

Supplementary Information

Chemicals. Acetanilide (99%, CAS no. 103-84-4), 5-methyl-1H-benzotriazole (CAS no. 136-85-6, 98%), prometon (CAS no. 1610-18-0; 99.3%), DEET (CAS no. 134-62-3; $\geq 99\%$), ethoprophos (CAS no. 13194-48-4; $\geq 99\%$), and cotinine (CAS no. 486-56-6; $\geq 98\%$) were purchased from Sigma Aldrich. Caffeine (CAS no. 58-08-2; $\geq 99\%$) was purchased from Fluka® Analytical. Florisil (60-100 mesh), silica (0.03-0.2 mm, 60 Å), diatomaceous earth (Celite® 545), basic alumina (Brockmann I, 50-200 μm , 60 Å), acidic alumina (Brockmann I, 58 Å), neutral alumina (Brockmann I, 58 Å), and Ottawa sand (20-30 mesh) were purchased from Acros Organics (Fisher Scientific).

Table S1. Adsorbent experiments to optimize tissue extraction.

Exp. #	Adsorbent experiments ^a	Adsorbent mass (g)	Adsorbent to sample ratio	Non-target Features	Target Compounds	Rate of false negatives (%)
1	Neutral Alumina (NA) ^b	10	10:1	3215	96	7
2	Basic Alumina (BA) ^b	10	10:1	3188	93	10
3	Acidic Alumina (AA) ^b	10	10:1	2096	98	5
4	Silica Gel ^b	10	10:1	1020	94	9
5	Florisil® ^b	10	10:1	1041	81	21
6	Diatomaceous Earth ^b	5	5:1	2761	98	5
7	Florisil®, Silica Gel, BA ^c	10,10,10	30:1	800	82	20
8	Florisil®, Silica Gel, AA ^c	10,10,10	30:1	1072	88	15
9	Florisil®, Silica Gel, BA ^c	10,15,5	30:1	872	77	25
10	Florisil®, Silica Gel, AA ^c	10,15,5	30:1	1007	85	17
11	Florisil®, Silica Gel, BA ^c	5,15,10	30:1	1611	84	18
12	Florisil®, Silica Gel, BA ^c	1,15,10	26:1	2155	89	14
13	Florisil®, Silica Gel, BA ^{c,d}	1,15,10	26:1	2312	87	16
14	Silica Gel, BA ^c	15,10	25:1	2819	95	8
15	Florisil®, Silica Gel, BA ^c	1,10,15	26:1	3188	93	10
16	Florisil®, Silica Gel, BA ^{c,d}	1,5,7.5	13.5:1	3635	89	14
17	Florisil®, Silica Gel, BA ^{b,d}	1,5,7.5	13.5:1	3606	92	11
18	Florisil®, Silica Gel, BA ^{b,d,e}	1,5,7.5	13.5:1	2251	92	11

^a Adsorbent order in ASE cell (bottom to top); Samples extracted with methanol and ran in Positive MS

^b Cell size: 33 mL

^c Cell size: 66 mL

^d Sample homogenized with 1 g DE

^e Extracted with acetonitrile

Table S2. Instrumental settings of LC-QTOF-MS.

LC conditions	
Column	Agilent ZORBAX Eclipse Plus C18 2.1×100mm, 1.8µm (p/n 959758-902)
Column temperature	45 °C
Injection volume	5 µL
Mobile phase	A) Water, 5mM NH ₄ acetate+0.1% acetic acid B) MeOH, 5mM NH ₄ acetate+0.1% acetic acid
Linear gradient	Time (min) %B 0 5 1 5 4 50 17 100 20 100 20.1 5 Stop time 22.5 min Post time 2 min
Flow rate	0.4 mL/min
Q-TOF conditions	
Ionization Mode	ESI positive (and negative), MS only and MS/MS
Drying gas temperature	175 °C
Drying gas flow	12 L/min
Sheath gas temperature	375 °C
Sheath gas flow	11 L/min
Nebulizer gas	35 psi
Capillary voltage	3500 V
Nozzle voltage	200 V (2000 V for negative)
Fragmentor	125 V
Acquisition speed	MS only: 3 spectra/s; MS/MS: 1 spectra/s
Reference mass ions	121.0509, 922.0098

