 5mM NH_4CH_3COO, pH 7.80; B= 0.05% NH_4CH_3COO in Acetonitrile)

0-0.5 min:	5% B	3.5-7.5 min:	100% B
0.5-1.5 min:	30% B	7.5-10 min:	100% B
1.5- 3.5 min:	70% B	10-14 min:	5% B

Ion Source: ESI negative (Dual Agilent Jet Stream)

Scan Mode: Auto MS/MS (Collision Energy: 3 (m/z)/ 100 +8)

Mass Range: 75-1000 amu (TOF); 75-1000 amu (MS/MS)

Scan Rate: MS- 10 scans/s; MS/MS- 2 scans/s (2 GHz, Extended Dynamic Range)

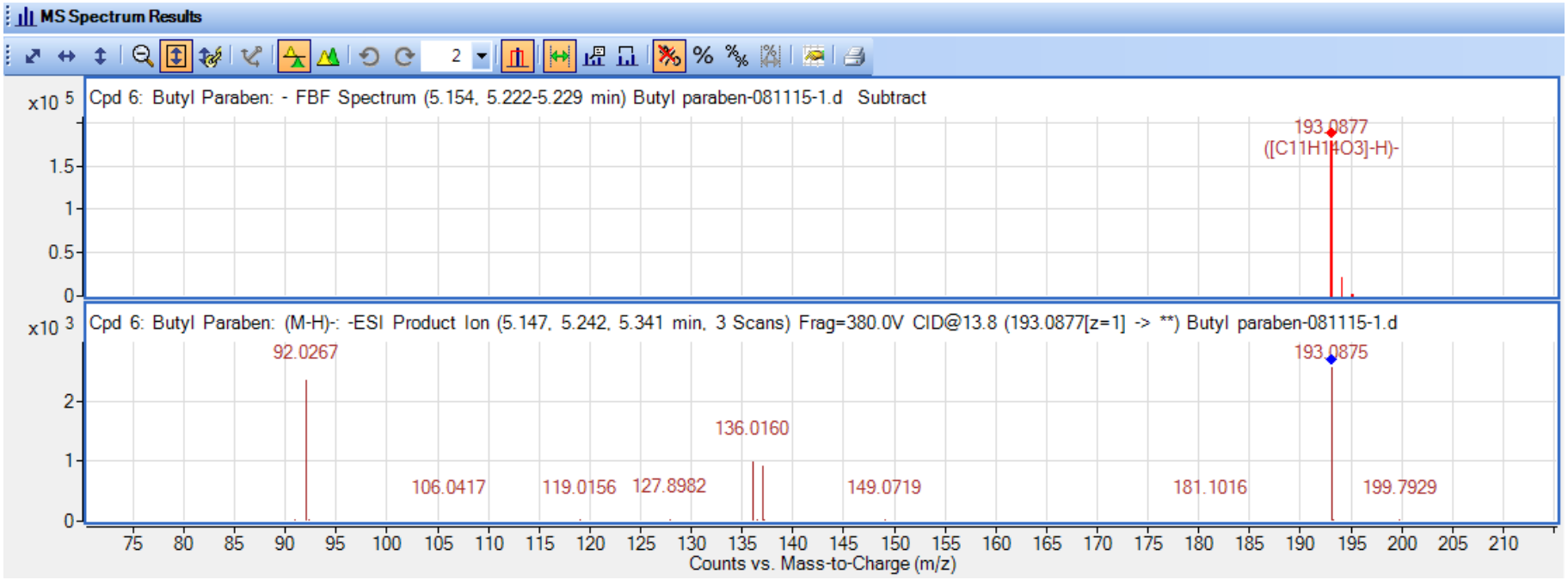
Data Analysis

- "Find by Formula"
  - Accurate mass
  - Isotope pattern
  - Retention time
  - Peak Shape
- "Identify Compound"
  - Accurate mass database library (EOA)
  - Peak Filters (Absolute Height > 100 counts; Relative Height > 0.5% largest peak
  - Minimum reverse score: 40

