Supporting Information

Identification of novel polyfluorinated ether sulfonates as PFOS alternatives in municipal sewage sludge in China

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Figure S1. Sampling locations, spatial concentrations and composition profiles of Cl-PFAES homologues in municipal sewage sludge samples in China (mean concentration values of each analyte were present for WWTP samples collected in the same provinces/municipalities).

Figure S2. MS^2 characteristic fragmentation ions of the 6:2 Cl-PFAES standard in the HCD mode (collision energy: 35%, spiked at 20 ng/mL).

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Figure S4. The score plots (PC1 versus PC2) of fluoroalkyl sulfonate concentrations (log-transformed data of PFOS, 6:2 FTS, 8:2 FTS, 6:2 Cl-PFAES and 8:2 Cl-PFAES, analytes with detection ratio less than 70% in the sludge samples were not taken into account) in the sewage sludge samples grouped by WWTP treatment techniques

(AAO, anaerobic-anoxic-oxic; AO, anoxic/oxic; OD, oxidation ditch; SBR, sequencing batch reactor) for further visualization of potential relationships from the principal component analysis (varimax rotation method, statistical significant level set as $p < 0.05$).

Figure S5. Separation and collection of the eluate fractions of the 6:2 and 8:2 Cl-PFAES in the Autopurification system. (A). Retention behavior of 8:2 Cl-PFAES in the LC column. (B). Retention behavior of 6:2 Cl-PFAES in the LC column. (C). Fraction collection window for each analyte in the purification cycles.

Figure S6. Detailed ^{19}F-NMR result of the laboratory-purified 6:2 Cl-PFAES standard.

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Experimental

Laboratory-purification of the 6:2 Cl-PFAES and 8:2 Cl-PFAES standards from the commercial F-53B mist suppressant product.

Purification of the 6:2 Cl-PFAES and 8:2 Cl-PFAES analytical standards from the commercial F-53B product (with 6:2 Cl-PFAES content of 77.6%) was performed on an Waters Autopurification HPLC/MS System (Waters Inc., Milford, MA), including a System Fluids Organizer, a 2545 Binary Gradient Module, a 2767 Sample Manager, a 515 Makeup Pump and an ACQUITY SQD single-quadrupole MS detector. An XBridge C18 column (Waters, 19 mm i.d. × 250 mm length, 5 μm) was chosen for the analyte separation. Flow gradient initiated at a composition of 30:70 (acetonitrile/water,v/v, both containing 1 g/L ammonium bicarbonate additive) with a flow rate of 20 mL/min and then linearly increased to 80% acetonitrile in 15 min. Product solution was prepared at 100 mg/mL, and 500 μL was injected in each separation cycle. The single-quadrupole MS detector was used to direct the fraction collection. Analyte eluates with the retention time in the range of 8.3−11.3 min and 12.8−13.8 min were confirmed and collected as the 6:2 Cl-PFAES and 8:2 Cl-PFAES fractions, respectively (Figure S5). Then, both the two eluate fractions were lyophilized, and white solid crystals were obtained. The structure and purity of the 6:2 Cl-PFAES and 8:2 Cl-PFAES standards were further validated by an nuclear magnetic resonance spectrometer (^{12}C-NMR and ^{19}F-NMR, Bruker AVANCE III 500WB, Billerica, MA) and a high performance liquid chromatography coupled with an evaporative light-scattering detector (Shimadzu LC20A-ELSD II, Kyoto, Japan) with detailed information shown in Figure S6-S11. No obvious organic impurity was found for the purified 6:2 Cl-PFAES standard (purity: ~ 100% from the LC-ELSD chromatography), while the major impurity of the 8:2 Cl-PFAES standard was the 6:2 Cl-PFAES component (~ 2.7%).

Table S1. Detailed information on the wastewater treatment plant characteristics.

Sampling ID	TOC [a] (%)	Processing Volume (10^4 m^3/d)	WWTP Biotreatment Techniques [b]	Sampling ID	TOC [a] (%)	Processing Volume (10^4 m^3/d)	WWTP Biotreatment Techniques [b]
BJ-1	35.5	40	AAO	HF-1	32.4	30	OD
BJ-2	18.5	1.0	SBR	HF-2	28.1	18	OD
BJ-3	37.0	35	OD	HF-3	25.8	6.0	SBR
BJ-4	40.6	8.0	SBR	GD-1	28.8	10	OD
BJ-5	39.1	60	AAO	XA-1	39.1	25	AAO
BJ-6	37.4	4.0	AO	XA-2	29.9	8.0	OD
SH-1	45.0	6.0	PASF	XY-1	36.0	10	OD
CQ-1	16.1	2.0	SBR	HN-1	34.4	7.0	OD
CS-1	43.3	4.0	OD	HN-2	27.0	9.0	AO
CS-2	37.9	3.0	OD	WH-1	13.6	15	AAO
CS-3	40.2	16	AO	WH-2	7.0	25	AO
TA-1	25.7	7.0	AAO	HEB-1	35.5	20	OD
TA-2	19.4	7.0	OD	HZ-1	25.5	20	AAO
JN-1	13.9	20	OD	HZ-2	36.1	40	AO
LW-1	21.5	4.0	AO	NB-1	16.8	3.0	AO
LW-2	20.0	3.0	AO	NB-2	23.7	10	AAO
LW-3	20.2	2.0	AO	HB-1	26.5	16	AO
DZ-1	10.4	2.0	AO	FJ-1	21.2	10	SBR
DZ-2	16.2	2.0	AAO	ZGE-1	12.9	2.0	CAST
QD-1	35.8	10	AAO	CD-1	28.9	10	AAO
QD-2	34.8	3.0	OD	QH-1	24.2	8.5	AAO
DY-1	30.7	3.0	AO	XJ-1	12.3	20	AAO
RZ-1	34.0	5.0	SBR	YN-1	23.0	4.0	OD
LY-1	29.6	10	OD	YN-2	26.0	10	AAO
LY-2	20.8	15	AAO	YN-3	20.2	4.3	OD
YT-1	24.0	25	A AO	YN-4	28.9	12	OD
YT-2	31.1	10	AAO	YN-5	24.5	7.2	AO
GS-1	31.1	20	AAO	YN-6	34.0	10	AAO

[a] total organic carbon content; [b] WWTP activated sludge biotreatment techniques. AAO: anaerobic-anoxic-oxic process, OD: oxidation ditch process, AO: anoxic/oxic process, SBR: sequencing batch reactor process, PASF: phosphorus and nitrogen removal combined with active sludge and biofilm process, CAST: cyclic activated sludge technology. PASF and CAST were treated as analogous AAO and SBR processes respectively in the statistics analysis.