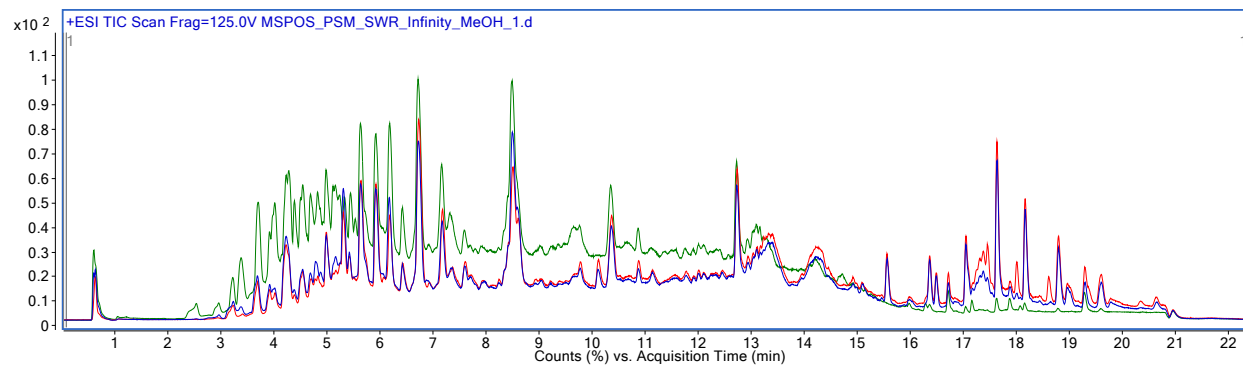


Figure S1. Total ion chromatography for different solid phase extraction methods. Blue – Infinity MeOH; red – Infinity DCM; green – HLB MeOH.

