Proteins as Adsorbents for PFAS Removal from Water

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Supporting Information

Materials & Methods

Proteins and Instrumentation. Proteins, PFAS analytes: PFOA (cas no. 335-67-1, 99%); PFOS (cas no. 45298-90-6, 99%); HFPO-DA (13252-13-6, 97%); PFBA (cas no. 375-22-4, 97%); PFBS (cas no. 375-73-5, 97%), buffer salts, and LCMS-grade MeCN were purchased from Sigma and Matrix Scientific. An Agilent 6530 Q-TOF coupled to an LC 1260 Infinity II system was used for mass spectrometry analysis.

Protein stock solutions. Stock solutions (5 mM and 160 μM) of BSA, casein, egg white albumin, lysozyme, and RNase A were prepared in each water 18.2 MΩ•cm (25°C) Milli-Q, tap (UC Berkeley Latimer Hall), Strawberry Creek (surface Berkeley, CA), Bis-Tris pH 7.2, Bis-Tris pH 8.0, Bis-Tris pH 7.2 (60 mg/mL NaCl), Bis-Tris pH 8.0 (60 mg/mL NaCl), and ocean shore (Thornton State Beach, CA). All water sources were filtered prior to adding proteins (Thermo Scientific Nalgene Disposable Filter Unit, 0.2 μM pore, polyethersulfone).

PFAS stock solutions. Stock solutions (1 ppm) of perfluorooctanoic acid (PFOA), ammonium perfluoro(2-methyl-3-oxahexanoate) (GenX), heptafluorobutyric acid (HFBA), and perfluoro butane sulfonic acid (PFBS) were prepared in each water matrix: Milli-Q, tap, Strawberry Creek, Bis-Tris pH 7.2, Bis-Tris pH 8.0, Bis-Tris pH 7.2 (60 mg/mL NaCl), Bis-Tris pH 8.0 (60 mg/mL NaCl), and ocean.

Incubation of proteins with PFAS. For each matrix, a stock solution (25 µL, 1 ppm) of PFOA was introduced (72.8 µL), followed by addition of protein (2.2 µL, 160 µM). Stock solutions (25 µL, 1 ppm) of HFBA, PFBS, or GenX were introduced (68 µL), followed by addition of protein (7 µL, 5mM). Immediately after preparation, the PFAS/protein solution was incubated at RT for 14 h.

Removal of protein from solution. Microcon centrifugal filters (10 kDa MWCO) were used to remove protein from solution. Sample reservoirs were first wetted with 500 µL of Bis-Tris pH 7.2. After capping the filter device, it was shaken manually to wet the sample reservoir sides, followed by centrifugation (4 min, 14.5 x 1000 rpm, Eppendorf miniSpin plus). Remaining buffer inside the sample reservoir was poured out. The reservoir was inverted and tapped against a paper towel to remove residual buffer. Buffer collected in the receiving vial was removed with a pipette.

Incubated protein/PFAS depletion samples were centrifugated and transferred to the pre-wetted Microcon sample reservoirs. Protein was removed from solution by centrifugation (30 min, 14.5 x 1000 rpm, RT, Eppendorf miniSpin plus). The collected filtrate was transferred to a separate spin

filter (Costar, Spin-X, 0.22 μM, cellulose acetate), and centrifuged to prepare for LCMS analysis (4 min, 14.5 x 1000 rpm, RT, Eppendorf miniSpin plus).

LCMS method for PFOA and GenX. The binary mobile phase consisted of ammonium acetate buffer (10 mM, pH 7) and LCMS-grade MeCN. Samples (18 μL) were injected onto a Zorbax Extend-C18 column (50 mm x 2.1 mm, 1.8 μm bead size) and eluted according the elution gradient listed in SI-Table 1 (0.4 mL/min, 42 °C). Vcap and fragmentor potentials were 2500 V and 100 V, respectively.

Time (min)	MeCN (% vol.)
0.0	5
4.0	50
8.0	95
10	5

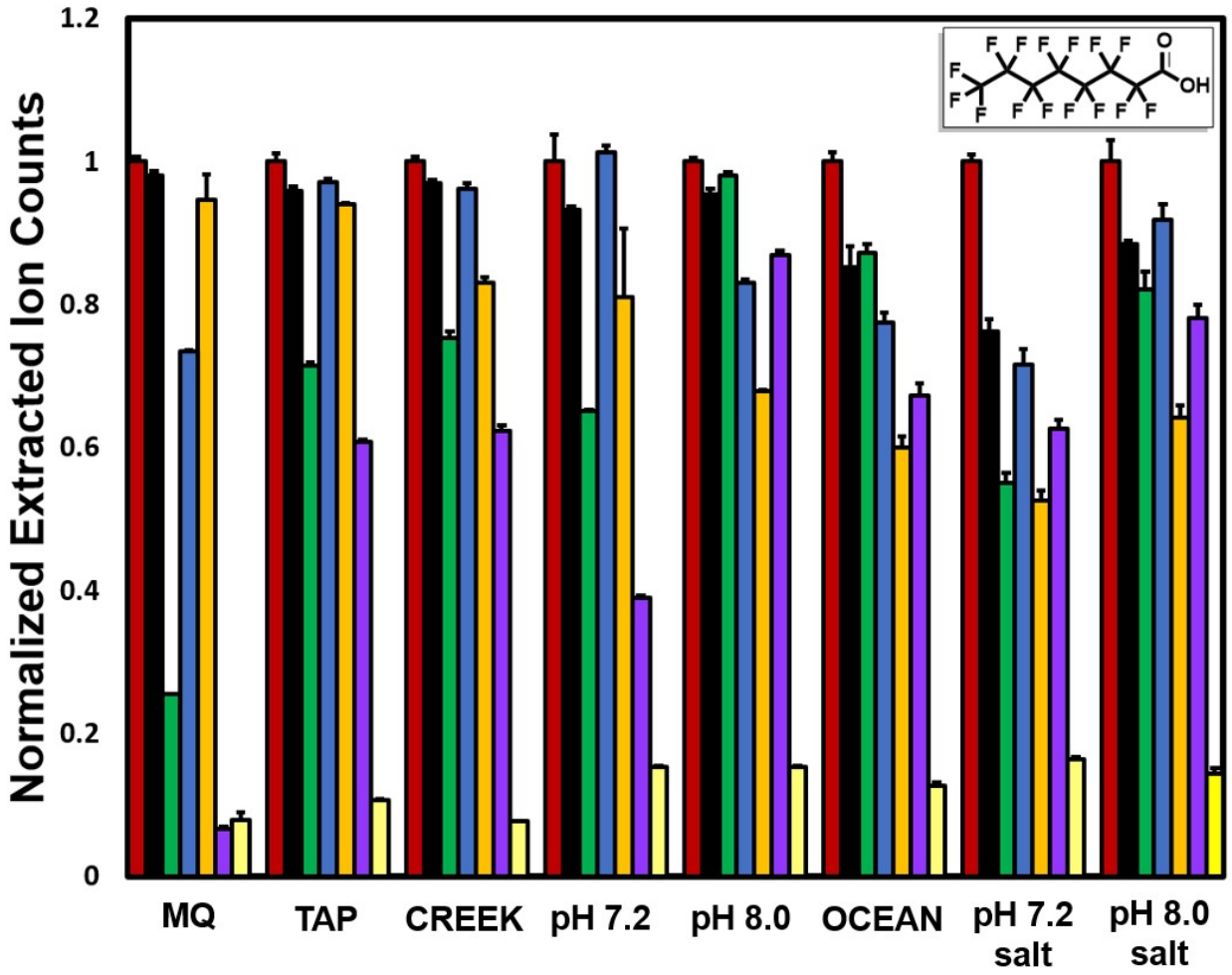
SI-Table 1. LC gradient for MS detection of PFOA and GenX.

LCMS method for C4 PFAS. The binary mobile phase consisted of ammonium citrate buffer (10 mM, pH 4) and LCMS-grade MeCN. Samples (18 μL) were injected onto a Thermo BioBasic AX column (50 mm x 2.1 mm, 5 μm bead size, 300 Å pore size) and eluted with an isocratic flow of 5% MeCN at 0.2 mL/min, 37 °C. Vcap and fragmentor potentials were 3500 V and 150 V, respectively.

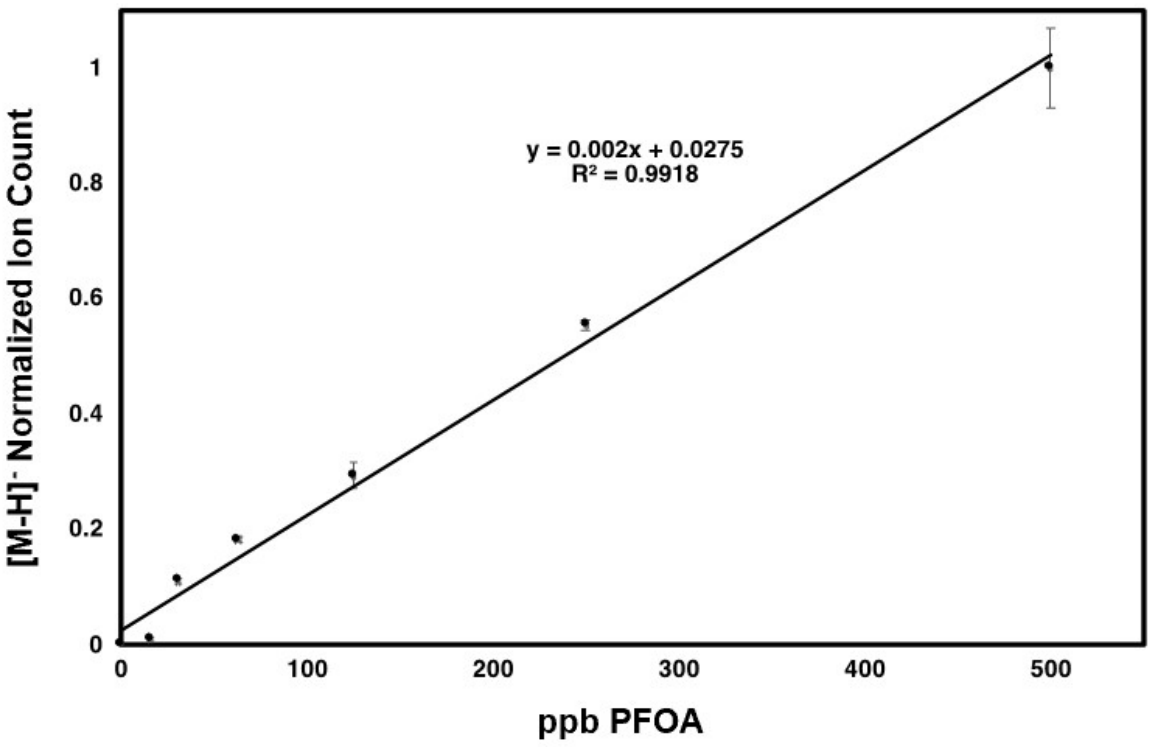
Data analysis of LCMS traces. The remaining analyte in solution was monitored via extracted ion count (EIC) of parent ion and fragment ions when available. **SI-Table 2** lists the analyte and m/z values monitored.

Ion	EIC Range (Da)	Expected Mass (Da)	Observed Mass (Da)	Elution Time (min)
PFOA $[M-H]^{-1}$	412-414	412.97	412.97	6.2
PFOA $[M-COOH]^{-1}$	368-370	368.98	368.98	6.2
GenX $[M-H]^{-1}$	328-330	328.97	328.97	5.3
GenX $[M-COOH]^{-1}$	284-286	284.98	284.98	5.3
GenX $[M-C_3F_4O_3H]^{-1}$	168-170	168.99	168.99	5.3
HFBA $[M-H]^{-1}$	212-214	212.98	212.98	1.5
HFBA $[M-COOH]^{-1}$	168-170	168.99	168.99	1.5
PFBS $[M-H]^{-1}$	298-300	298.99	298.95	1.8

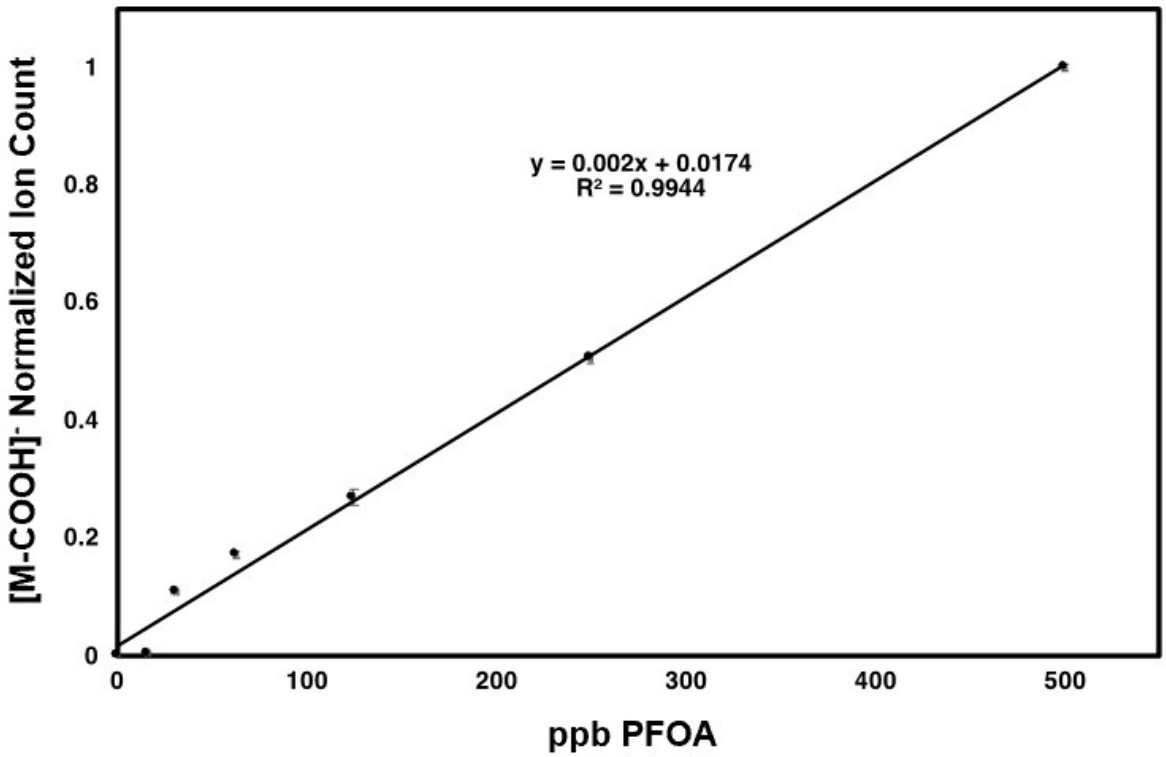
SI-Table 2. PFAS ions monitored during MS analysis.