Table S2. Measured accurate mass and corresponding theoretical mass of the molecular ion and characteristic fragmentation ion for each PFAES analogues in the sludge samples by liquid chromatography-high resolution mass spectrometry.

Acronym	Accurate mass (m/z)	Theoretical mass (m/z)	Error (ppm)
Chlorinated polyfluoroalkyl ether sulfonates (Cl-PFAESs):			
Molecular Ion [M(^{35}Cl)]$^-$:			
4:2 Cl-PFAES	N.D.[a]	430.90197	N.D.
6:2 Cl-PFAES	530.89557	530.89558	-0.021
8:2 Cl-PFAES	630.88950	630.88919	0.485
10:2 Cl-PFAES	730.88482	730.88281	2.754
Characteristic Fragmentation Ion [M(^{35}Cl)-$C_2F_4SO_3$]$^-$:			
4:2 Cl-PFAES	N.D.	250.95154	N.D.
6:2 Cl-PFAES	350.94516	350.94515	0.017
8:2 Cl-PFAES	450.93869	450.93877	-0.171
10:2 Cl-PFAES	550.93250	550.93238	0.218
Perfluoroalkyl ether sulfonates (F-PFAESs):			
Molecular Ion [M]$^-$:			
4:2 F-PFAES	N.D.	414.93152	N.D.
6:2 F-PFAES	N.D.	514.92513	N.D.
8:2 F-PFAES	N.D.	614.91874	N.D.
10:2 F-PFAES	N.D.	714.91236	N.D.
Characteristic Fragmentation Ion [M-$C_2F_4SO_3$]$^-$:			
4:2 F-PFAES	N.D.	234.98109	N.D.
6:2 F-PFAES	N.D.	334.97470	N.D.
8:2 F-PFAES	N.D.	434.96832	N.D.
10:2 F-PFAES	N.D.	534.96193	N.D.

[a] N.D.: Not detected.

Table S3. LC-MS/MS instrument parameters and method quantification limits for the separation and quantification of the target analytes.

Instrument:	Ultimate 3000 ultrahigh performance liquid chromatography (Thermo Fisher Scientific Inc., Waltham, MA) with API 5500 triple-quadrupole mass spectrometer (AB SCIEX Inc., Framingham, MA) equipped with an electrospray source. The mass spectrometer was operated in the negative ion multiple reaction-monitoring mode.
Analytical Column:	ACQUITY BEH C18 analytical column (2.1 mm i.d. × 100 mm length, 1.7 um, Agilent)
Column Temperature:	35°C
Mobile Phases:	A: 1mM NH_4Ac in methanol B: 1mM NH_4Ac in H_2O

Gradient Profile:

Time (min)	Percentage A (%)	Percentage B (%)	Flow Rate (mL/min)
0.0	60	40	0.30
1.0	60	40	0.30
6.0	100	0	0.30
8.0	100	0	0.30
8.1	60	40	0.30
13.0	60	40	0.30

Injection Volume:	5μL

Monitored Ion Transitions:

Analytes	Ion Transitions[a]	Declustering Potential (V)	Collision Energy (V)	Surrogate Standard	MQL[b] (pg/g dw)	IDL[c] (pg/mL)
<u>Perfluoroalkyl sulfonates (PFSAs):</u>						
PFBS	299>99	-63	-64			
	299>80 (*)	-63	-37	MPFHxS	135	13
PFHxS	399>99	-59	-90			
	399>80 (*)	-59	-76	MPFHxS	125	7.6
PFHpS	449>99	-88	-110			
	449>80 (*)	-88	-83	MPFOS	143	13
PFOS	499>99	-60	-117			
	499>80 (*)	-60	-95	MPFOS	127	8.1
PFDS	599>99	-62	-141			
	599>80 (*)	-62	-127	MPFOS	101	8.0
<u>Chlorinated polyfluoroalkyl ether sulfonates (Cl-PFAESs):</u>						
6:2 Cl-PFAES	531>83	-67	-65			
	531>351 (*)	-67	-37	MPFOS	28.2	3.6

	8:2 Cl-PFAES	631>83	-94	-93			
		631>451 (*)	-94	-43	MPFOS	65.2	5.2
	Fluorotelomer sulfonates (FTSs):						
	4:2 FTS	327>81	-103	-53			
		327>307 (*)	-103	-29	M2-6:2 FTS	43.2	3.8
	6:2 FTS	427>81	-129	-77			
		427>407 (*)	-129	-31	M2-6:2 FTS	25.2	3.0
	8:2 FTS	527>81	-129	-95			
		527>507 (*)	-129	-39	M2-6:2 FTS	31.6	7.2
	Isotope-labeled internal standards:						
	MPFHxS	403>99	-59	-90			
		403>80 (*)	-59	-76	--	--	--
	MPFOS	503>99	-60	-117			
		503>80 (*)	-60	-95	--	--	--
	M2-6:2 FTS	429>81	-129	-77			
		429>409 (*)	-129	-31	--	--	--
	M3PFHxS	402 >99	-59	-90			
		402 >80 (*)	-59	-76	--	--	--
	M8PFOS	507>99	-60	-117			
		507>80 (*)	-60	-95	--	--	--
	[a] The symbol (*) represents the quantitative ion transition of the corresponding analyte. [b] MQL: Method quantification limits, calculated as the signal/noise ratio at 10 for each analyte. [c] IDL: Instrument detection limits, calculated as the signal/noise ratio at 3 for each analyte at 100 pg/mL spiked level.						
LC/MS/MS Analog Parameters:	Ion Spray Voltage: -4500 V; Curtain Gas: 40 psi; Collision Gas: 7 psi; Temperature: 500°C; Ion Source Gas 1: 45 psi; Ion Source Gas 2: 50 psi						

Table S4. Details of the "fragment constant" methodology in the KOWWIN model (PFOS as negative sulfonate ion), which clearly illustrate the contributions of the functional groups in the molecular structure to the total hydrophobic property of the target chemical.

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFFICIENT	VALUE
Frag	8	C [aliphatic carbon - No H, not tert]	0.9723	7.7784
Frag	17	-F [fluorine, aliphatic attach]	-0.0031	-0.0527
Frag	1	-SO2-O [sulfonate, aliph att]	-0.7250	-0.7250
Frag	1	SO-C(polyhalo) structure correction	1.4790	1.4790
Frag	1	S-O-{Na,K,Li}, [coef*(1+0.3*(NUM-1))]	-4.5800	-4.5800
Frag	6	-CF2(-CF2)(-CF2) (linear -CF2- core)	-0.2970	-1.7820
Const		Equation Constant		0.2290
			Log Kow:	**2.3467**

Table S5. Details of the "fragment constant" methodology in the KOWWIN model (6:2 Cl-PFAES as negative sulfonate ion), which clearly illustrate the contributions of the functional groups in the molecular structure to the total hydrophobic property of the target chemical.