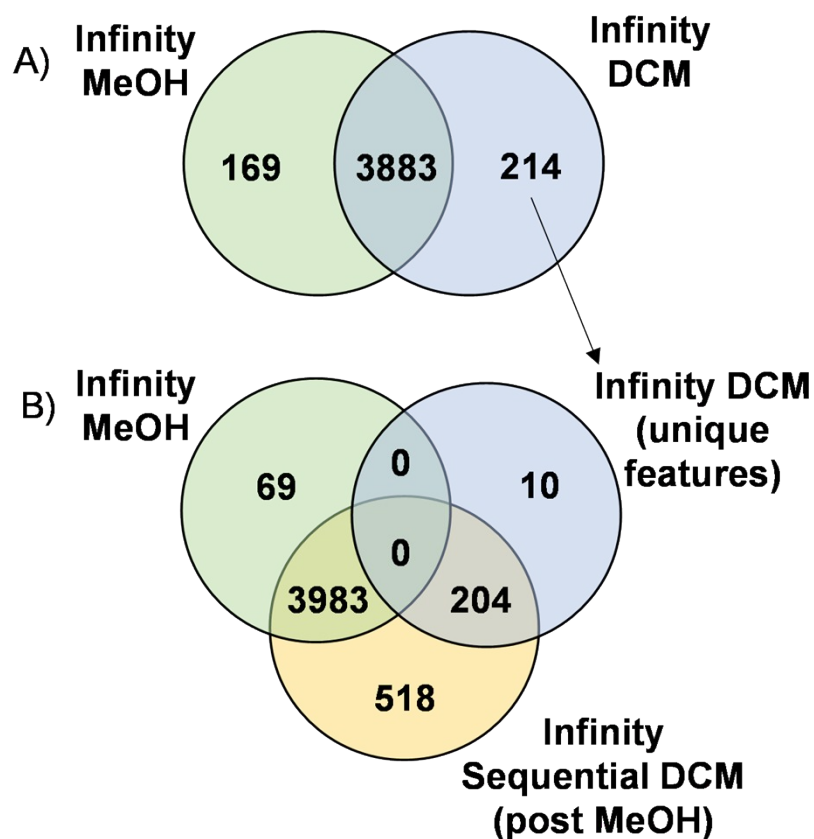


Figure S2. Comparison of solvent elution for optimizing non-target features in highway runoff Infinity SPE extracts, in positive ionization mode. A) Unique and concurrent features for two different solvents (MeOH and DCM); B) Comparison of unique and concurrent features across Infinity MeOH (all 4052 features from panel A), Infinity DCM (the 214 unique features from panel A), and Infinity Sequential DCM (post-MeOH) (features eluted with DCM after elution of the Infinity cartridge with MeOH).

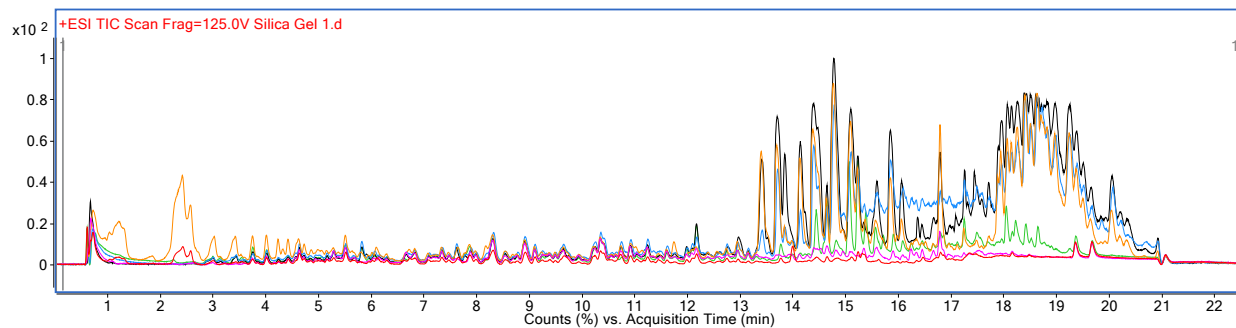


Figure S3. Total ion chromatography extracted with the individual adsorbent. Blue – neutral alumina; black – basic alumina; orange – diatomaceous earth; green – acidic alumina; pink – florisil®; red – silica gel.