	Values to match	Mass
Formula Matching	Mass tolerance	+/- 10ppm
Negative Ions	Charge carrier	-H
Retention Time Matching	RT Tolerance	+/- 0.1 min
Scoring	Mass score contribution	100
	Isotope abundance score contribution	60
	Isotope spacing score contribution	50
	Retention time score contribution	100
	Expected MS mass variation	2.0mDa + 5.6ppm
	Expected MS isotope abundance variation	7.5%
Result Filters	Only generate compounds for matched formula	Yes
	Warn if the unobserved 2nd ion's abundance is expected to be	>50
	Do not match if the unobserved 2nd ion's abundance is expected to be	>200

Reference Standards in Neat Solvent

- Unable to establish RT for 5 of 70 Ref Stds (Glyphosate, AMPA, DMP, DMTP, DMDTP)
- Collected RT and MS/MS spectra for 65 Ref Stds



Reference Standards in Neat Solvent

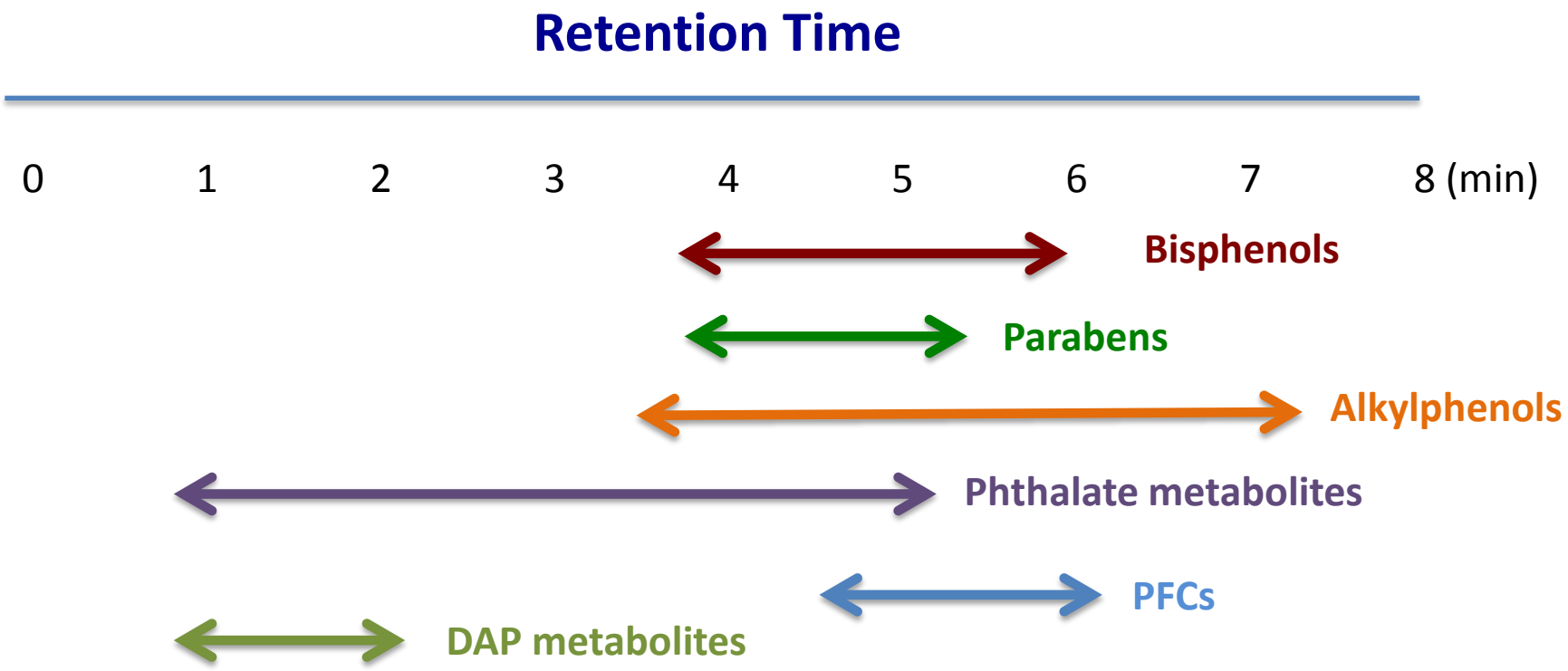
- Created Compound Database Library for 65 Reference Standards