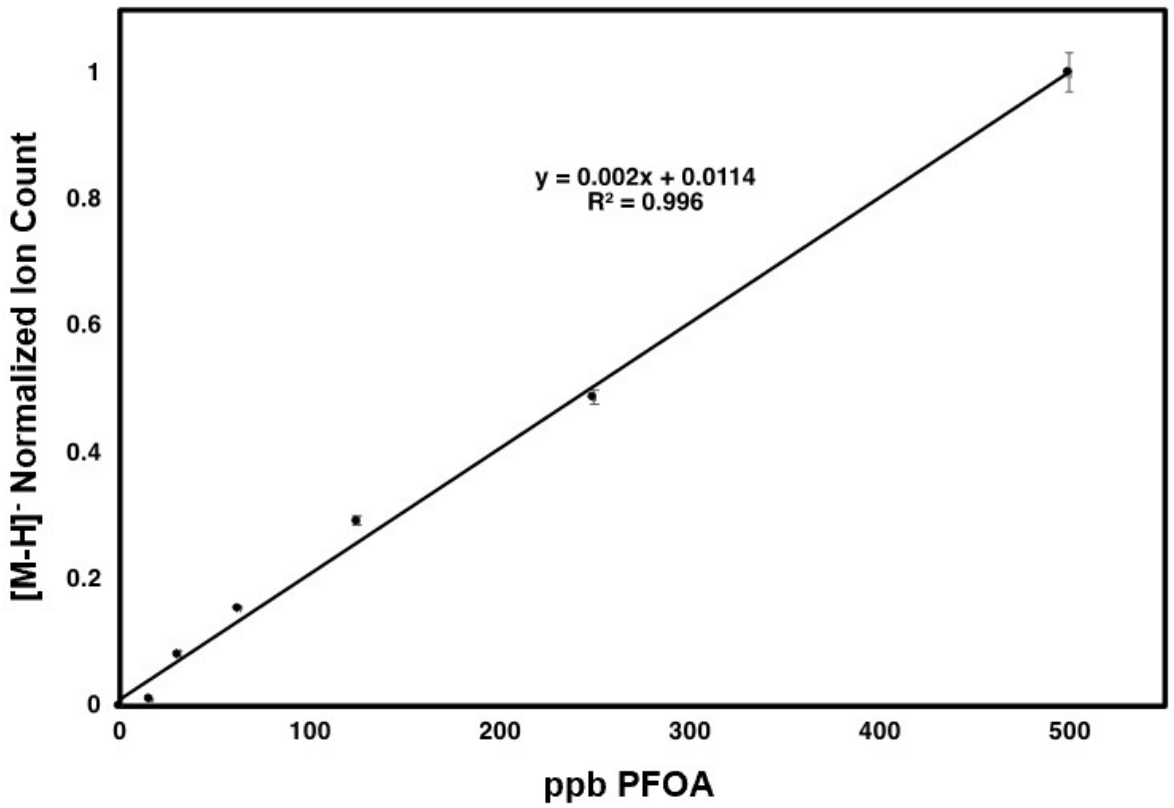
SI-Figure 1. Normalized extracted ion count (EIC) for PFOA $[M\text{-}COOH]^-$: 368-370 Da. Starting concentrations were 250 ppb. Red: starting sample, no protein (no filter); Black: MWCO filter only, no protein; Green: RNase A; Blue: casein; Orange: egg white albumin; Purple: lysozyme; Yellow: BSA. EICs reflected the amount of PFOA present in solution after incubating with a protein and passing solution through the MWCO filtration device. Normalized to mean ion counts of starting sample (no filter), the control where PFOA did not pass through the filtration device (std. error, n=3).



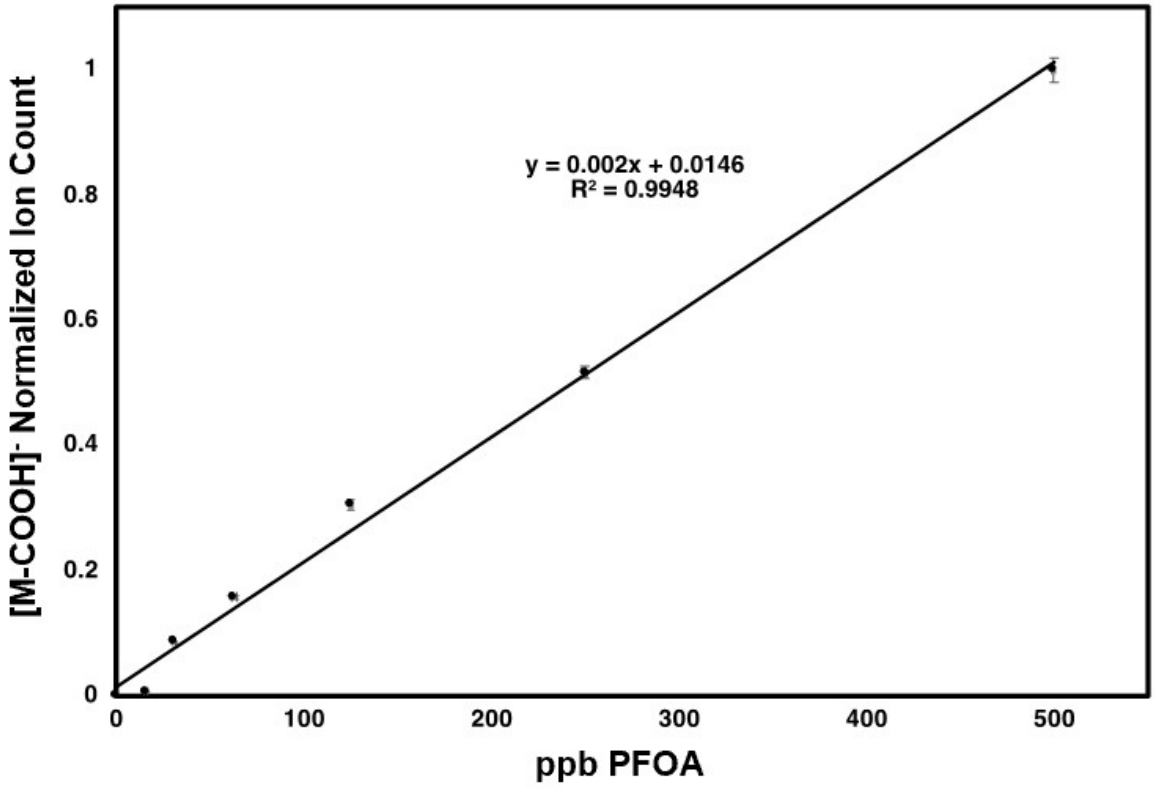
SI-Figure 2. Calibration curve for PFOA in Milli-Q water. Extracted ion count $[M-H]^-$: 412-414 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFOA.



SI-Figure 3. Calibration curve for PFOA in Milli-Q water. Extracted ion count $[M\text{-}COOH]^-$: 368-370 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFOA.



SI-Figure 4. Calibration curve for PFOA in tap water. Extracted ion count $[M-H]^-$: 412-414 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFOA.



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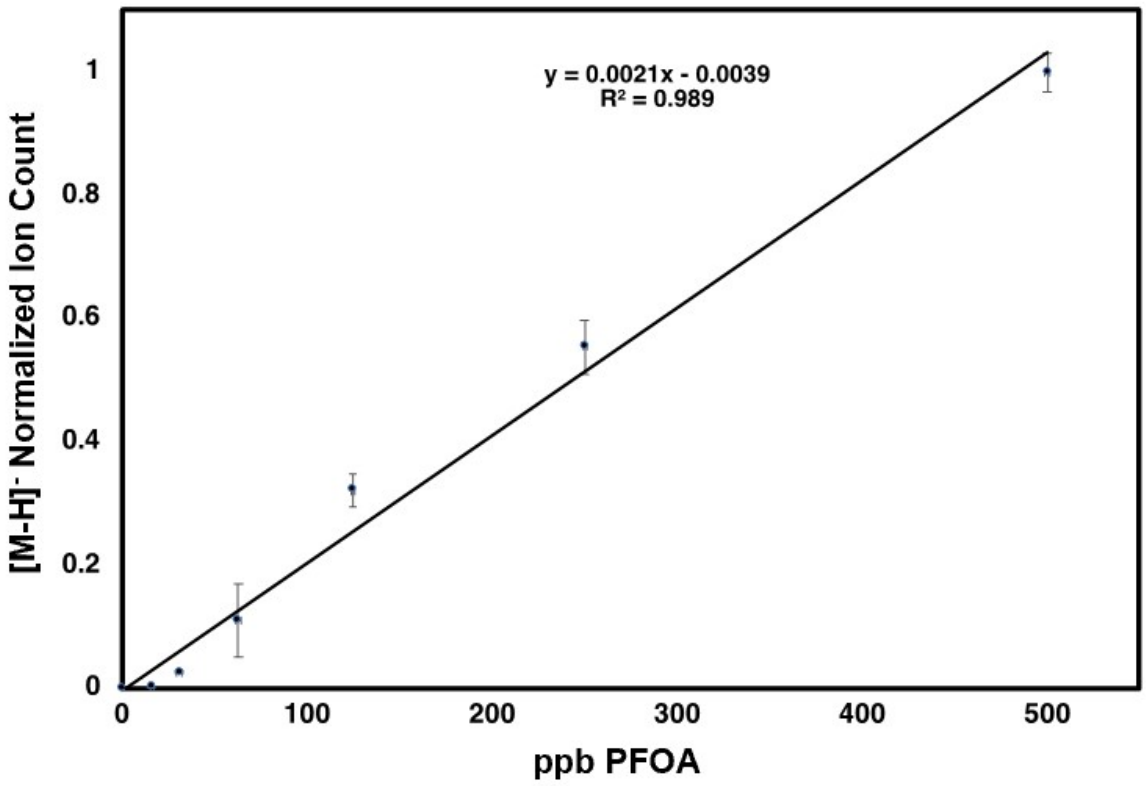
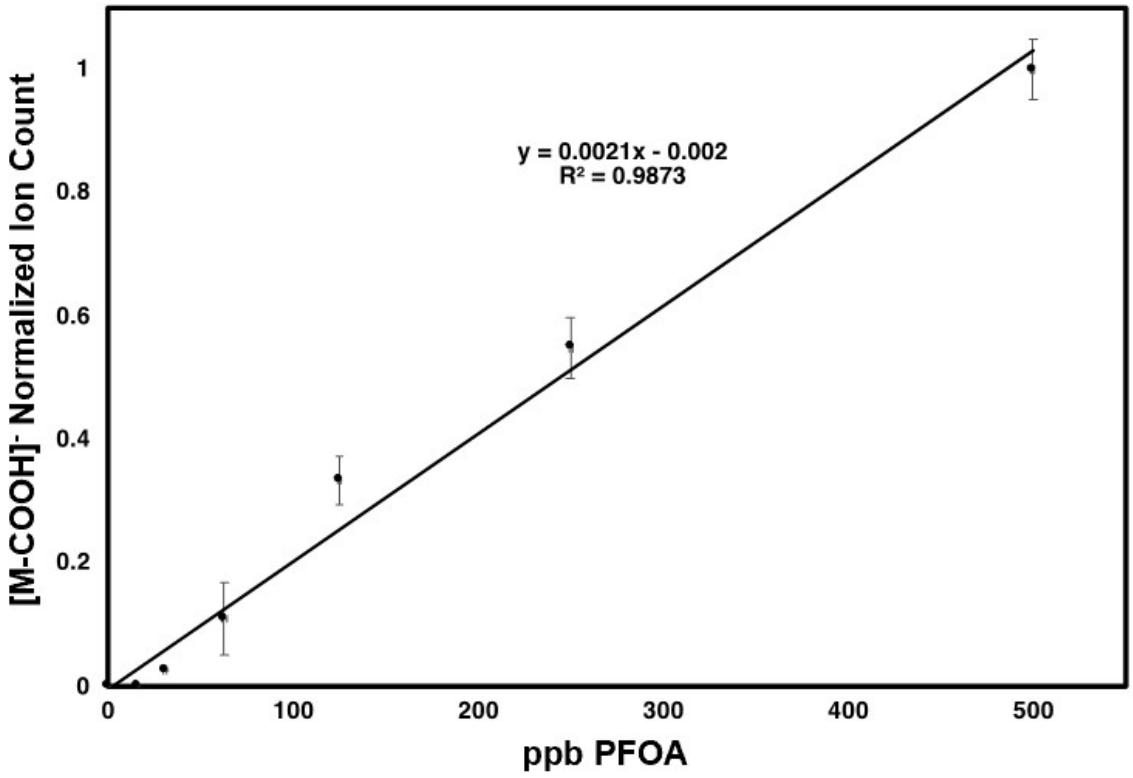
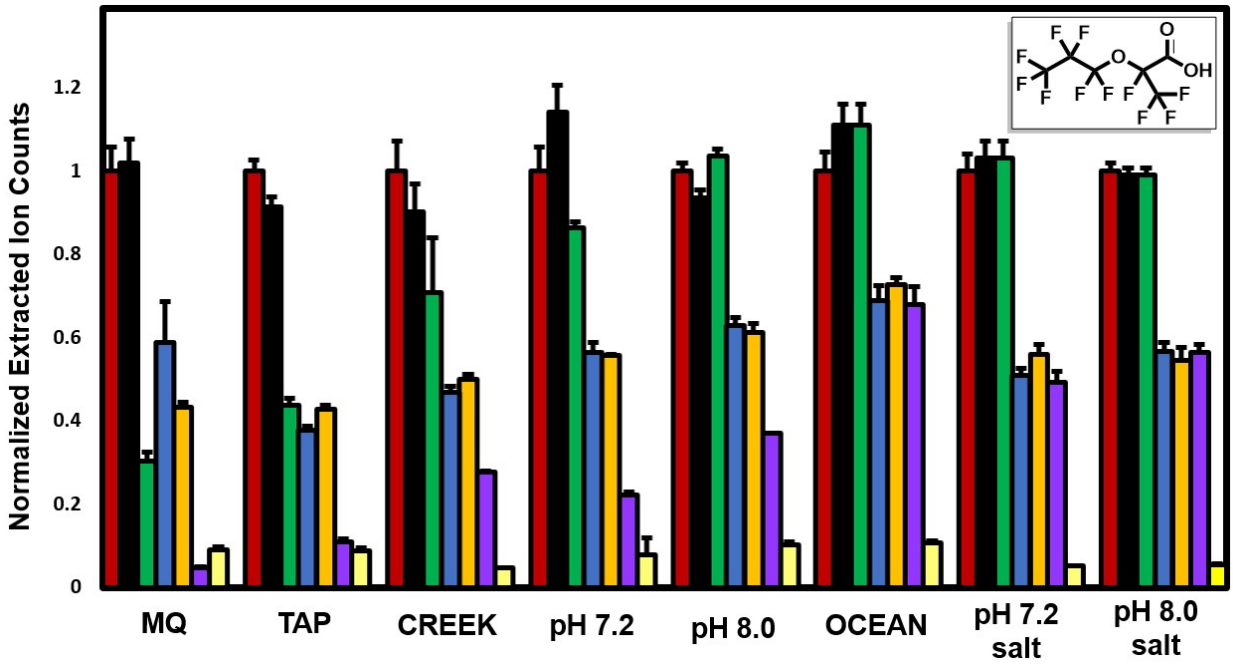
I-Figure 5. Calibration curve for PFOA in tap water. Extracted ion count $[M\text{-}COOH]^-$: 368-370 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFOA.