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFFICIENT	VALUE
Frag	8	C [aliphatic carbon - No H, not tert]	0.9723	7.7784
Frag	1	-O- [oxygen, aliphatic attach]	-1.2566	-1.2566
Frag	1	-CL[chlorine, aliphatic attach]	0.3102	0.3102
Frag	16	-F [fluorine, aliphatic attach]	-0.0031	-0.0496
Frag	1	-SO2-O [sulfonate, aliph att]	-0.7250	-0.7250
Factor	1	SO-C(polyhalo) structure correction	1.4790	1.4790
Factor	2	-O-C(F)For-S-C(F)Fcorrection	0.5500	1.1000
Frag	1	S-O-{Na,K,Li}, [coef*(1+0.3*(NUM-1))]	-4.5800	-4.5800
Factor	4	-CF2(-CF2)(-CF2) (linear -CF2- core)	-0.2970	-1.1880
Const		Equation Constant		0.2290
			Log Kow:	**3.0974**

Figure S1. Sampling locations, spatial concentrations and composition profiles of Cl-PFAES homologues in municipal sewage sludge samples in China (mean concentration values of each analyte were present for WWTP samples collected in the same provinces/municipalities).

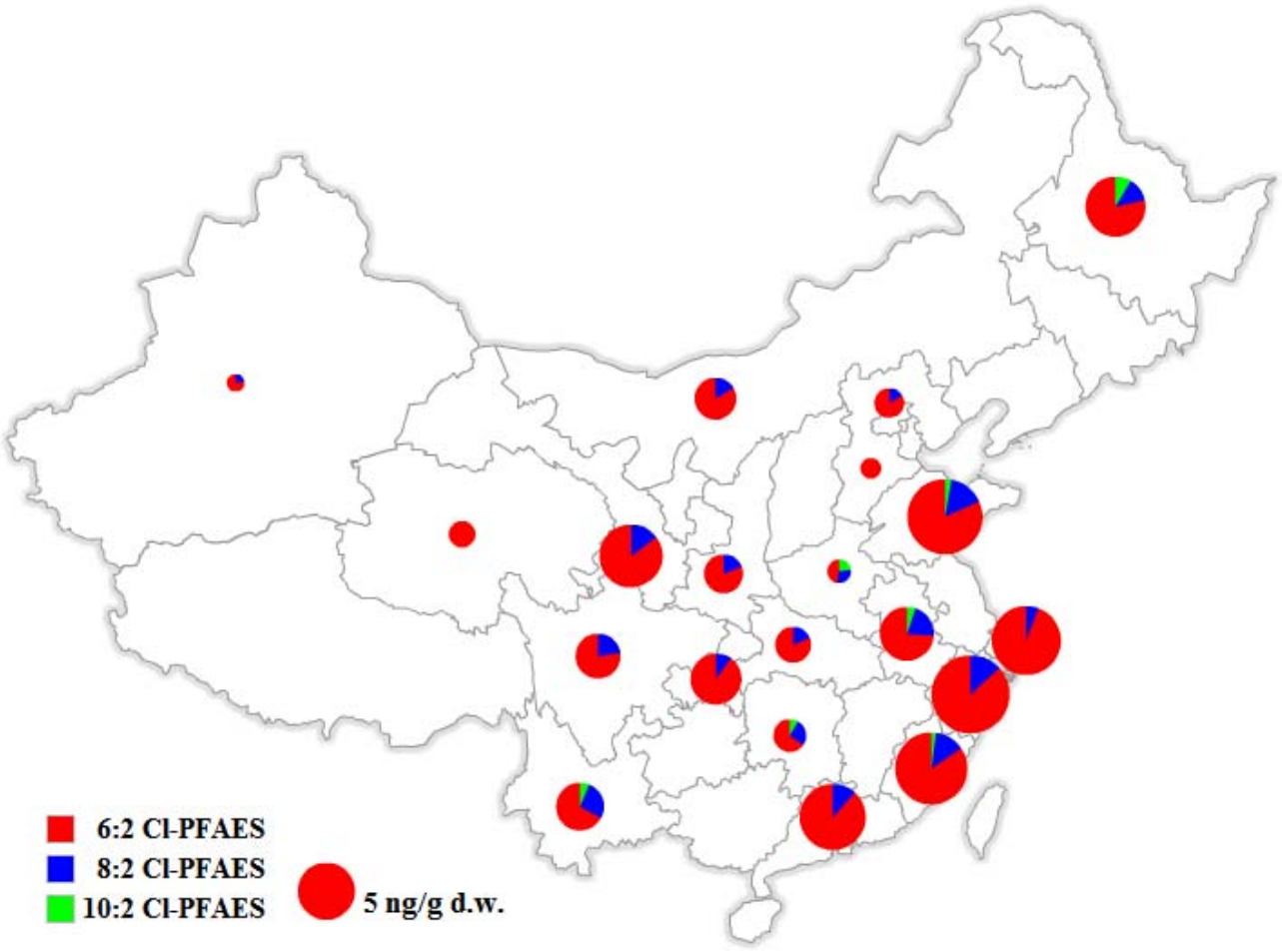


Figure S2. MS^2 characteristic fragmentation ions of the 6:2 Cl-PFAES standard in the HCD mode (collision energy: 35%, spiked at 20 ng/mL).