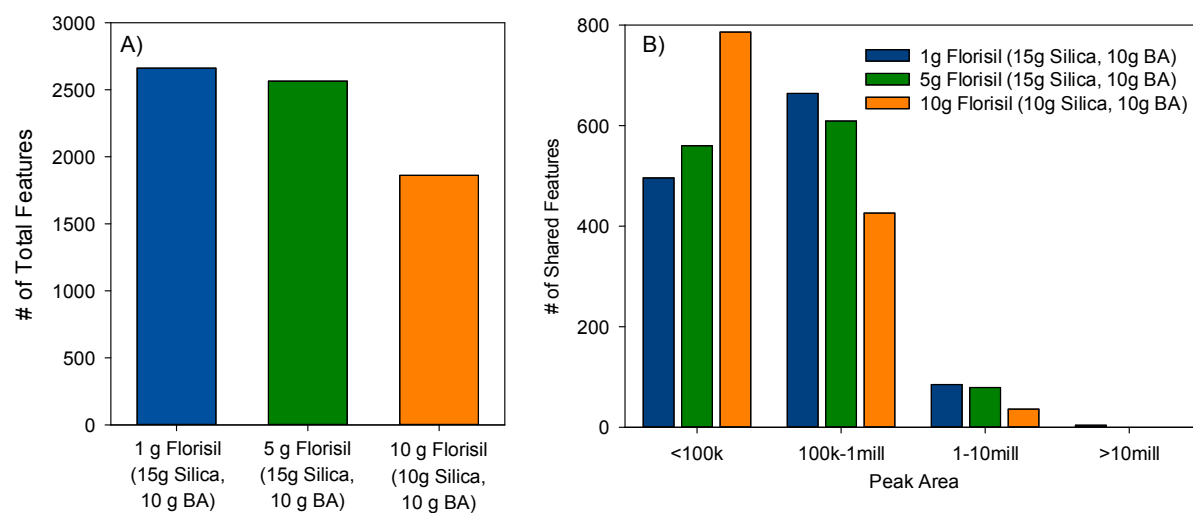


Figure S4. Comparison of accelerated solvent extraction (ASE) of coho salmon liver samples with differing amounts of Florisil® (1 g, 5 g, or 10 g; in combination with silica gel and basic alumina) for (A) total observed features and (B) peak area of features common to extracts of all Florisil® combinations (i.e., shared features).