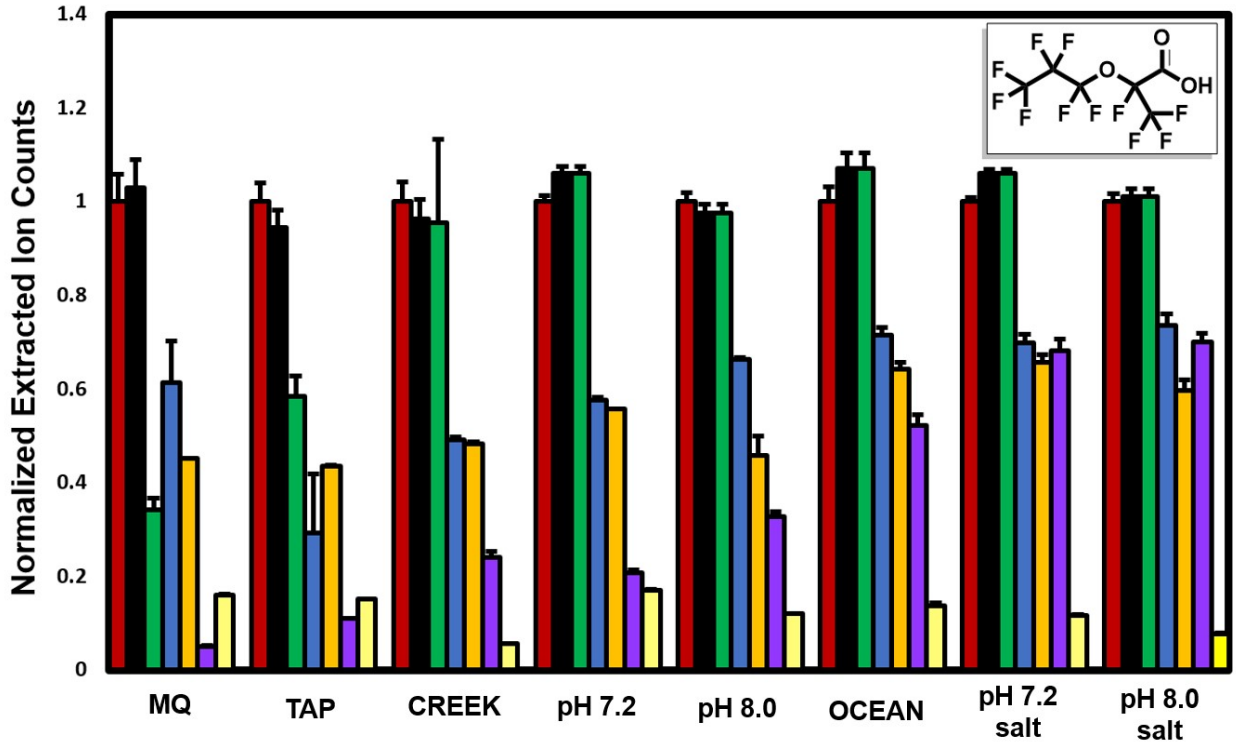
SI-Figure 6. Calibration curve for PFOA in creek water. Extracted ion count $[M-H]^-$: 412-414 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFOA.



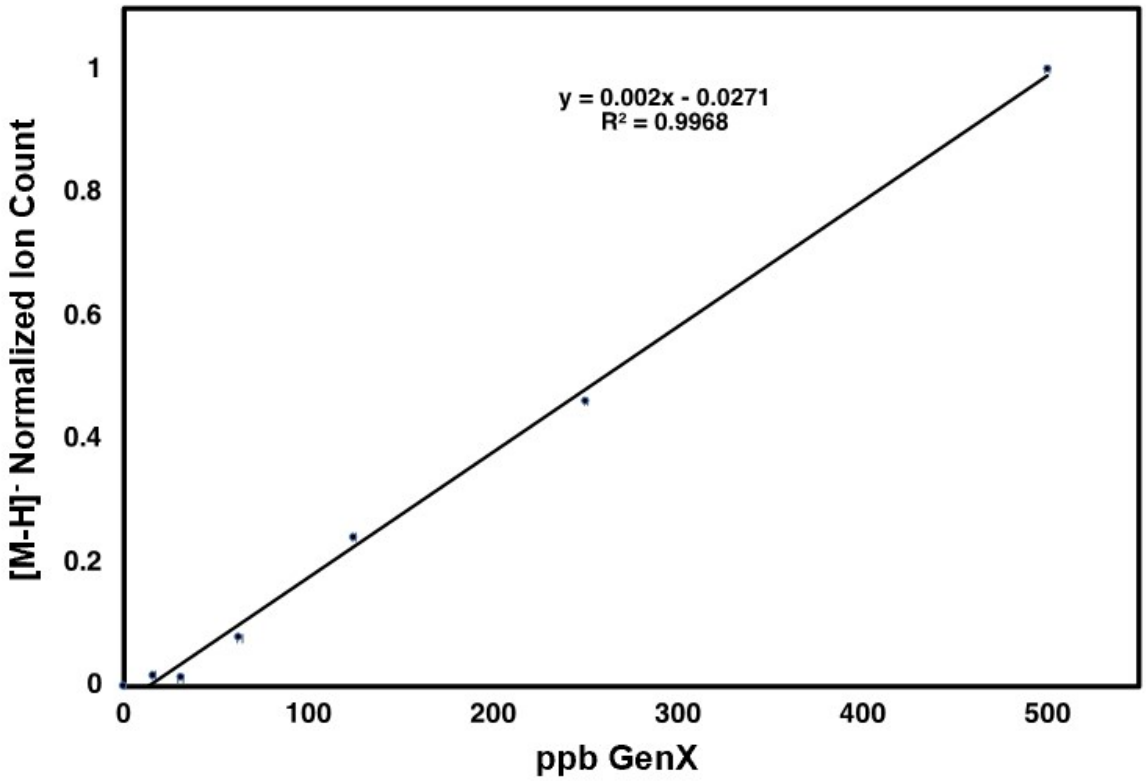
SI-Figure 7. Calibration curve for PFOA in creek water. Extracted ion count $[M\text{-}COOH]^-$: 368-370 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFOA.



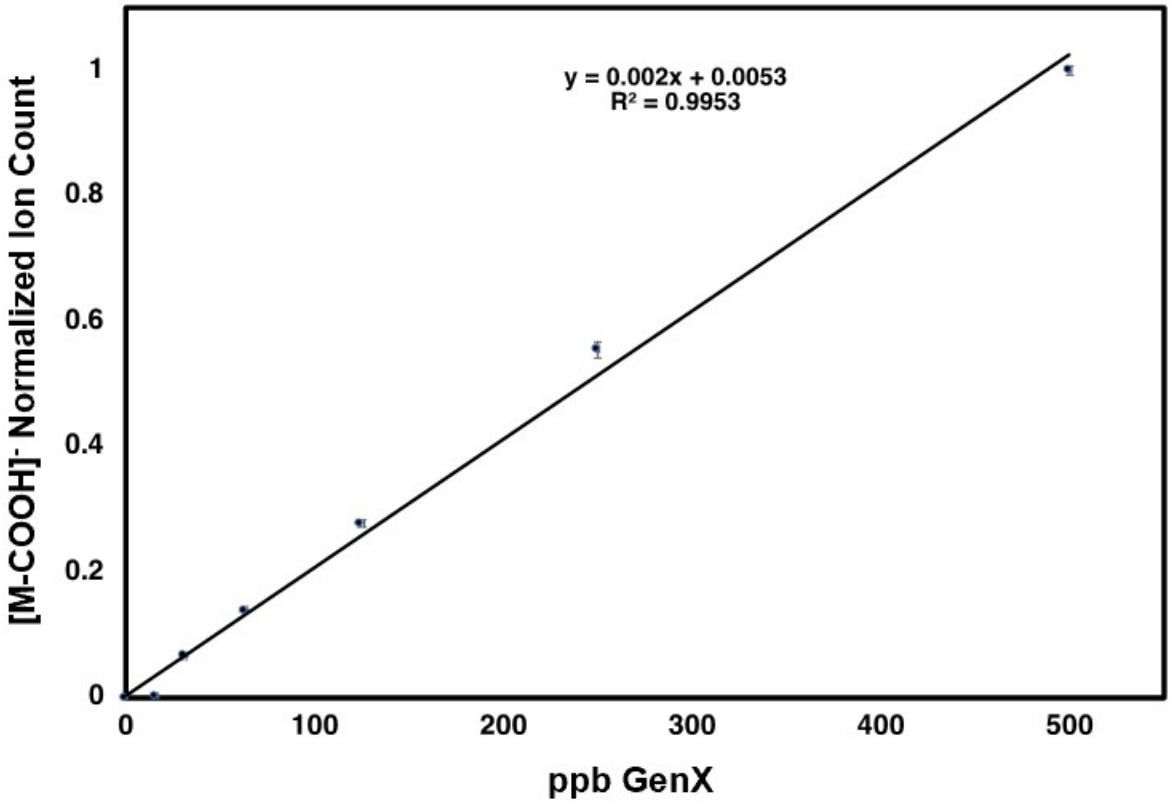
SI-Figure 8. Extracted ion count (EIC) for GenX $[M\text{-}COOH]^-$: 284-286 Da. Starting concentrations were 250 ppb. Red: starting sample, no protein (no filter); Black: MWCO filter only, no protein; Green: RNase A; Blue: casein; Orange: egg white albumin; Purple: lysozyme; Yellow: BSA. EICs reflected the amount of GenX present in solution after incubating with a protein and passing solution through the MWCO filtration device. Normalized to mean ion counts of starting sample (no filter), the control where GenX did not pass through the filtration device (std. error, n=3).