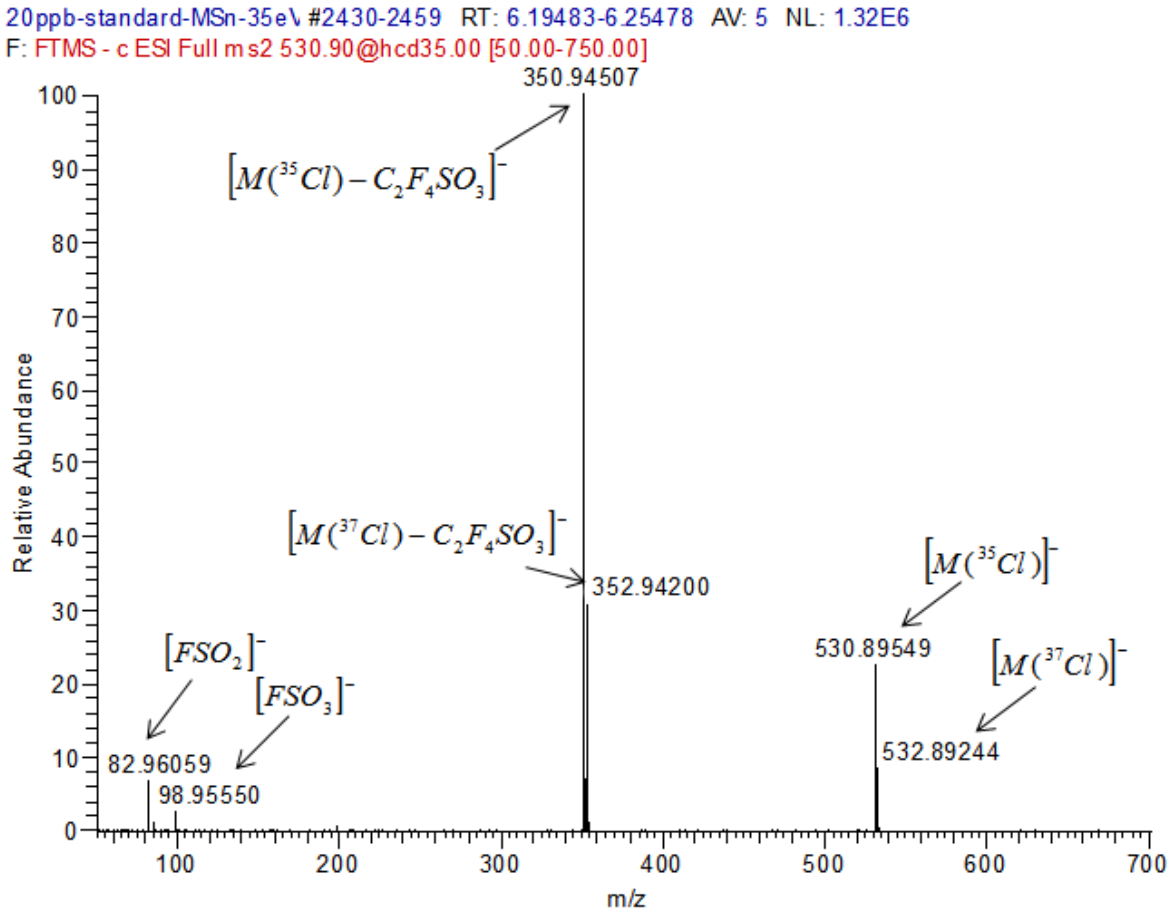


Figure S3. Instrument responses (n = 3) of $[M(^{35}Cl)]^-$ molecular ions for 6:2 and 8:2 Cl-PFAESs in the HRMS full scan spectrum at spiked calibration concentrations (0.1−10 ng/mL).

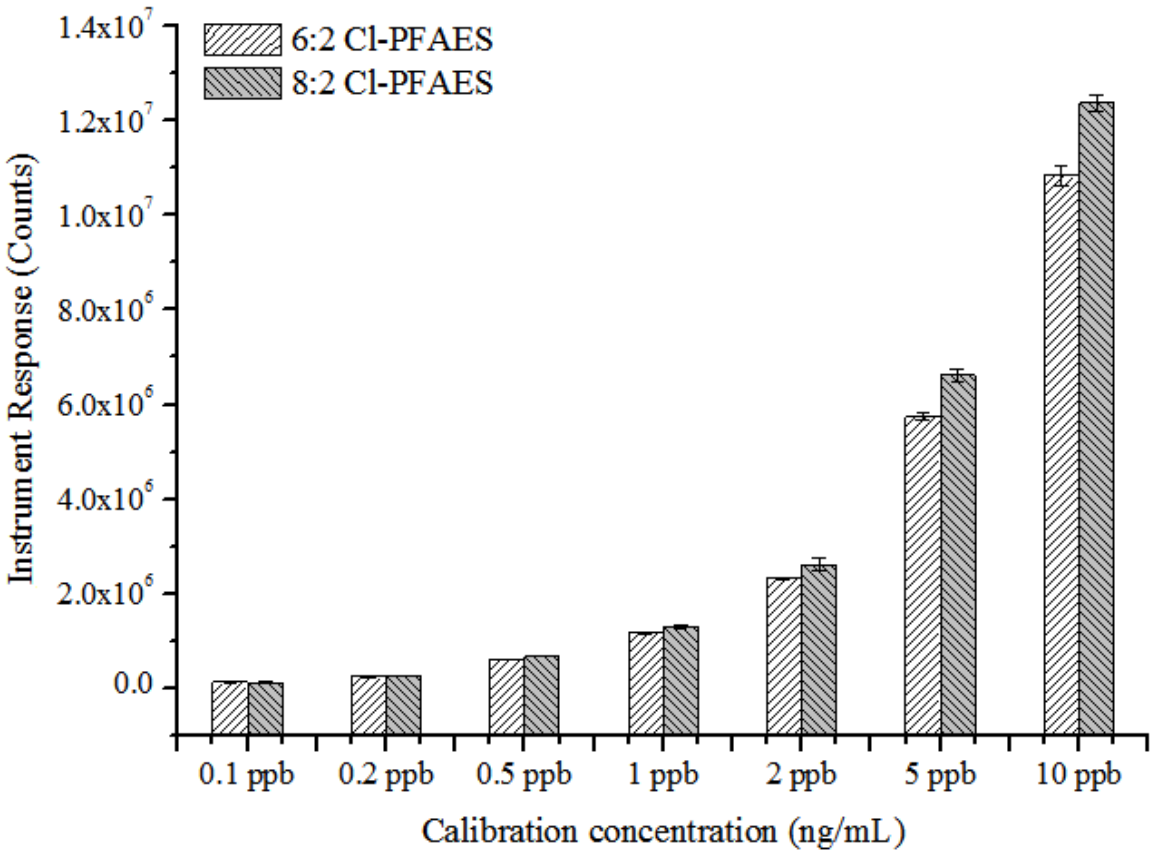


Figure S4. The score plots (PC1 versus PC2) of fluoroalkyl sulfonate concentrations (log-transformed data of PFOS, 6:2 FTS, 8:2 FTS, 6:2 Cl-PFAES and 8:2 Cl-PFAES, analytes with detection ratio less than 70% in the sludge samples were not taken into account) in the sewage sludge samples grouped by WWTP treatment techniques (AAO, anaerobic-anoxic-oxic; AO, anoxic/oxic; OD, oxidation ditch; SBR, sequencing batch reactor) for further visualization of potential relationships from the principal component analysis (varimax rotation method, statistical significant level set as $p < 0.05$).