Compound	Formula	Mass	RT	Num Spectra
2,4-Dichlorophenoxyacetic acid	C8H6Cl2O3	219.9694	3.70	1
4-Nonylphenol	C15H24O	220.1827	7.48	1
Benzophenone-1	C13H10O3	214.0629	5.00	1
Bisphenol B	C16H18O2	242.1306	5.10	1
Bisphenol S	C12H10O4S	250.0299	3.85	1
Enterodiol	C18H22O4	302.1518	4.16	1
Ethylparaben	C9H10O3	166.0629	4.40	1
Mono (2-ethyl-5-hydroxyhexyl) phthalate	C16H22O5	294.1647	3.87	1
Monobenzyl phthalate	C15H12O4	256.0735	3.82	1
Monobutyl phthalate	C12H14O4	222.0892	3.58	1
Perfluorooctanesulfonic acid	C8HF17O3S	499.9374	5.45	1
Perfluorooctanoic acid	C8HF15O2	413.9737	4.94	1
Triclosan	C12H7Cl3O2	287.9511	6.23	1

Assessing False Positives in Charcoal-Stripped Serum

- Stringencies in Matching Criteria
  - Accurate mass, Isotope pattern, Target Score, Peak shape
  - + Retention time plausibility
  - + Retention time
  - + Mass spectral Library

Expected Retention Times of Analytes



- Expected RTs of analyte class can be used as criteria in RT plausibility

False Positives in Charcoal-Stripped Serum

- About 1/3 of EOAs in the library come up as false positive in GSS

Compound Class	Criteria		
	Accurate Mass, Isotope Pattern, Target Score, Peak Shape	AM,IP,TS,PS, RT Plausibility	AM,IP,TS,PS, RT Match
Environmental Phenol (27)	15 (56%)	9 (33%)	1 (4%)
Pesticdes and metabolites (7)	0	0	0
PFC (11)	6 (55%)	2 (18%)	1 (9%)
Phthalate metabolites (15)	12 (80%)	7 (47%)	3 (20%)
Phytoestrogen metabolites (5)	2 (40%)	2 (40%)	0
Total (65)	35 (54%)	24 (31%)	5 (8%)

False Positives- Bzp-1, PFOSA, MBP, MHxP, MiNP

False Positives in Charcoal-Stripped Serum

- Specific examples of results obtained from GSS

	Compound	Criteria		
		Accurate Mass, Isotope Pattern, Target Score, Peak Shape	AM,IP,TS,PS, RT Plausibility	AM,IP,TS,PS, RT Match
	2,4-Dichlorophenoxyacetic acid	x	x	x
	4-Nonylphenol	+	x	x
*	Benzophenone-1	+	+	+
	Bisphenol B	+	x	x
*	Bisphenol S	x	x	x
	Enterodiol	+	+	x
*	Ethylparaben	+	+	x
	Mono (2-ethyl-5-hydroxyhexyl) phthalate	+	x	x
	Monobenzyl phthalate	+	+	x
	Monobutyl phthalate	+	+	+
	Perfluorooctanesulfonic acid	+	x	x
	Perfluorooctanoic acid	x	x	x
	Triclosan	x	x	x