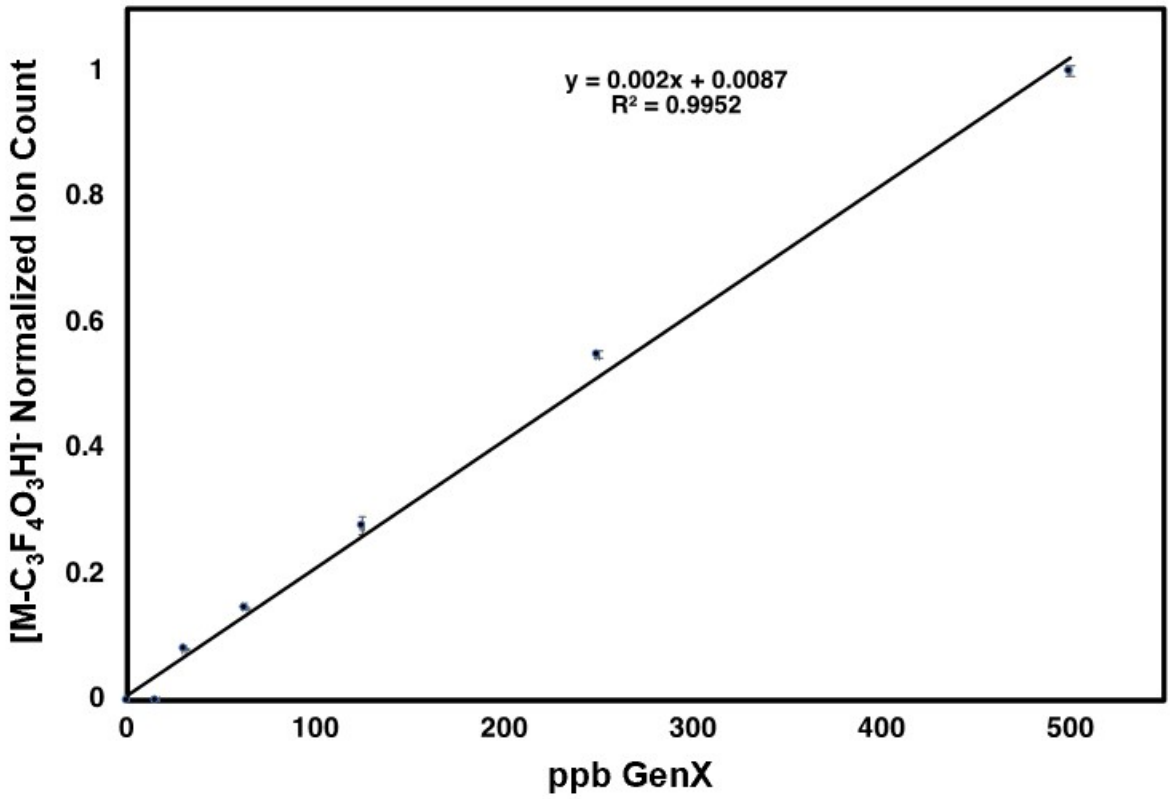
SI-Figure 9. Extracted ion count (EIC) for GenX $[M\text{-}C_3F_4O_3H]^-$: 168-170 Da. Starting concentrations were 250 ppb. Red: starting sample, no protein (no filter); Black: MWCO filter only, no protein; Green: RNase A; Blue: casein; Orange: egg white albumin; Purple: lysozyme; Yellow: BSA. EICs reflected the amount of GenX present in solution after incubating with a protein and passing solution through the MWCO filtration device. Normalized to mean ion counts starting sample (no filter), the control where GenX did not pass through the filtration device (std. error, n=3).



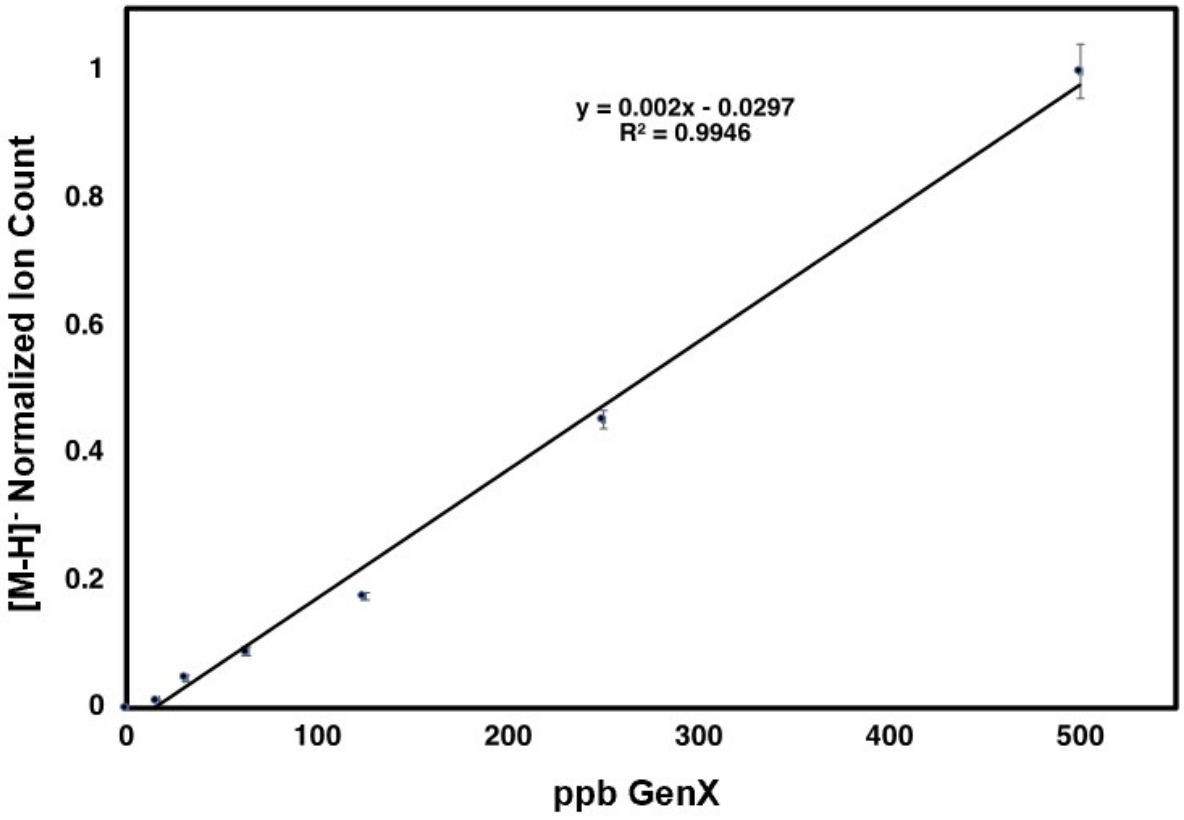
SI-Figure 10. Calibration curve for GenX in Milli-Q water. Extracted ion count $[M-H]^-$: 328-330 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



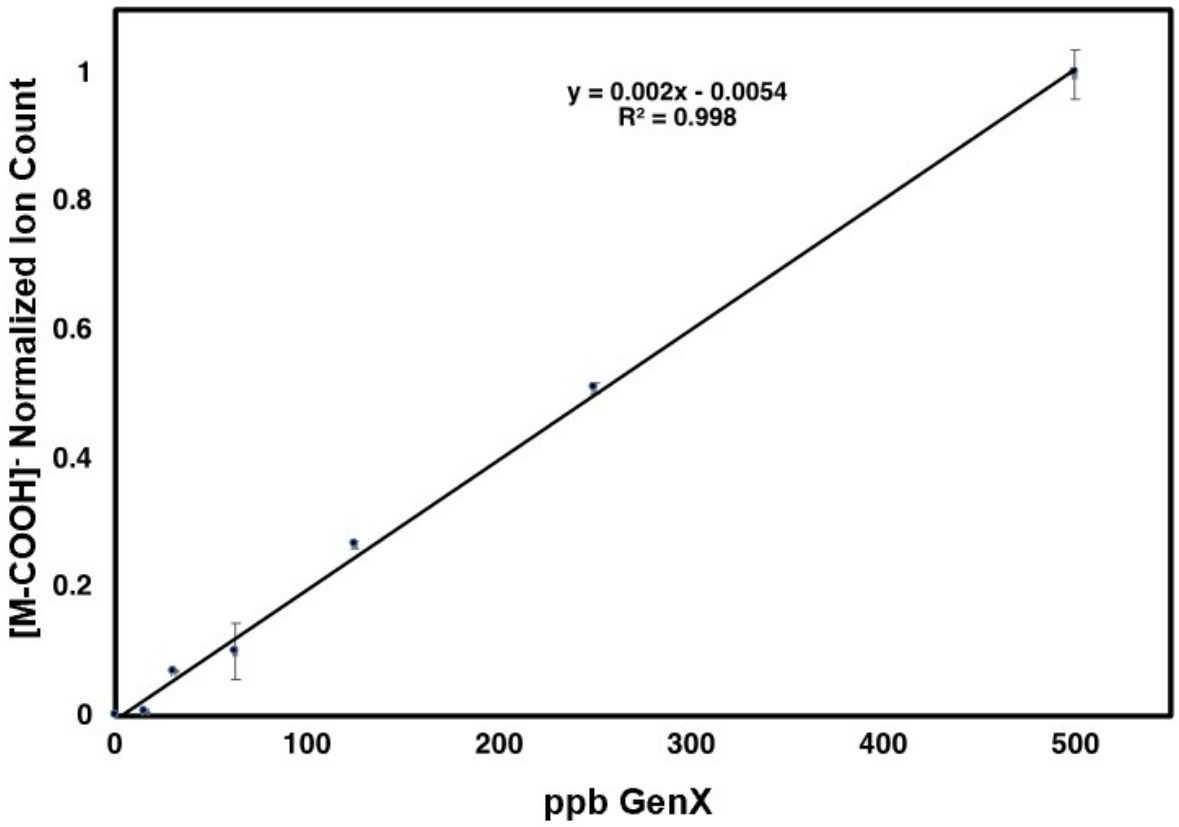
SI-Figure 11. Calibration curve for GenX in Milli-Q water. Extracted ion count $[M\text{-}COOH]^-$: 284-286 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



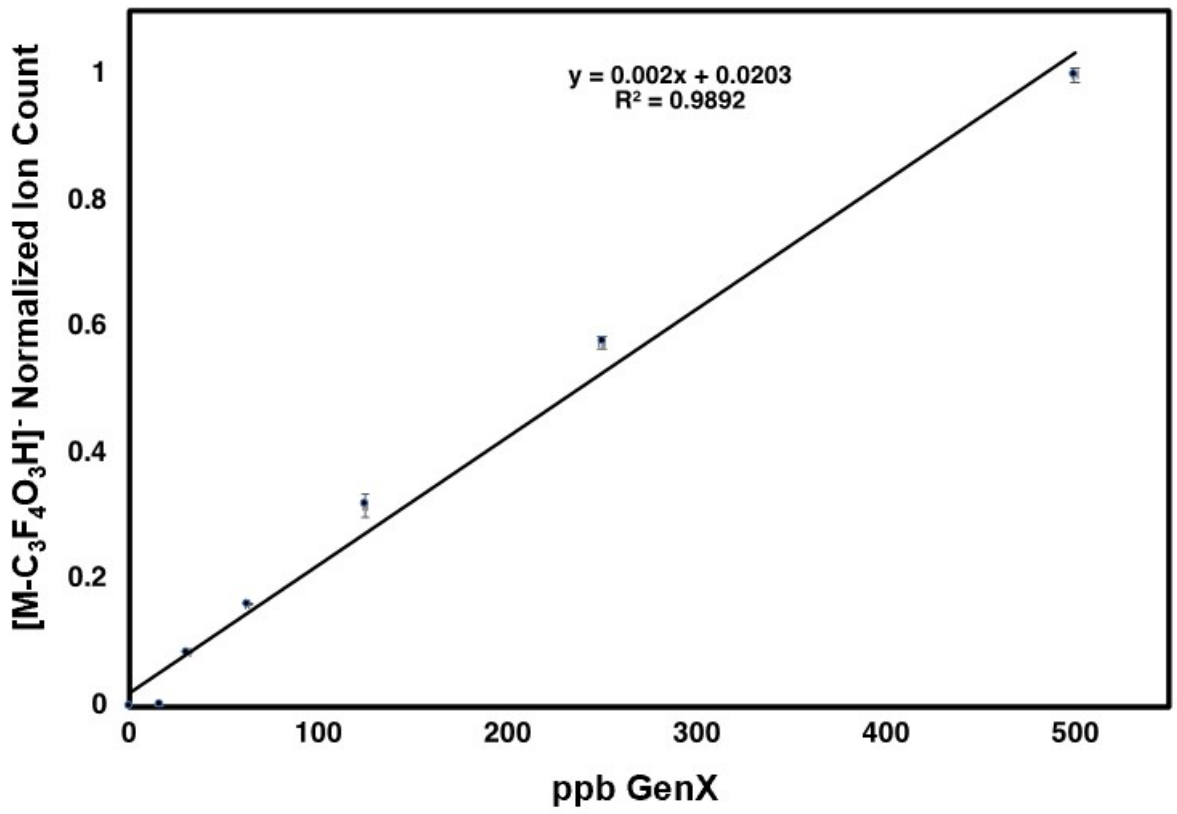
SI-Figure 12. Calibration curve for GenX in Milli-Q water. Extracted ion count $[M\text{-}C_3F_4O_3H]^-$: 168-170 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