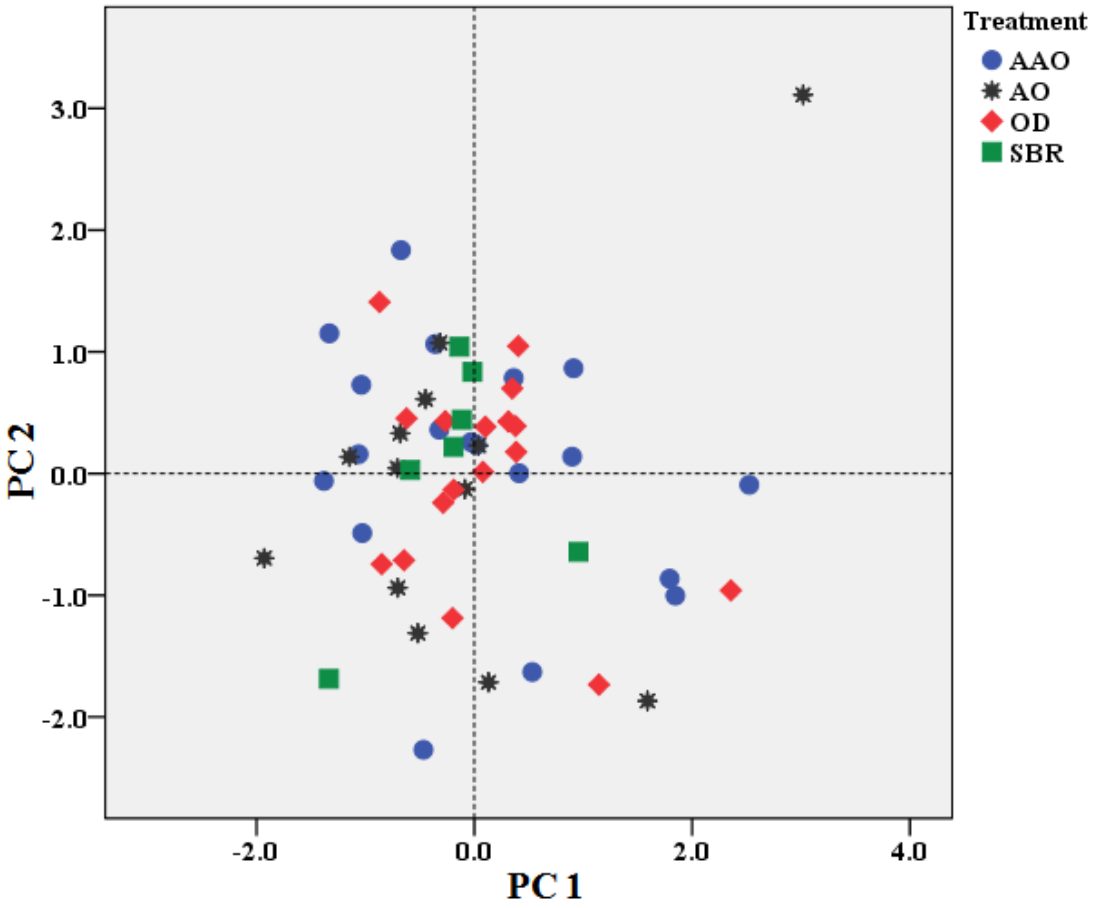


Figure S5. Separation and collection of the eluate fractions of the 6:2 and 8:2 Cl-PFAES in the Autopurification system. (A). Retention behavior of 8:2 Cl-PFAES in the LC column. (B). Retention behavior of 6:2 Cl-PFAES in the LC column. (C). Fraction collection window for each analyte in the purification cycles.