Spectral Library Matching

- Spectral library matching significantly eliminates false positives in GSS

	Compound	Criteria			Spectral Library
		Accurate Mass, Isotope Pattern, Target Score, Peak Shape	AM,IP,TS,PS, RT Plausibility	AM,IP,TS,PS, RT Match	
	2,4-Dichlorophenoxyacetic acid	x	x	x	x
	4-Nonylphenol	+	x	x	x
*	Benzophenone-1	+	+	+	x
	Bisphenol B	+	x	x	x
	Bisphenol S	x	x	x	x
	Enterodiol	+	+	x	x
	Ethylparaben	+	+	x	x
	Mono (2-ethyl-5-hydroxyhexyl) phthalate	+	x	x	x
	Monobenzyl phthalate	+	+	x	x
*	Monobutyl phthalate	+	+	+	+
	Perfluorooctanesulfonic acid	+	x	x	x
	Perfluorooctanoic acid	x	x	x	x
	Triclosan	x	x	x	x

Limits of Detection

- LODs for QTOF/MS (Agilent 6550) are 10-100x higher than established LODs in LC-MS/MS (AB- Sciex 5500)
- LODs for 50% of EOAs are similar to the reported GM by NHANES

	Compound	LOD		
		QTOF/MS	Gerona Lab LC-MS/MS	NHANES 2011-12
*	Benzophenone-1	0.1	0.01	
	Benzphenone-3	5.0		0.4 U
*	Bisphenol B	5.0	0.05	
*	Bisphenol S	0.5	0.01	
	Ethylparaben	0.2		1.0 U
	Methylparaben	0.1		1.0 U
*	Monobutyl phthalate	1.0	0.05	0.4 U
*	Mono (2-ethyl-5-hydroxyhexyl) phthalate	2.5	0.05	0.2 U
*	Perfluorooctanoic acid	1.0	0.10	0.1
	Perfluorooctanesulfonic acid	0.5	0.10	0.2
	Perfluorooctanesulfonamide	0.1	0.02	0.1
	Triclosan	5.0	0.05	2.3 U

Pregnant Women's Serum Suspect Compounds

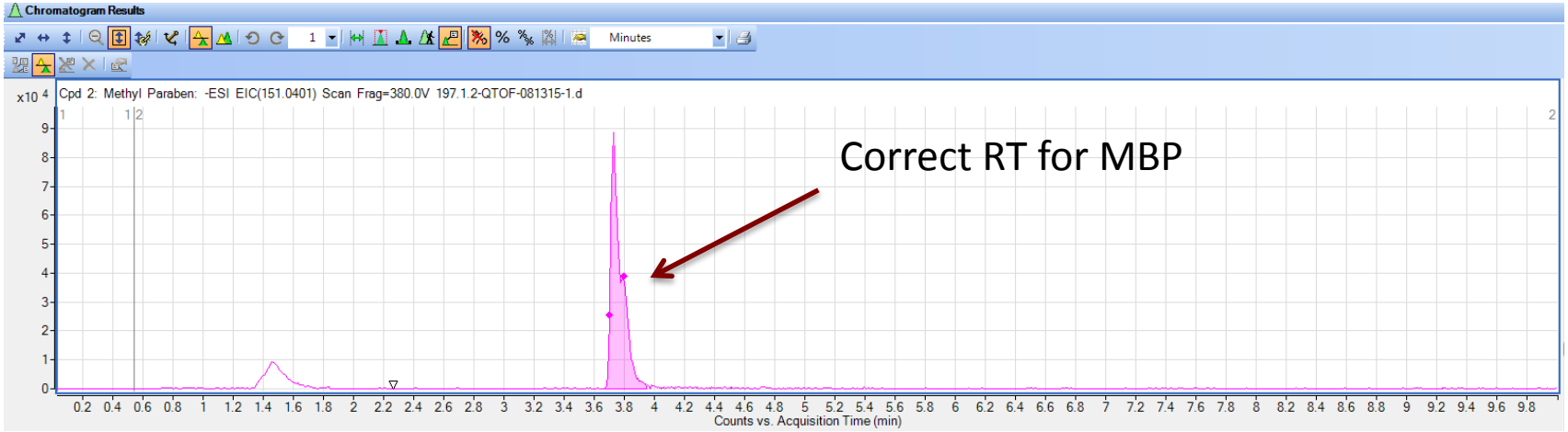
- Results of General Suspect Screening compared to Targeted Screening