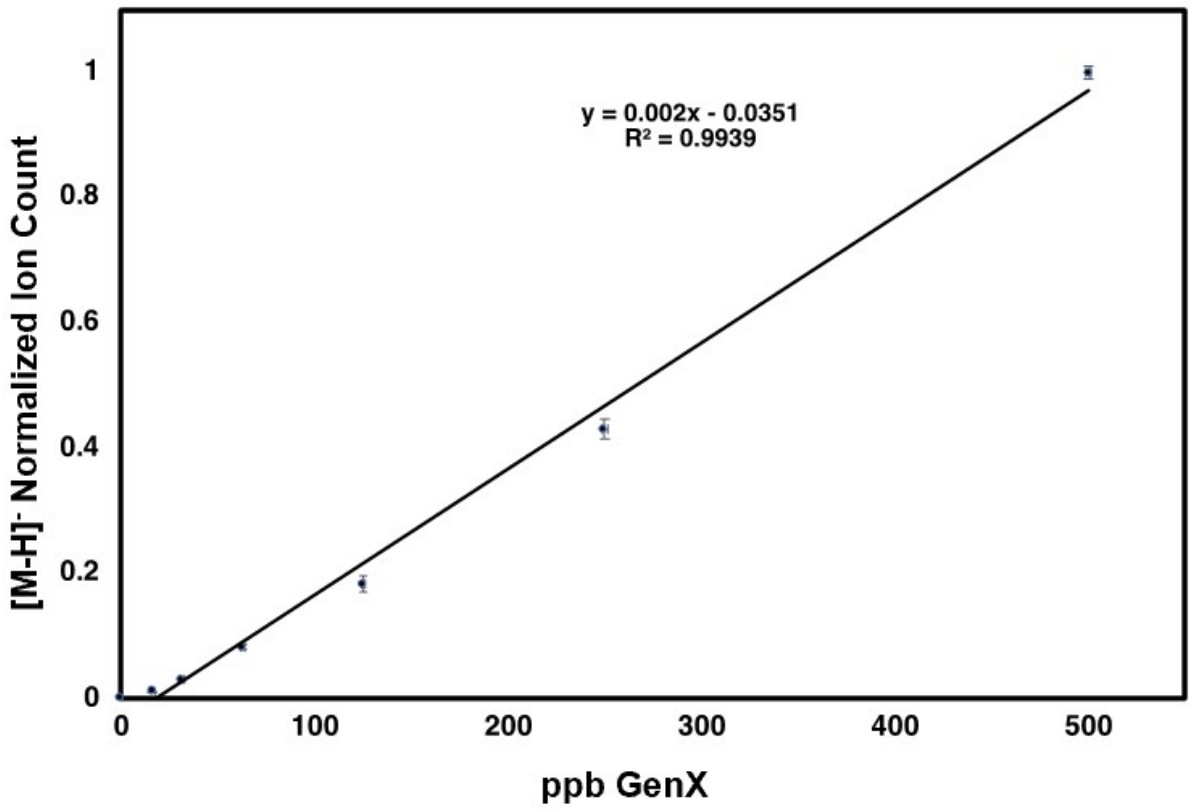
SI-Figure 13. Calibration curve for GenX in tap water. Extracted ion count $[M-H]^-$: 328-330 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



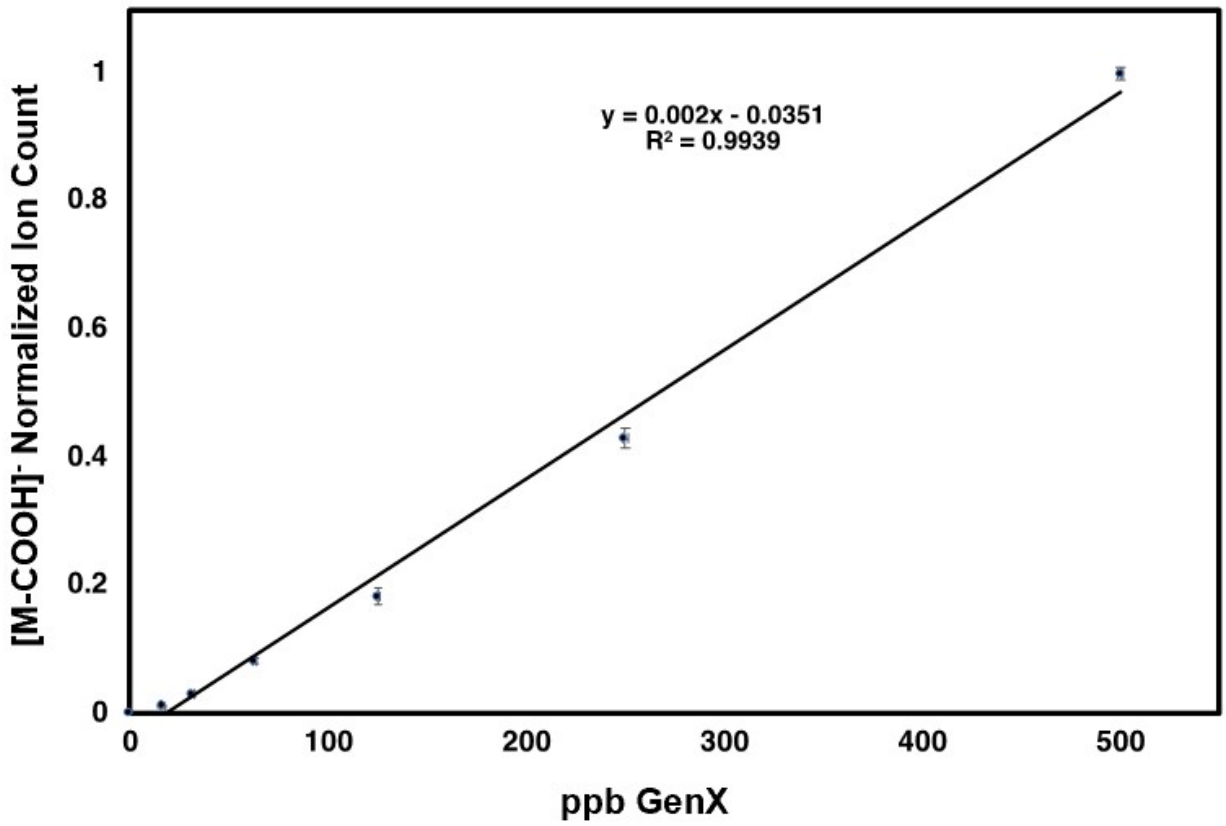
SI-Figure 14. Calibration curve for GenX in tap water. Extracted ion count $[M\text{-}COOH]^-$: 284-286 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



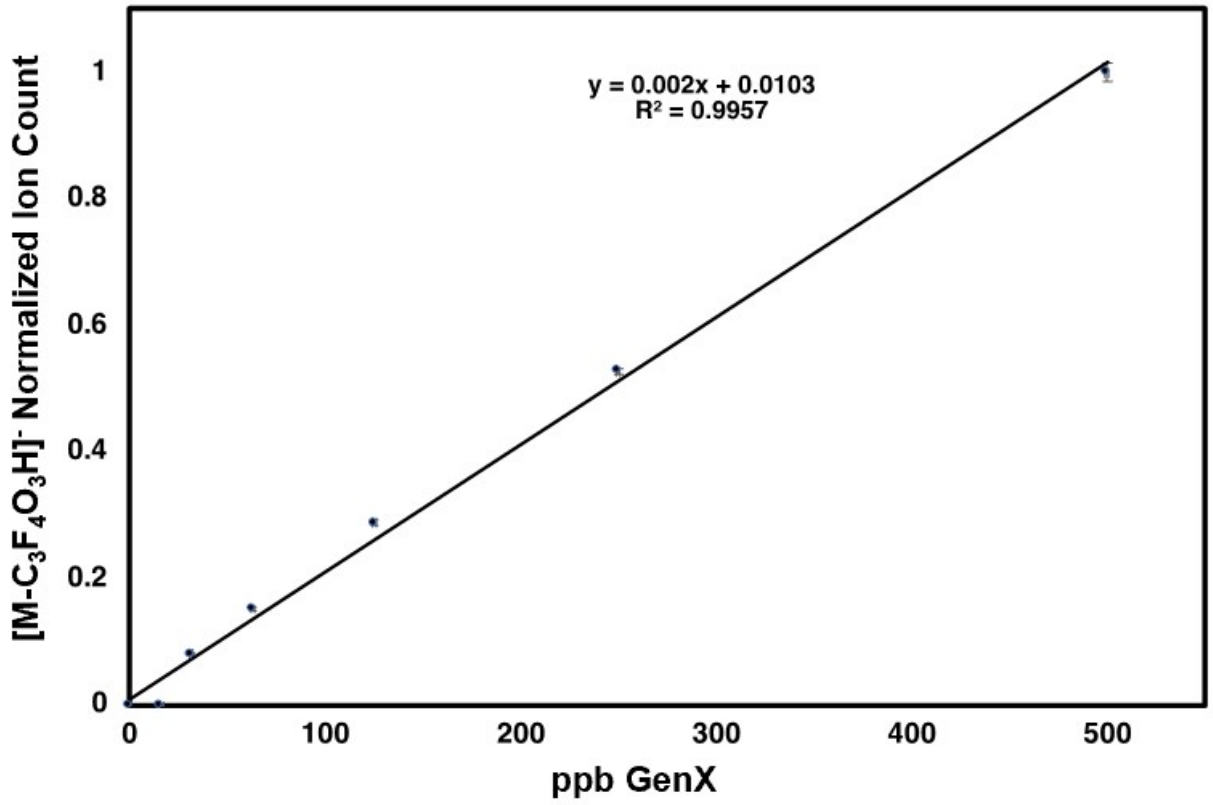
SI-Figure 15. Calibration curve for GenX in tap water. Extracted ion count $[M\text{-}C_3F_4O_3H]^-$: 168-170 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



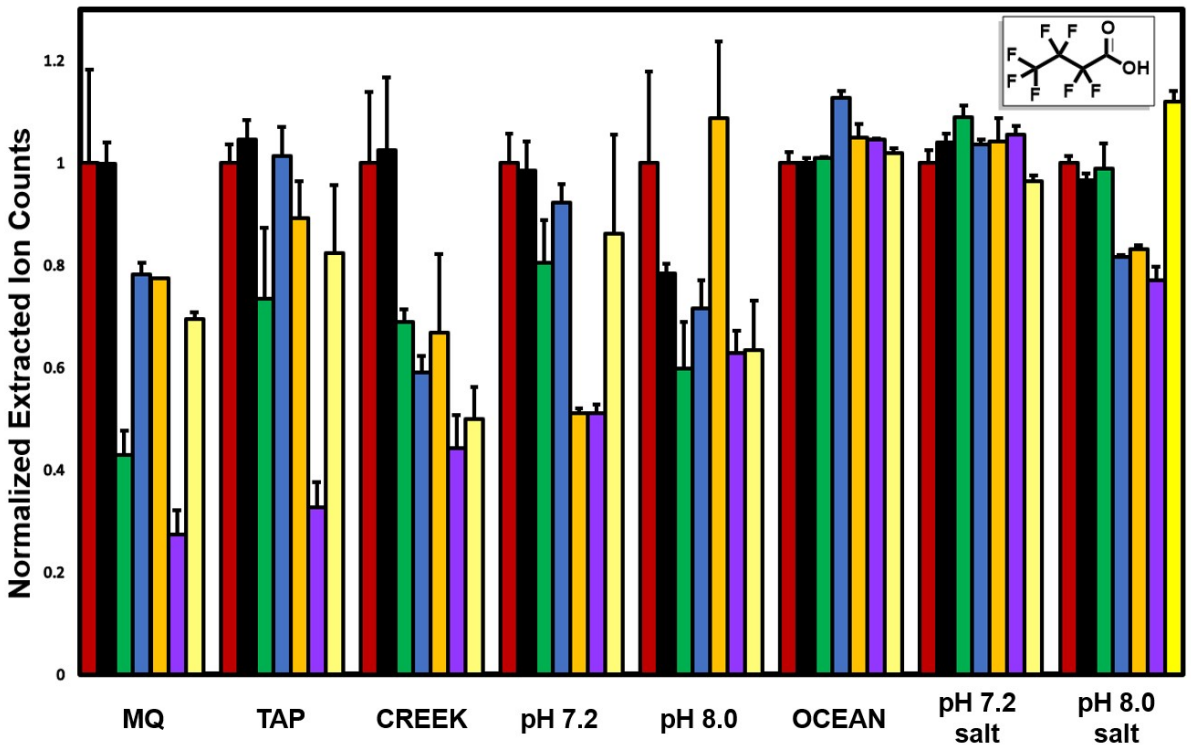
SI-Figure 16. Calibration curve for GenX in creek water. Extracted ion count $[M-H]^-$: 328-330 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