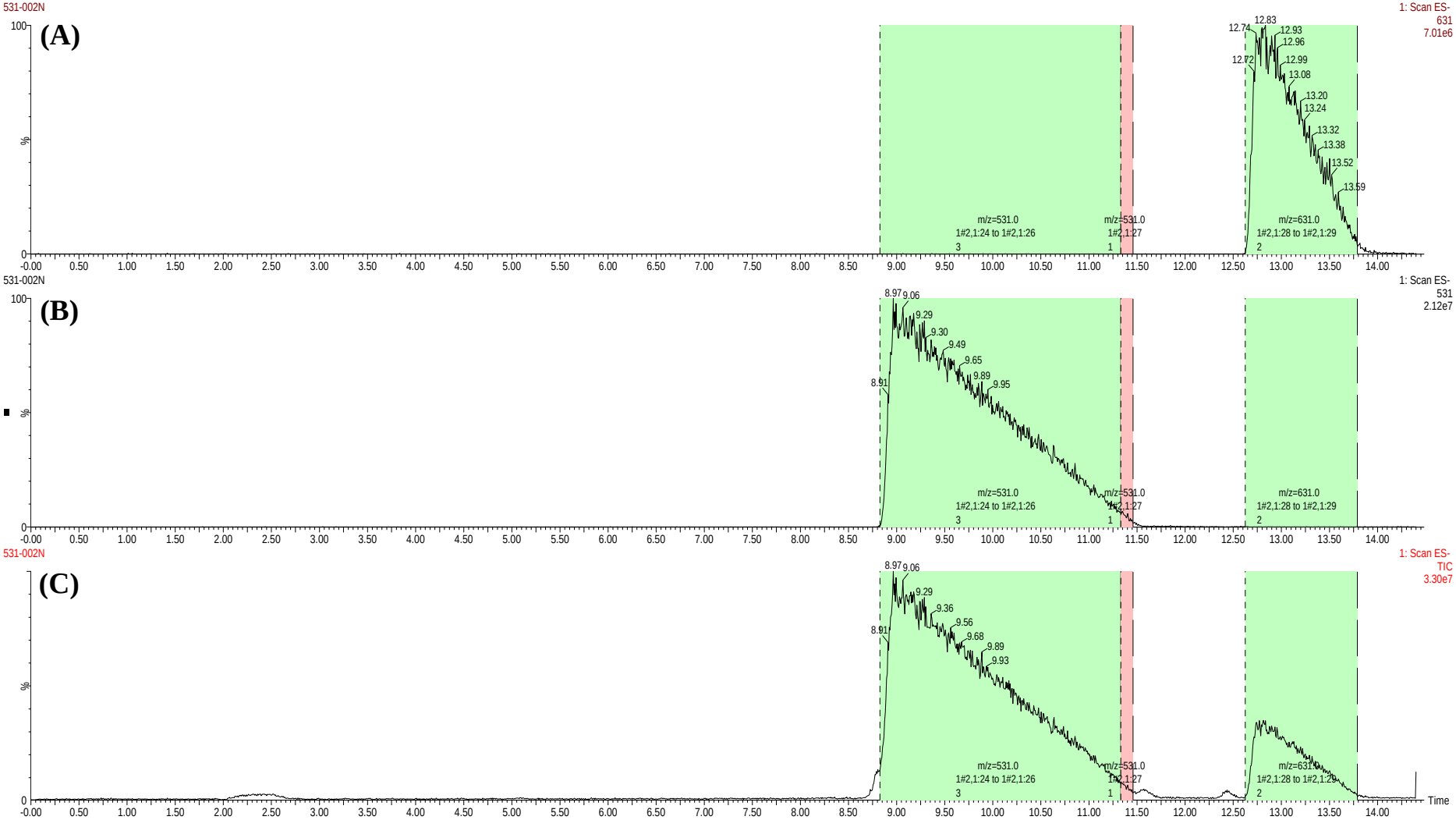
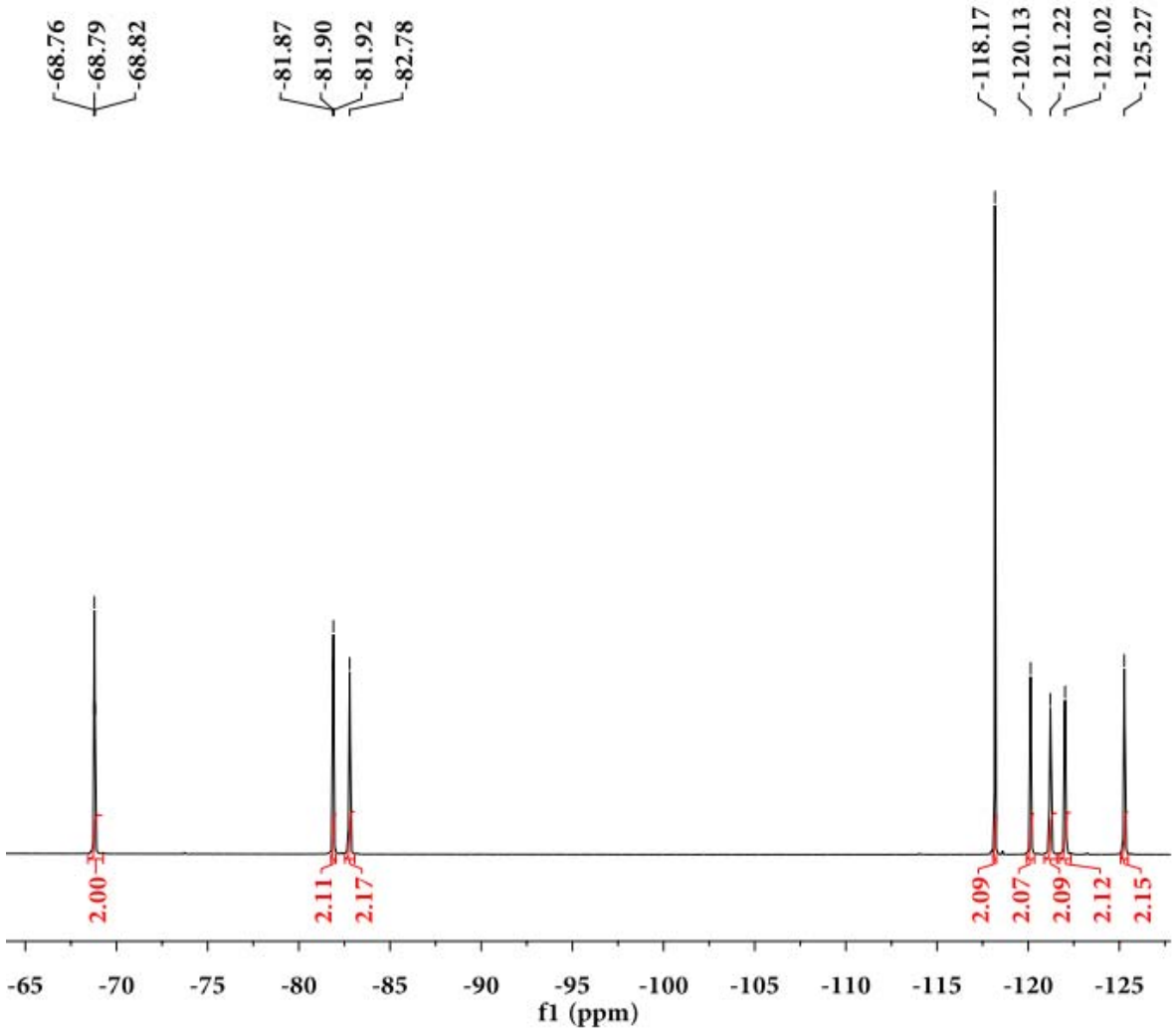


Figure S6. Detailed ^{19}F-NMR result of the laboratory-purified 6:2 Cl-PFAES standard.

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D12            0.00002000 sec
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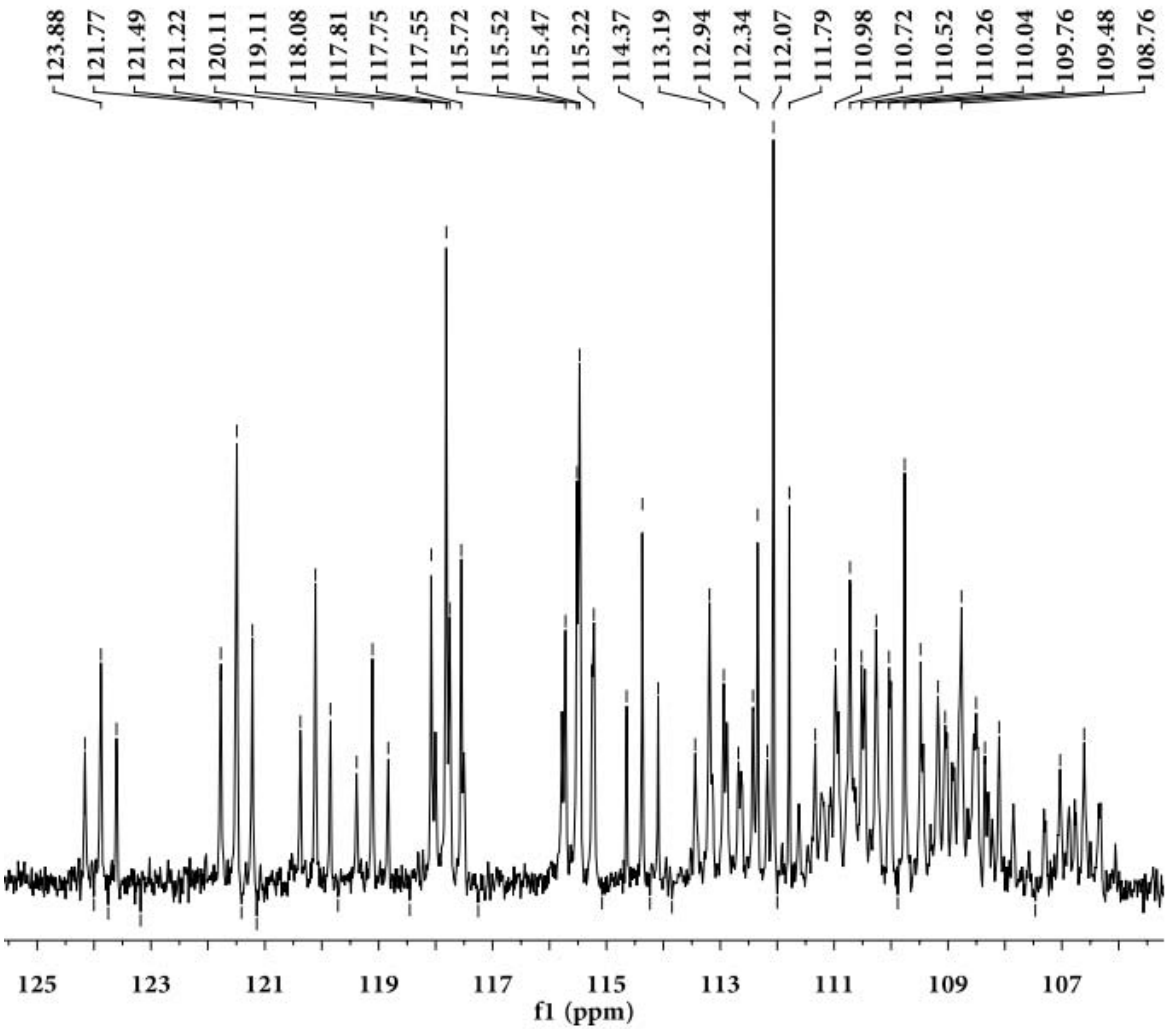
Figure S7. Detailed ^{12}C-NMR result of the laboratory-purified 6:2 Cl-PFAES standard.