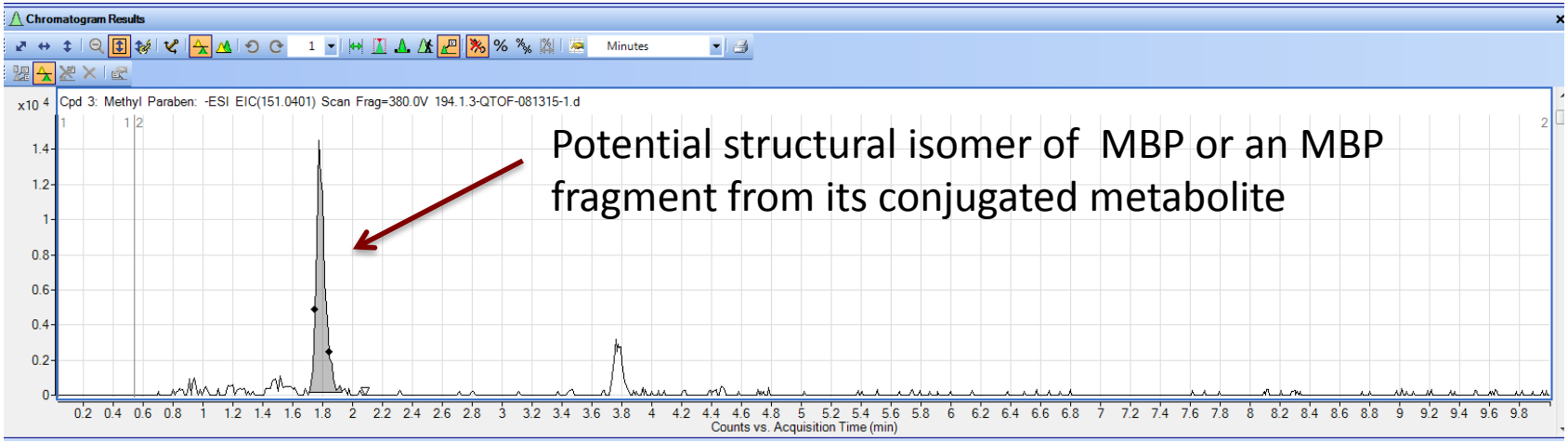
Compound	LOD QTOF/MS	NHANES GM 2011-2012	LC-MS/MS Confirmed Compounds*	QTOF/MS Suspect Compounds	
Benzophenone-1	0.1		1	5	*
Benzphenone-3	5.0	23.20	10	10	*
Bisphenol B	5.0		ND	ND	*
Bisphenol S	0.5		1	1	*
Ethylparaben	0.2	<LOD	1	2	
Methylparaben	0.1	40.30	2	7	*
Monobutyl phthalate	1.0	7.61	7	9	*
Mono (2-ethyl-5-hydroxyhexyl) phthalate	2.5	7.91	2	8	*
Perfluorooctanoic acid	1.0	2.08	3	3	*
Perfluorooctanesulfonic acid	0.5	6.31	9	9	*
Perfluorooctanesulfonamide	0.1	<LOD	ND	ND	*
Triclosan	5.0	11.80	ND	ND	*

* This number only represents **those that are confirmed from the suspect compounds**; in most cases the detection frequency in LC-MS/MS is higher than LC-QTOF/MS