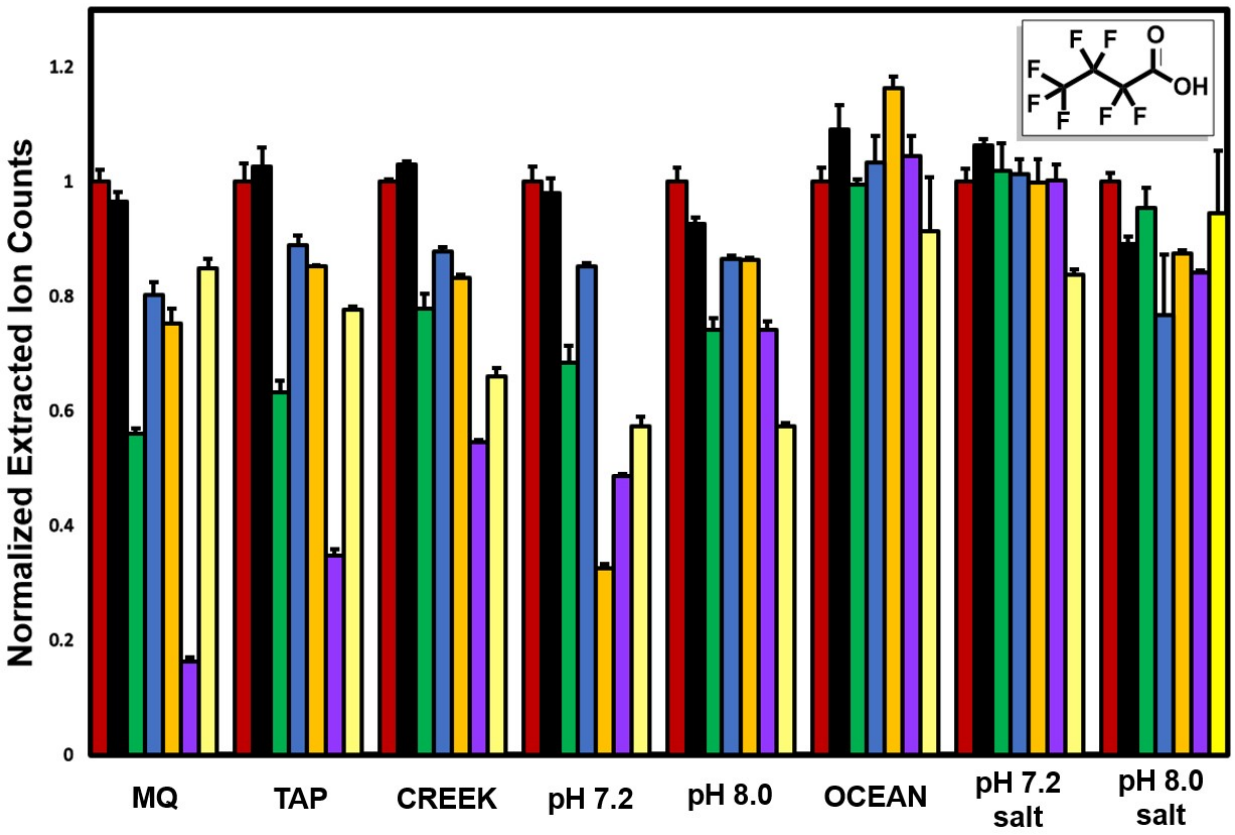
SI-Figure 17. Calibration curve for GenX in creek water. Extracted ion count $[M\text{-}COOH]^-$: 284-286 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



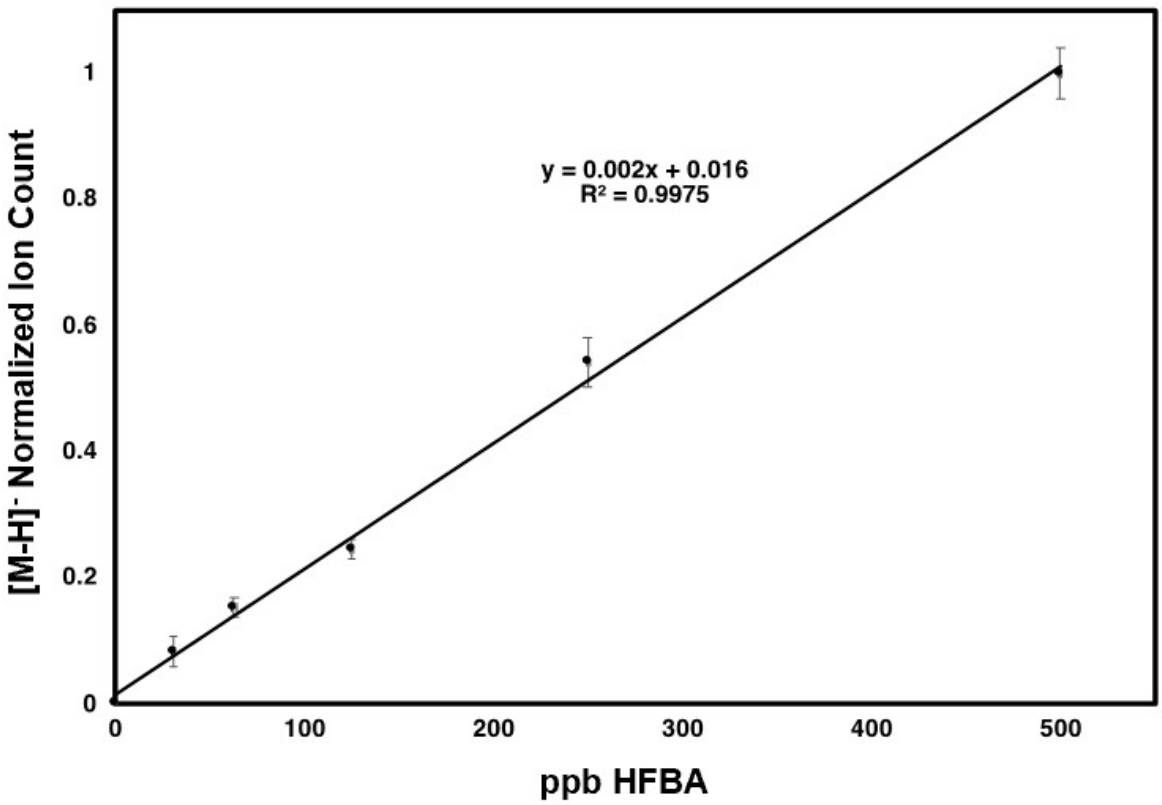
SI-Figure 18. Calibration curve for GenX in creek water. Extracted ion count $[M\text{-}C_3F_4O_3H]^-$: 168-170 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of GenX.



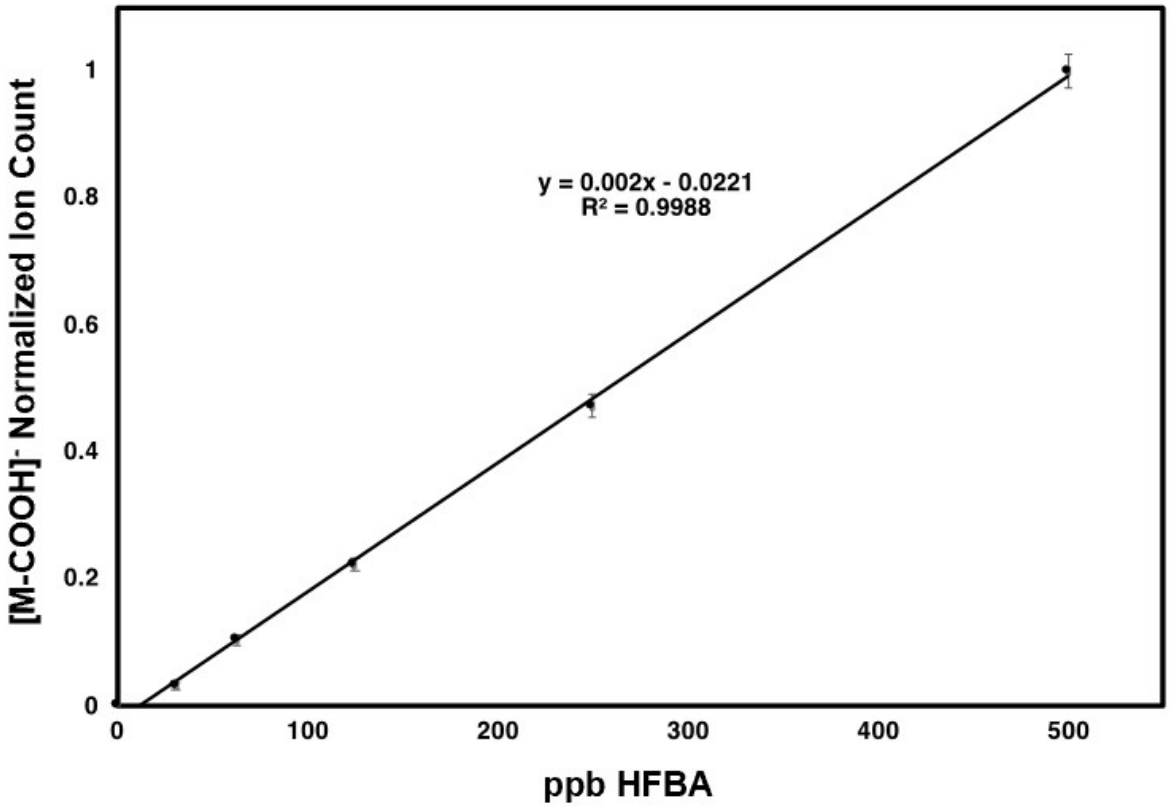
SI-Figure 19. Normalized extracted ion count (EIC) for HFBA $[M-H]^-$: 212-214 Da. Starting concentrations were 250 ppb with 350 μM protein. Red: starting sample, no filter (no filter); Black: MWCO filter only, no protein; Green: RNase A; Blue: casein; Orange: egg white albumin; Purple: lysozyme; Yellow: BSA. EICs reflected the amount of HFBA present in solution after incubating with a protein and passing solution through the MWCO filtration device. Normalized to mean ion counts of starting sample (no filter), the control where HFBA did not pass through the filtration device (std. error, n=3).



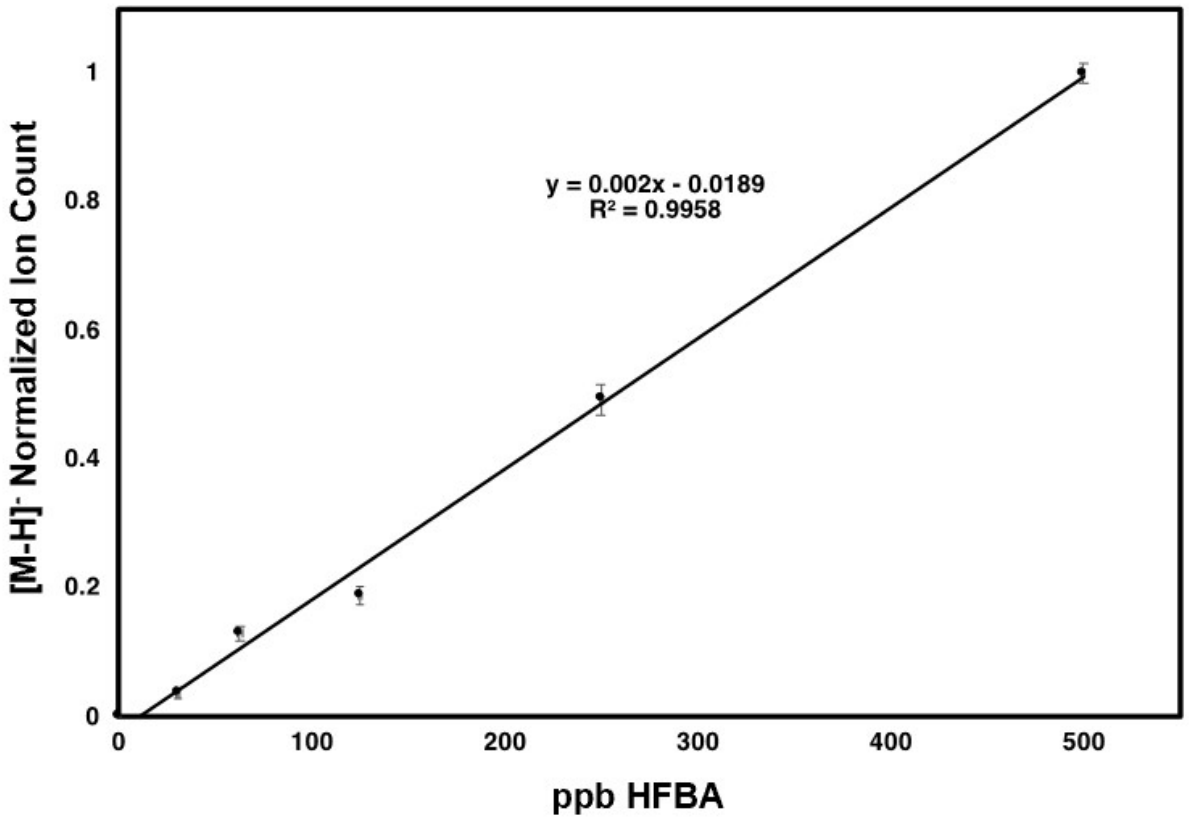
SI-Figure 20. Extracted ion count (EIC) for HFBA $[M\text{-}COOH]^-$: 168-170 Da. Starting concentrations were 250 ppb. Red: starting sample, no protein (no filter); Black: MWCO filter only, no protein; Green: RNase A; Blue: casein; Orange: egg white albumin; Purple: lysozyme; Yellow: BSA. EICs reflected the amount of HFBA present in solution after incubating with a protein and passing solution through the MWCO filtration device. Normalized to mean ion counts of starting sample (no filter), the control where HFBA did not pass through the filtration device (std. error, n=3).