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PLW13         0.09772400 W

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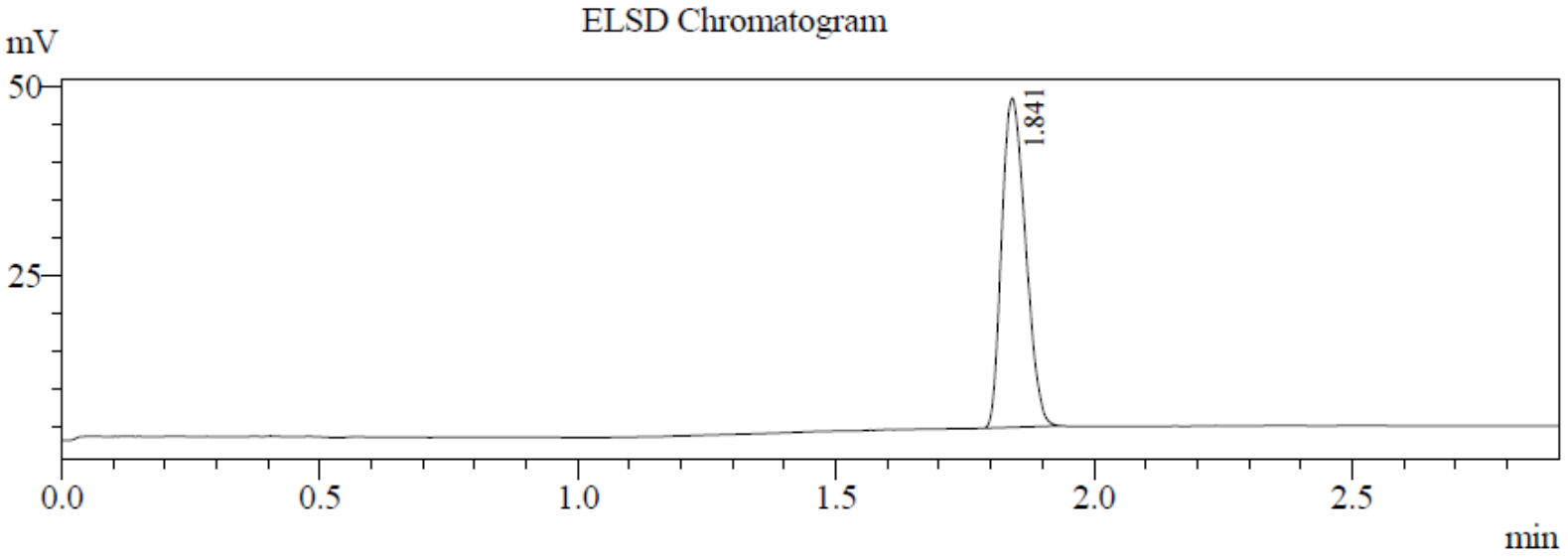
Figure S8. Detailed information on the purity of the 6:2 Cl-PFAES standard based on the LC-ELSD method.