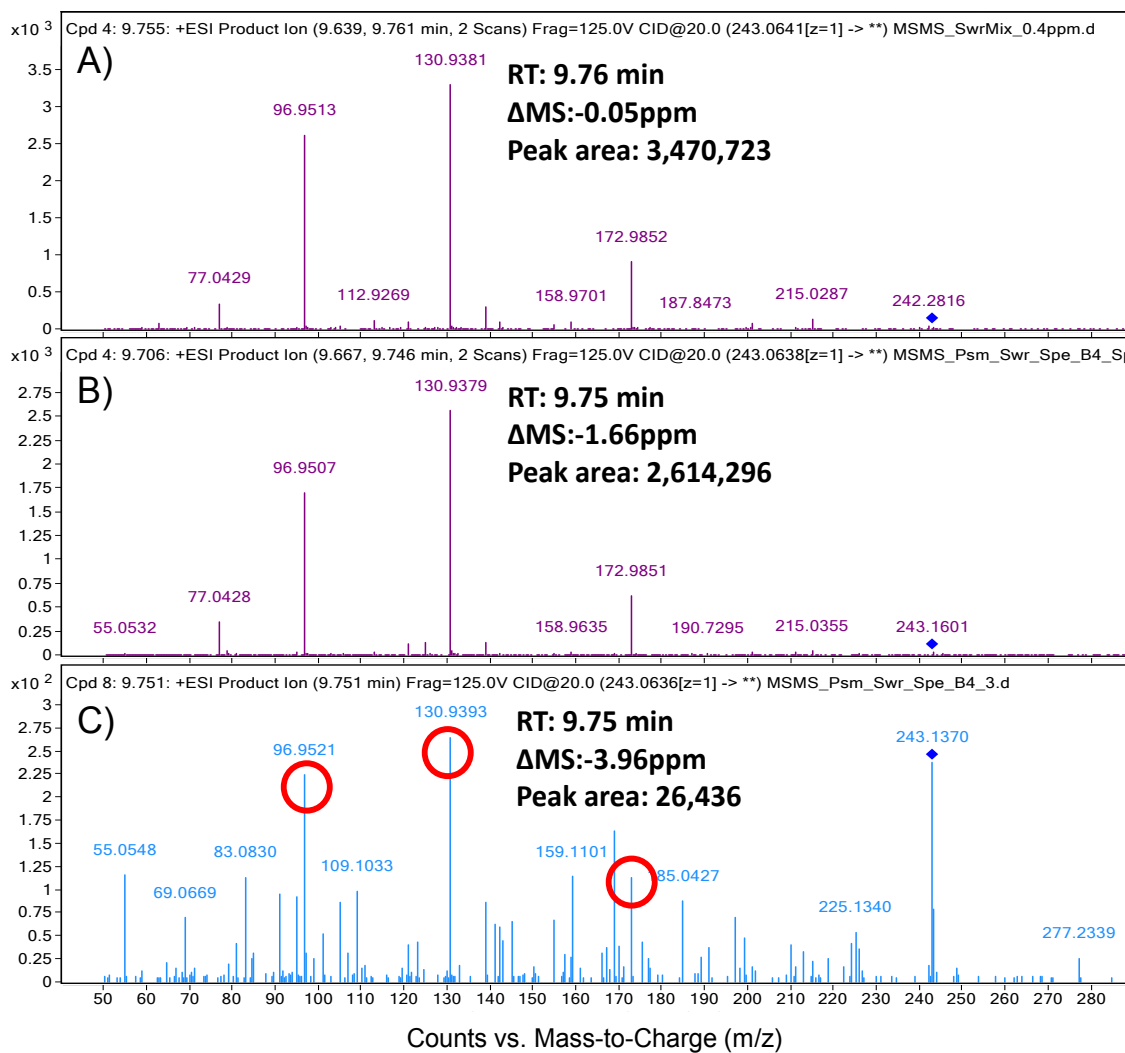


Figure S5. Comparison of fragmentation patterns (@20 eV) of ethoprophos among A) reference standard of 400 ng/mL, B) matrix spike, and C) highway runoff.

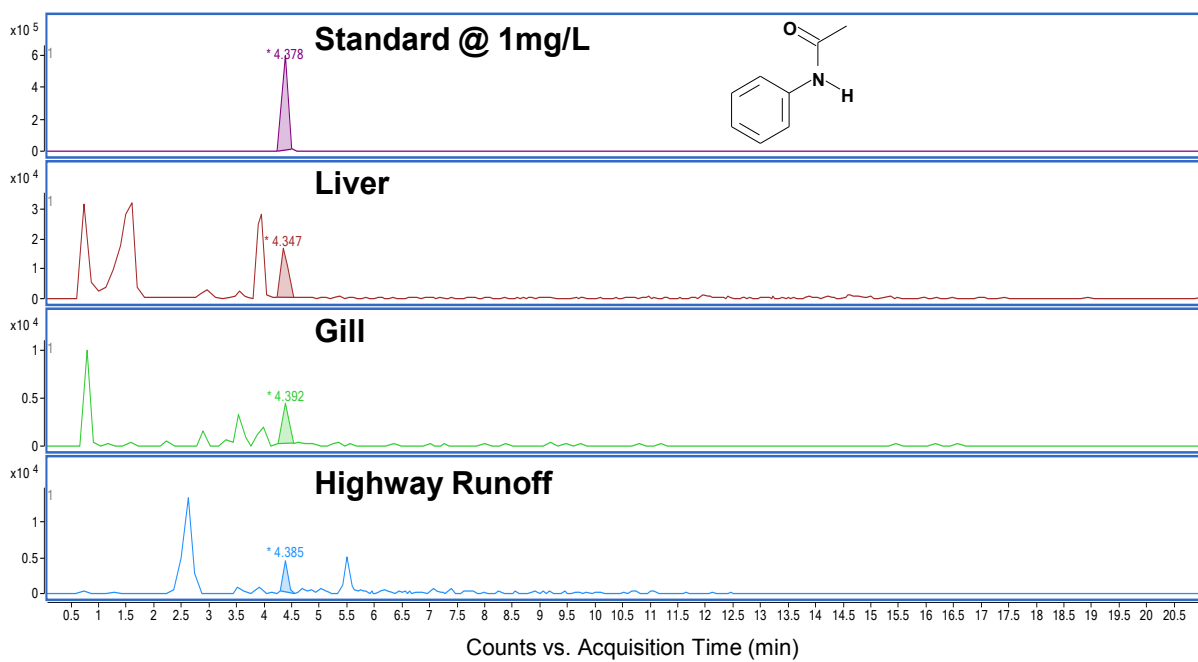


Figure S6. Comparison of extracted ion chromatograms of acetanilide in the reference standard of 1 mg/L, liver, gill, and highway runoff.