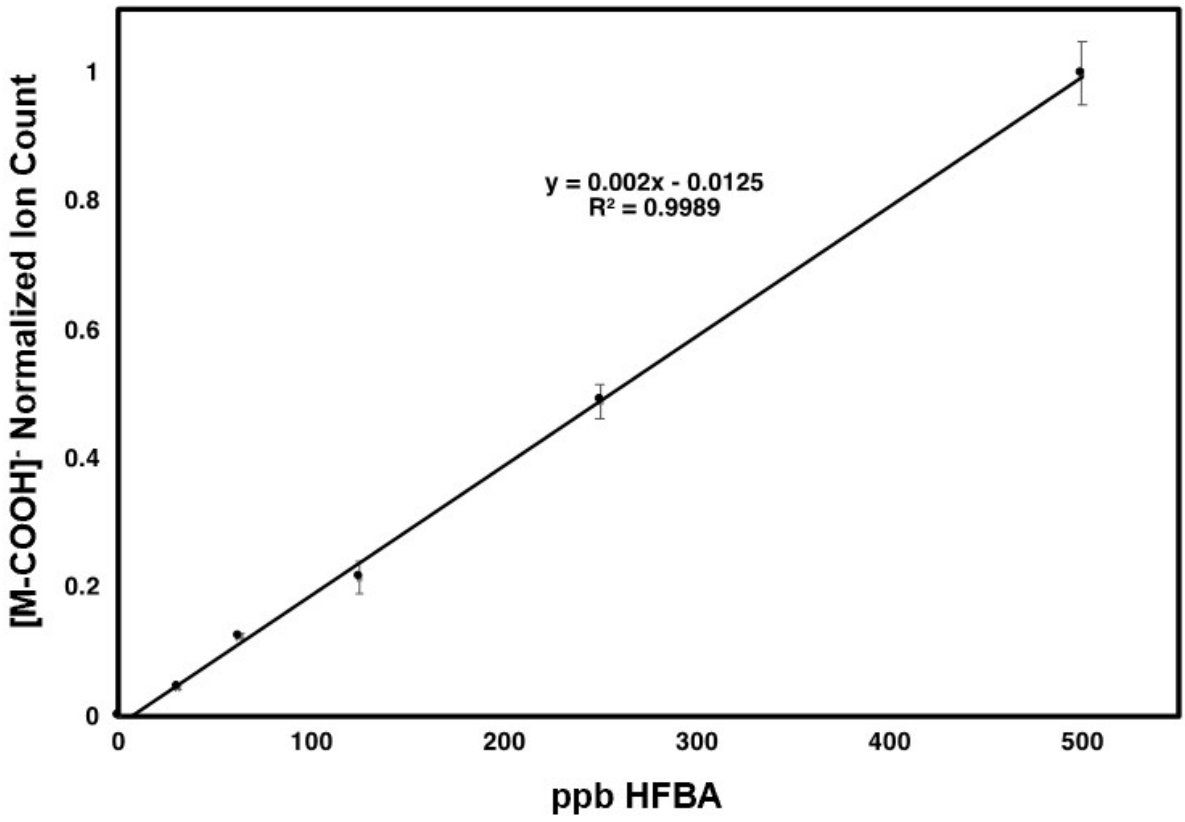
SI-Figure 21. Calibration curve for HFBA in Milli-Q water. Extracted ion count $[M-H]^-$: 212-214 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of HFBA.



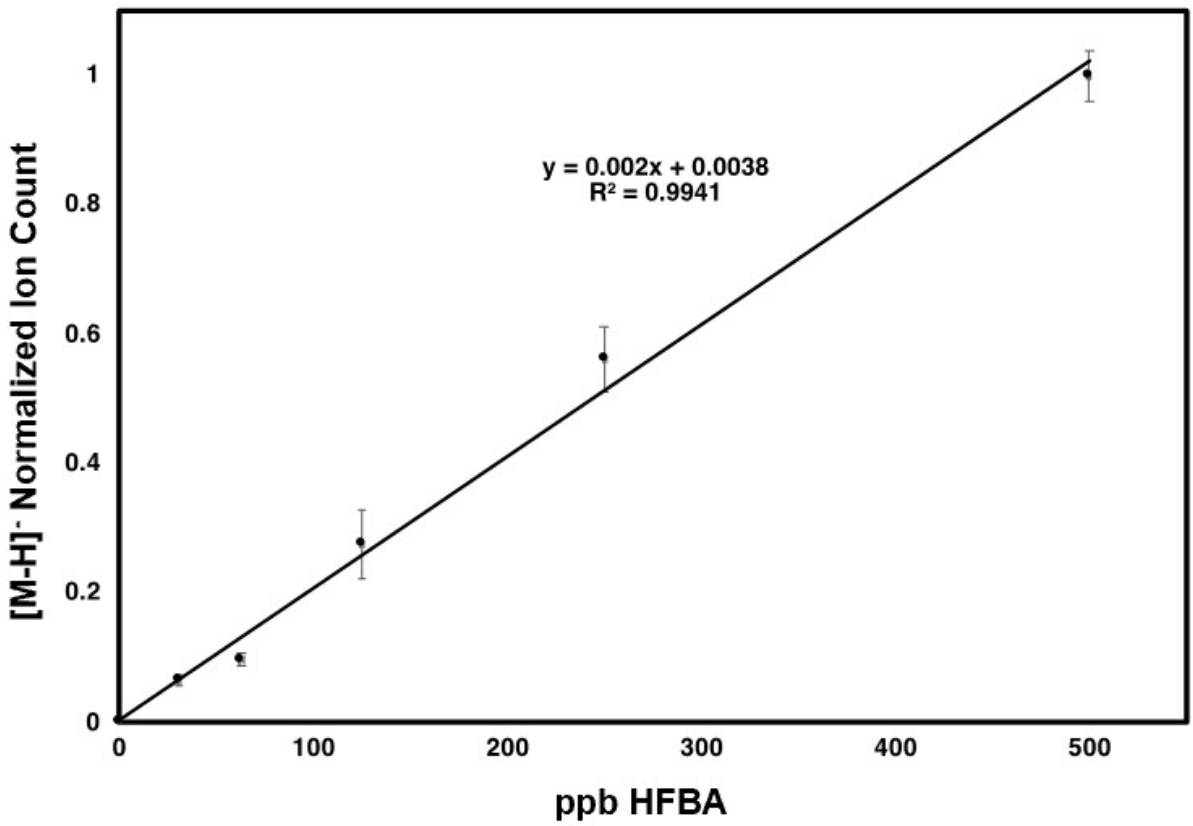
SI-Figure 22. Calibration curve for HFBA in Milli-Q water. Extracted ion count $[M\text{-}COOH]^-$: 168-170 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of HFBA.



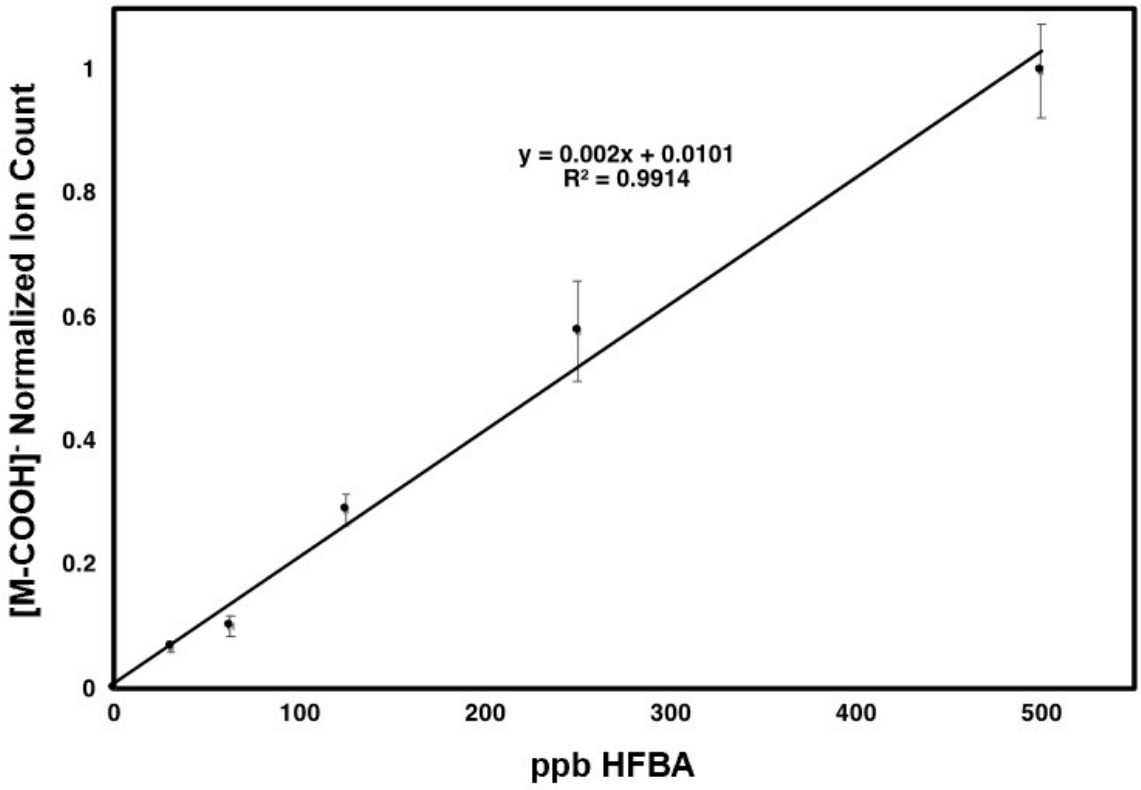
SI-Figure 23. Calibration curve for HFBA in tap water. Extracted ion count $[M-H]^-$: 212-214 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of HFBA.



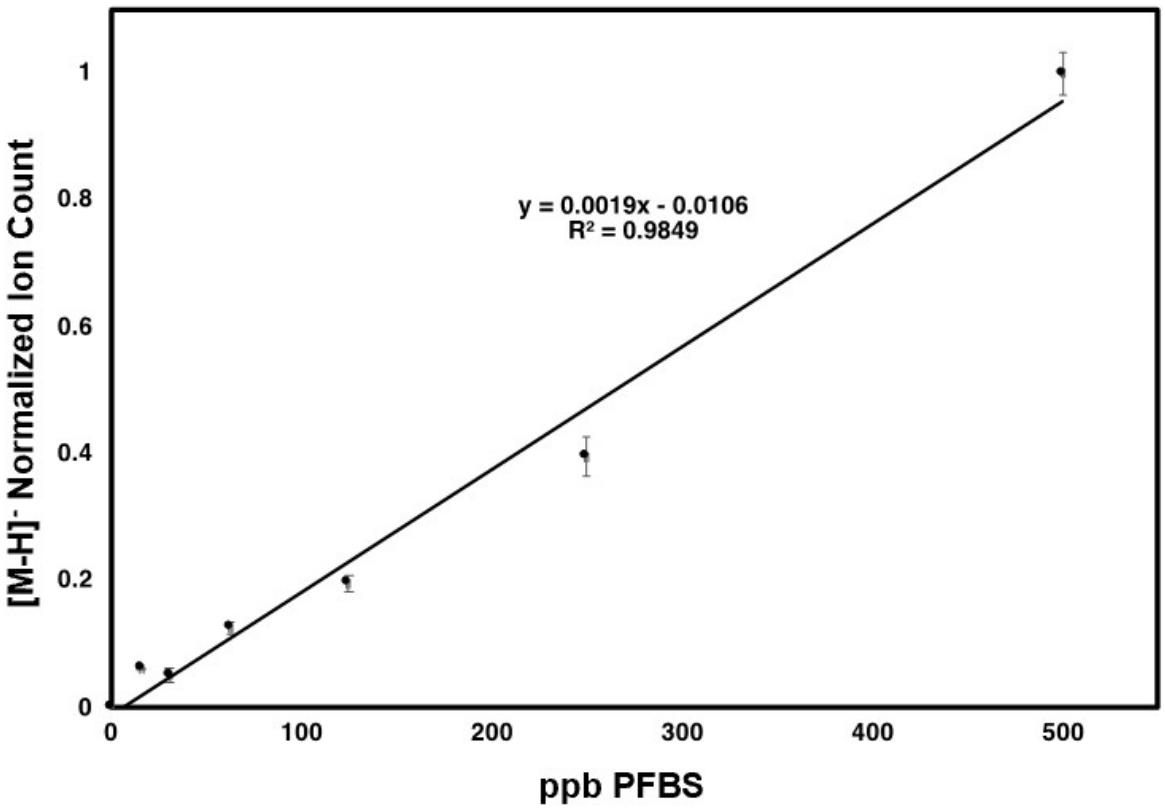
SI-Figure 24. Calibration curve for HFBA in tap water. Extracted ion count $[M\text{-}COOH]^-$: 168-170 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of HFBA.