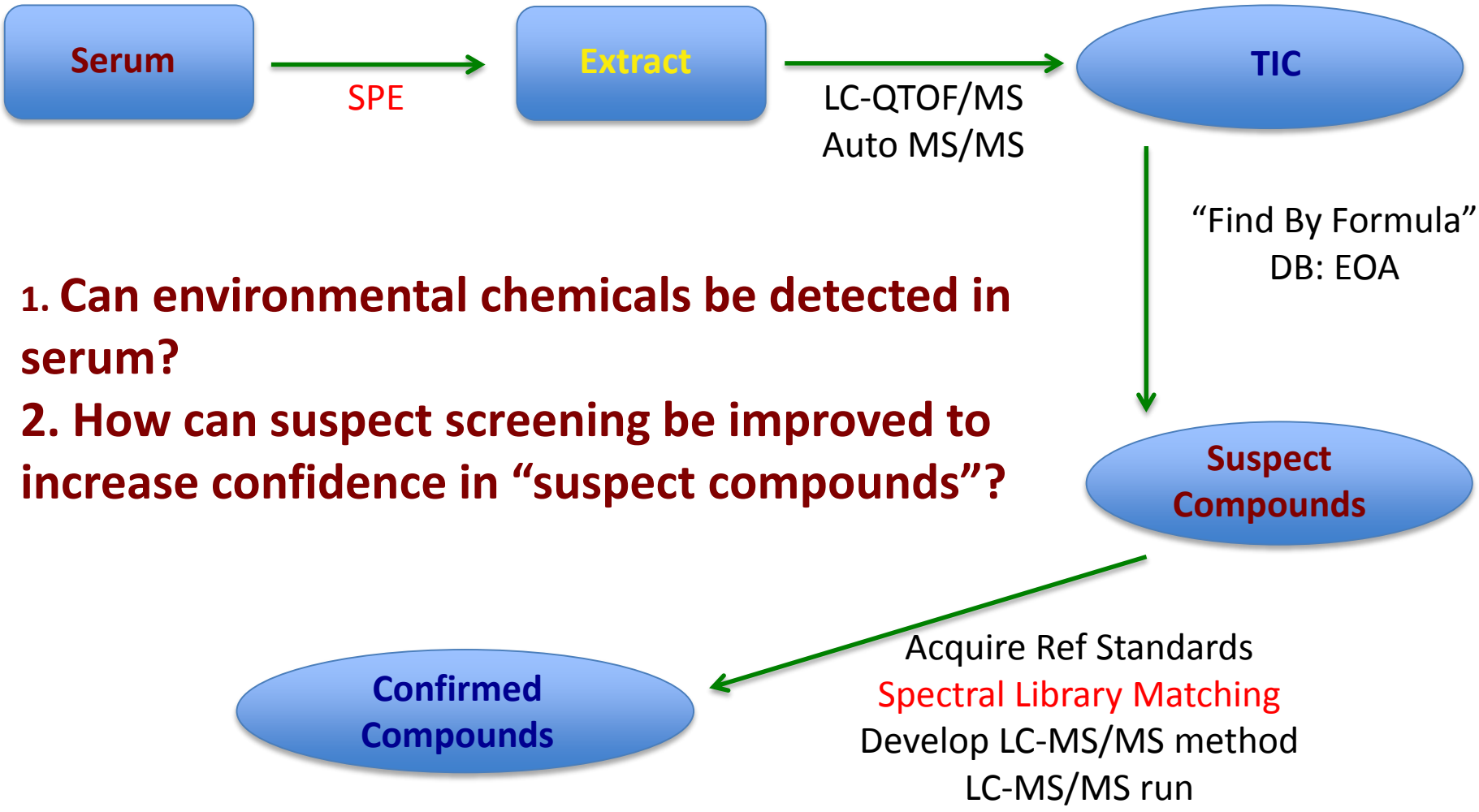
Sample Result from GSS

- Structural isomers pose a big challenge in GSS





Workflow



1. Can environmental chemicals be detected in serum?

2. How can suspect screening be improved to increase confidence in "suspect compounds"?

Conclusions

- EOAs can be detected in serum through non-targeted data acquisition
  - 50% of the 65 reference standards can be detected in serum matrix with LODs similar to the GMs reported by NHANES
  - False positives may account for about 1/3 of GSS results
- Suspect screening can provide correct information on more abundant xenobiotics in serum
- Prior to compound confirmation suspect screening will benefit a lot from spectral library matching

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