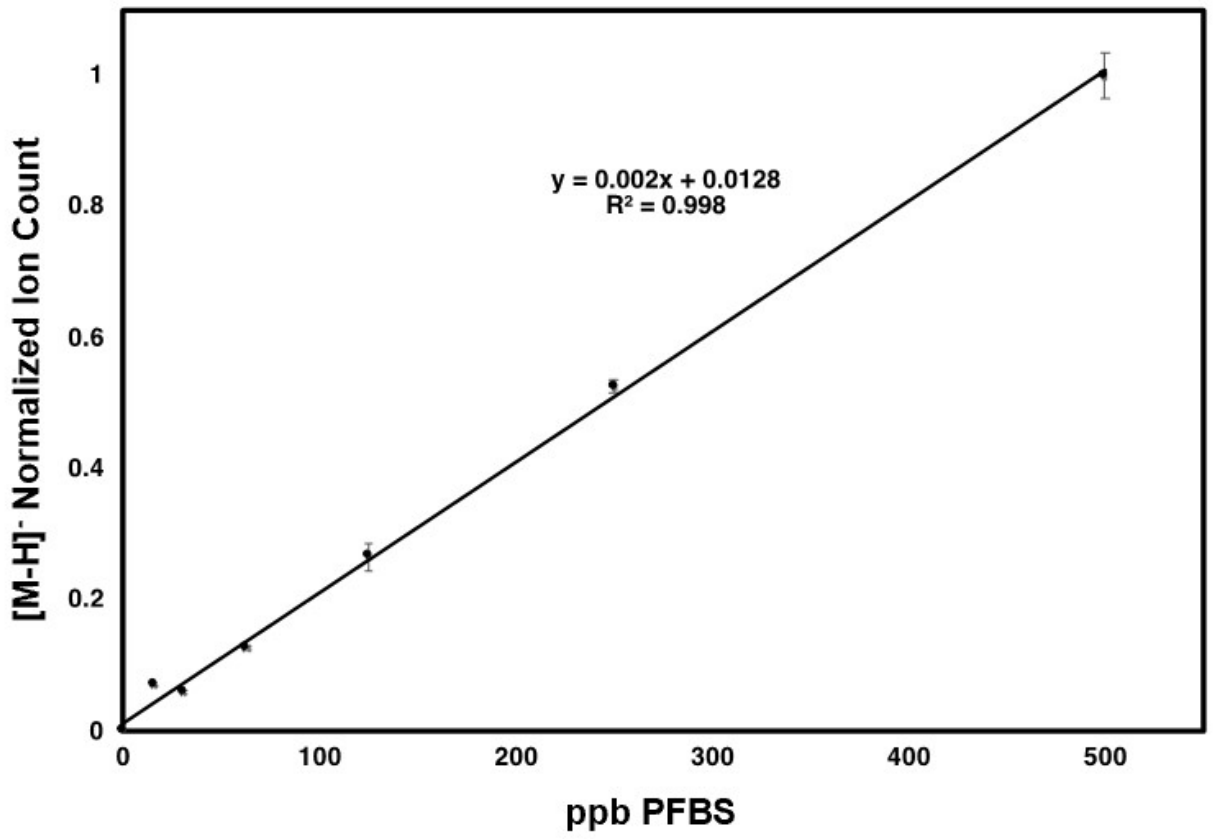
SI-Figure 25. Calibration curve for HFBA in creek water. Extracted ion count $[M-H]^-$: 212-214 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of HFBA.



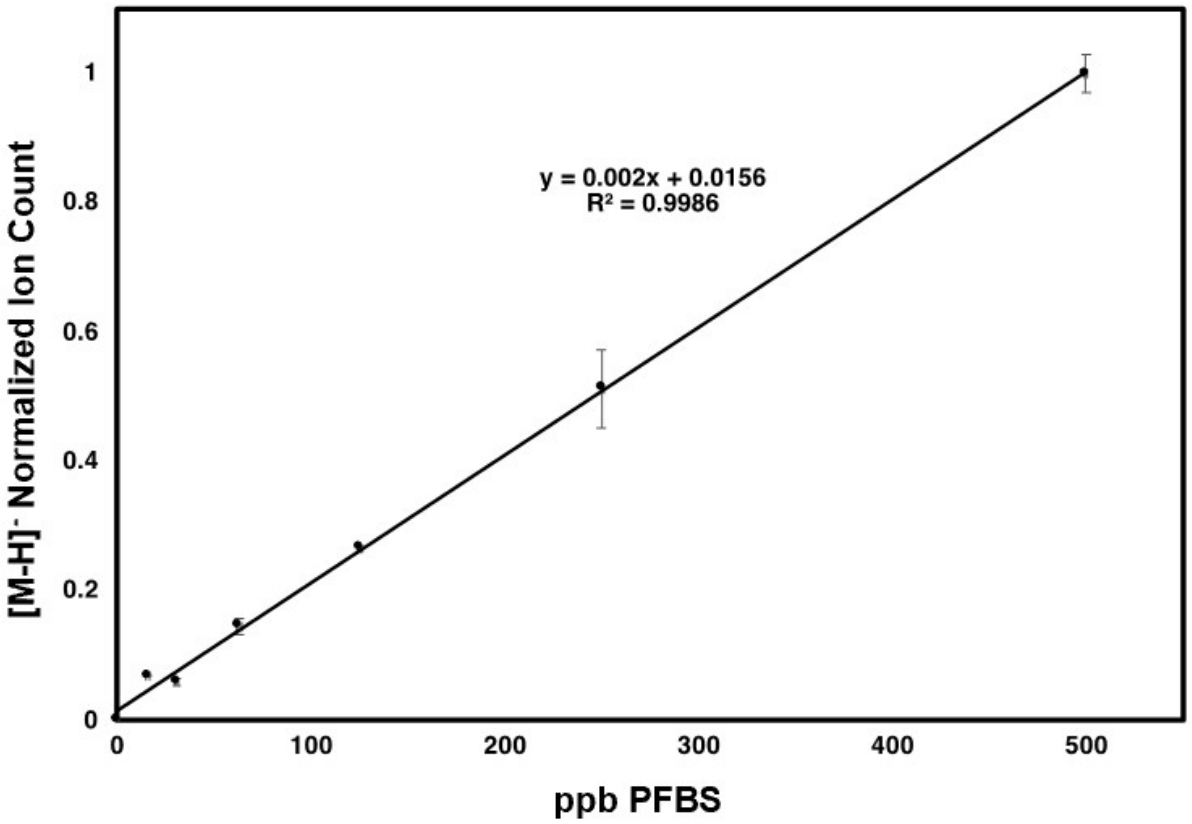
SI-Figure 26. Calibration curve for HFBA in creek water. Extracted ion count $[M\text{-}COOH]^-$: 168-170 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of HFBA.



SI-Figure 27. Calibration curve for PFBS in Milli-Q water. Extracted ion count $[M-H]^-$: 298-300 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFBS.



SI-Figure 28. Calibration curve for PFBS in tap water. Extracted ion count $[M-H]^-$: 298-300 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFBS.



SI-Figure 29. Calibration curve for PFBS in creek water. Extracted ion count $[M-H]^-$: 298-300 Da. Normalized to mean ion counts (std. error, n=3) of a 500 ppb solution of PFBS.

SI-Table 30. Tabulated percent removal of PFAS from water. Average and standard errors are calculated from three measurement replicates.

	PFOA (412-414)		PFOA (368-370)			GenX (328-330)		GenX (284-286)		GenX (168-170)			HFBA (212-214)		HFBA (168-170)			PFBS (2
		STD ERROR		AVG	STD ERROR		STD ERROR		STD ERROR		AVG	STD ERROR		STD ERROR		AVG	STD ERROR	
MQ_MWCO (-)	0%	0%	0%	0%	0%		0%		0%		0%	0%		0%		0%	0%	
MQ_MWCO (+)	-8%	5%	2%	-3%	5%		6%		-2%		-3%	3%		0%		3%	2%	
MQ_RnaseA	75%	0%	74%	75%	0%		71%		70%		66%	2%		57%		44%	50%	
MQ_Casein	26%	0%	27%	26%	0%		46%		41%		39%	2%		22%		20%	21%	
MQ_Egg White Albumin	5%	0%	5%	5%	0%		59%		57%		55%	1%		23%		25%	24%	
MQ_Lysozyme	93%	0%	93%	93%	0%		98%		95%		95%	1%		73%		84%	78%	
MQ_Bovine Serum Albumin	92%	0%	92%	92%	0%		97%		91%		84%	4%		30%		15%	23%	
Tap_MWCO (-)	0%	0%	0%	0%	0%		0%		0%		0%	0%		0%		0%	0%	
Tap_MWCO (+)	3%	1%	4%	3%	1%		5%		9%		6%	1%		-5%		-3%	-4%	
Tap_RnaseA	28%	0%	29%	28%	0%		60%		56%		42%	6%		27%		37%	32%	
Tap_Casein	3%	0%	3%	3%	0%		65%		62%		71%	3%		-1%		11%	5%	
Tap_Egg White Albumin	6%	0%	6%	6%	0%		54%		57%		57%	1%		11%		15%	13%	
Tap_Lysozyme	39%	0%	39%	39%	0%		94%		89%		89%	2%		67%		65%	66%	
Tap_Bovine Serum Albumin	89%	0%	89%	89%	0%		97%		91%		85%	3%		18%		22%	20%	
Creek_MWCO (-)	0%	0%	0%	0%	0%		0%		0%		0%	0%		0%		0%	0%	
Creek_MWCO (+)	5%	1%	3%	4%	1%		9%		10%		4%	2%		-2%		-3%	-3%	
Creek_RnaseA	26%	0%	25%	25%	0%		33%		29%		4%	9%		31%		22%	27%	
Creek_Casein	6%	1%	4%	5%	1%		45%		53%		51%	2%		41%		12%	26%	
Creek_Egg White Albumin	19%	0%	17%	18%	1%		40%		50%		52%	4%		33%		17%	25%	
Creek_Lysozyme	39%	0%	38%	38%	1%		72%		72%		76%	1%		56%		45%	51%	
Creek_Bovine Serum Albumin	93%	0%	92%	92%	0%		99%		95%		94%	1%		50%		34%	42%	
pH7.2_MWCO (-)	0%	0%	0%	0%	0%		0%		0%		0%	0%		0%		0%	0%	
pH7.2_MWCO (+)	9%	1%	7%	8%	1%		-14%		-14%		-6%	3%		1%		2%	2%	
pH7.2_RnaseA	39%	2%	35%	37%	2%		3%		14%		-6%	6%		19%		32%	26%	
pH7.2_Casein	0%	0%	-1%	-1%	0%		17%		44%		42%	9%		8%		15%	11%	
pH7.2_Egg White Albumin	11%	4%	19%	15%	4%		19%		44%		44%	9%		49%		67%	58%	
pH7.2_Lysozyme	63%	1%	61%	62%	1%		58%		78%		79%	7%		49%		51%	50%	
pH7.2_Bovine Serum Albumin	85%	0%	85%	85%	0%		94%		92%		83%	3%		14%		43%	28%	
pH8.0_MWCO (-)	0%	0%	0%	0%	0%		0%		0%		0%	0%		0%		0%	0%	
pH8.0_MWCO (+)	8%	2%	5%	6%	2%		9%		6%		2%	2%		22%		7%	7%	
pH8.0_RnaseA	4%	1%	2%	3%	1%		9%		-4%		2%	4%		40%		26%	33%	
pH8.0_Casein	19%	1%	17%	18%	1%		2%		37%		34%	11%		28%		13%	21%	
pH8.0_Egg White Albumin	33%	1%	32%	33%	1%		-2%		39%		54%	17%		-9%		14%	3%	
pH8.0_Lysozyme	15%	1%	13%	14%	1%		55%		63%		67%	4%		37%		26%	32%	
pH8.0_Bovine Serum Albumin	86%	1%	85%	85%	1%		86%		90%		88%	1%		37%		43%	40%	
Ocean Shore_MWCO (-)	0%	0%	0%	0%	0%		0%		0%		0%	0%		0%		0%	0%	
Ocean Shore_MWCO (+)	13%	1%	15%	14%	1%		-5%		-11%		-7%	2%		0%		-9%	-5%	
Ocean Shore_RnaseA	13%	0%	13%	13%	0%		-5%		-11%		-7%	2%		-1%		1%	0%	
Ocean Shore_Casein	17%	3%	22%	20%	3%		2%		31%		28%	9%		-13%		-3%	-8%	
Ocean Shore_Egg White Albumin	40%	0%	40%	40%	0%		-12%		27%		36%	2%		-5%		-16%	-11%	
Ocean Shore_Lysozyme	33%	0%	33%	33%	0%		11%		32%		48%	11%		-5%		-5%	-5%	
Ocean Shore_Bovine Serum Albumin	87%	1%	87%	87%	0%		96%		89%		86%	3%		-2%		9%	3%	
pH7.2_NaCl_MWCO (-)	0%	0%	0%	0%	0%	n/a		0%		0%	0%	0%		0%		0%	0%	
pH7.2_NaCl_MWCO (+)	22%	1%	24%	23%	1%	n/a		-3%		-6%	-5%	2%		-4%		-6%	-5%	
pH7.2_NaCl_RnaseA	46%	1%	45%	45%	1%	n/a		-3%		-6%	-5%	2%		-9%		-2%	-5%	
pH7.2_NaCl_Casein	28%	0%	28%	28%	0%	n/a		49%		30%	40%	10%		-4%		-1%	-2%	
pH7.2_NaCl_Egg White Albumin	46%	1%	48%	47%	1%	n/a		44%		34%	39%	5%		-4%		0%	-2%	
pH7.2_NaCl_Lysozyme	35%	1%	37%	36%	1%	n/a		51%		32%	41%	9%		-6%		0%	-3%	
pH7.2_NaCl_Bovine Serum Albumin	83%	0%	84%	83%	0%	n/a		95%		88%	92%	3%		4%		16%	10%	
pH8.0_NaCl_MWCO (-)	0%	0%	0%	0%	0%	n/a		0%		0%	0%	0%		0%		0%	0%	
pH8.0_NaCl_MWCO (+)	12%	1%	11%	12%	0%	n/a		1%		-1%	0%	1%		3%		11%	7%	
pH8.0_NaCl_RnaseA	20%	1%	18%	19%	1%	n/a		1%		-1%	0%	1%		1%		5%	3%	
pH8.0_NaCl_Casein	11%	1%	8%	10%	1%	n/a		43%		26%	35%	8%		18%		23%	21%	
pH8.0_NaCl_Egg White Albumin	38%	1%	36%	37%	1%	n/a		45%		40%	43%	3%		17%		12%	15%	
pH8.0_NaCl_Lysozyme	27%	3%	22%	25%	3%	n/a		44%		30%	37%	7%		23%		16%	19%	
pH8.0_NaCl_Bovine Serum Albumin	85%	0%	86%	85%	0%	n/a		95%		92%	93%	1%		-12%		5%	-3%	