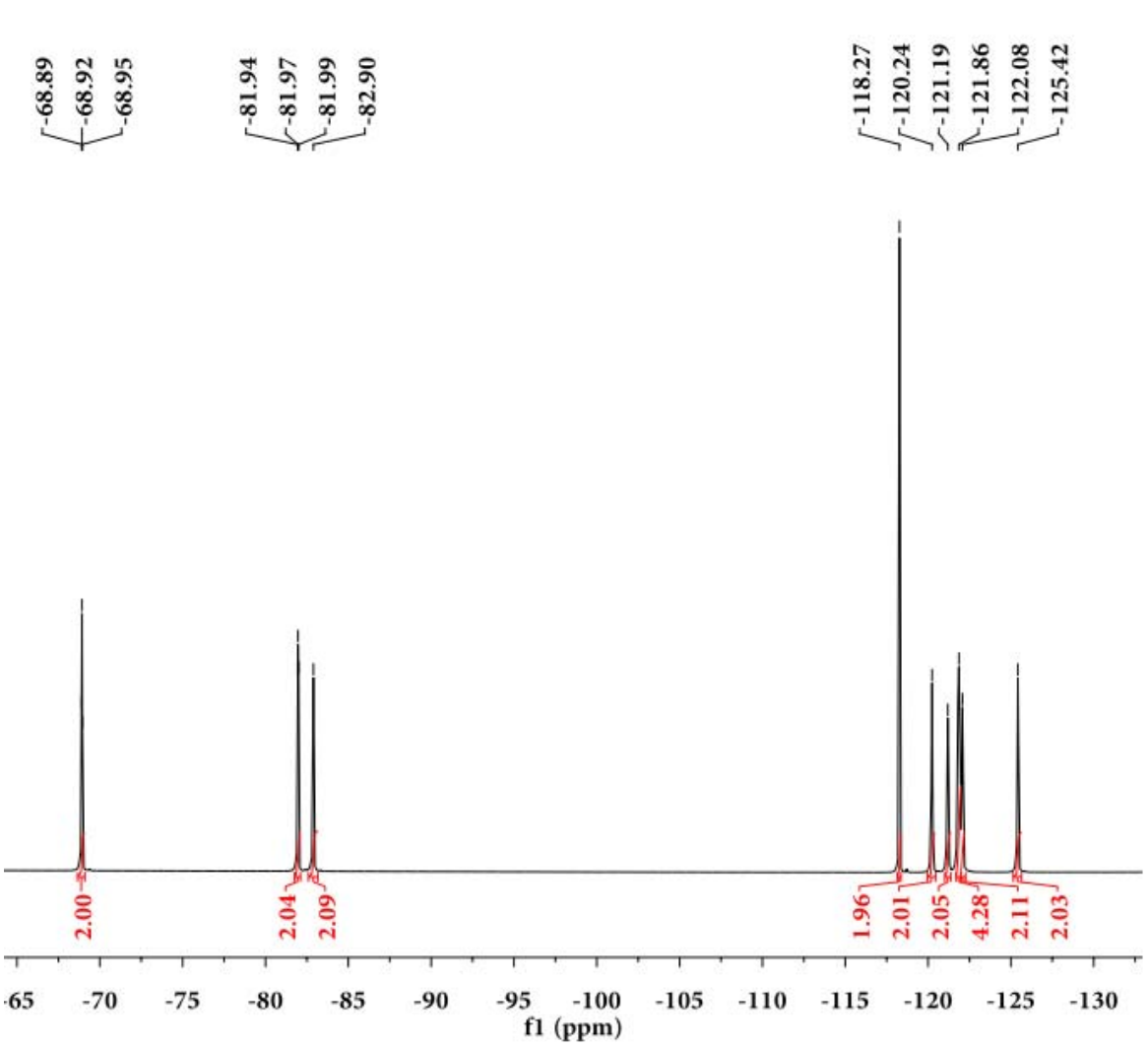


PeakTable

ELSD Ch1

Peak#	Ret. Time	Height	Height %	Area	Area %
1	1.84	42853	100.00	139980	100.00
Total		42853	100.00	139980	100.00

Figure S9. Detailed ^{19}F-NMR result of thelaboratory-purified 8:2 Cl-PFAES standard.

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D11             0.03000000 sec
D12             0.00002000 sec
TD0                      1

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P1                   12.50 usec
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NUC2                   1H
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PCPD2                70.00 usec
PLW2           9.77239990 W
PLW12          0.19944000 W

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PC                   1.00
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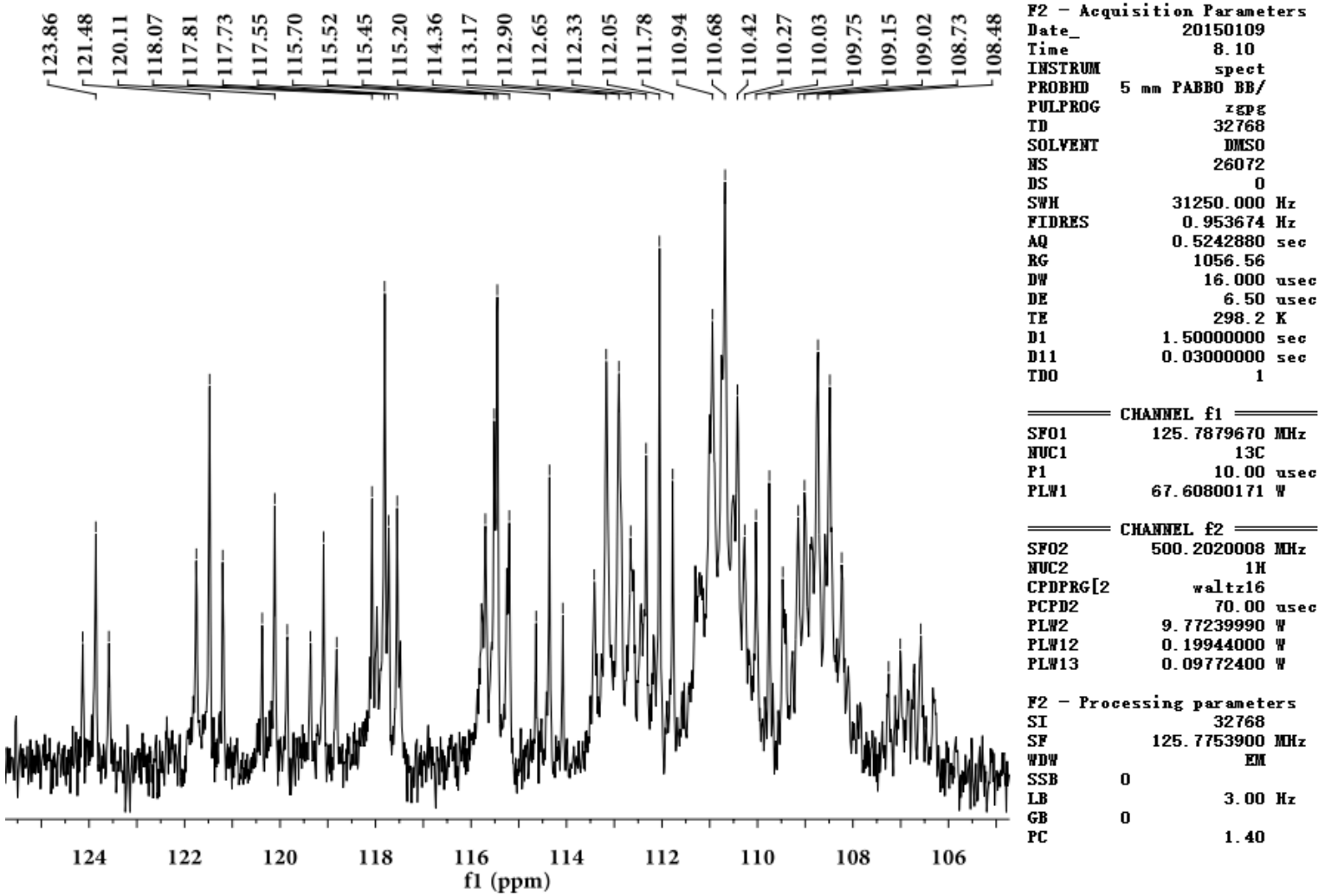
Figure S10. Detailed ^{12}C-NMRresult of the laboratory-purified 8:2 Cl-PFAES standard.



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Date_	20150109	
Time	8.10	
INSTRUM	spect	
PROBHD	5 mm PABBO BB/	
PULPROG	zgpg	
TD	32768	
SOLVENT	DMSO	
NS	26072	
DS	0	
SWH	31250.000	Hz
FIDRES	0.953674	Hz
AQ	0.5242880	sec
RG	1056.56	
DW	16.000	usec
DE	6.50	usec
TE	298.2	K
D1	1.50000000	sec
D11	0.03000000	sec
TD0	1	

CHANNEL f1

SFO1	125.7879670	MHz
NUC1	13C	
P1	10.00	usec
PLW1	67.60800171	W

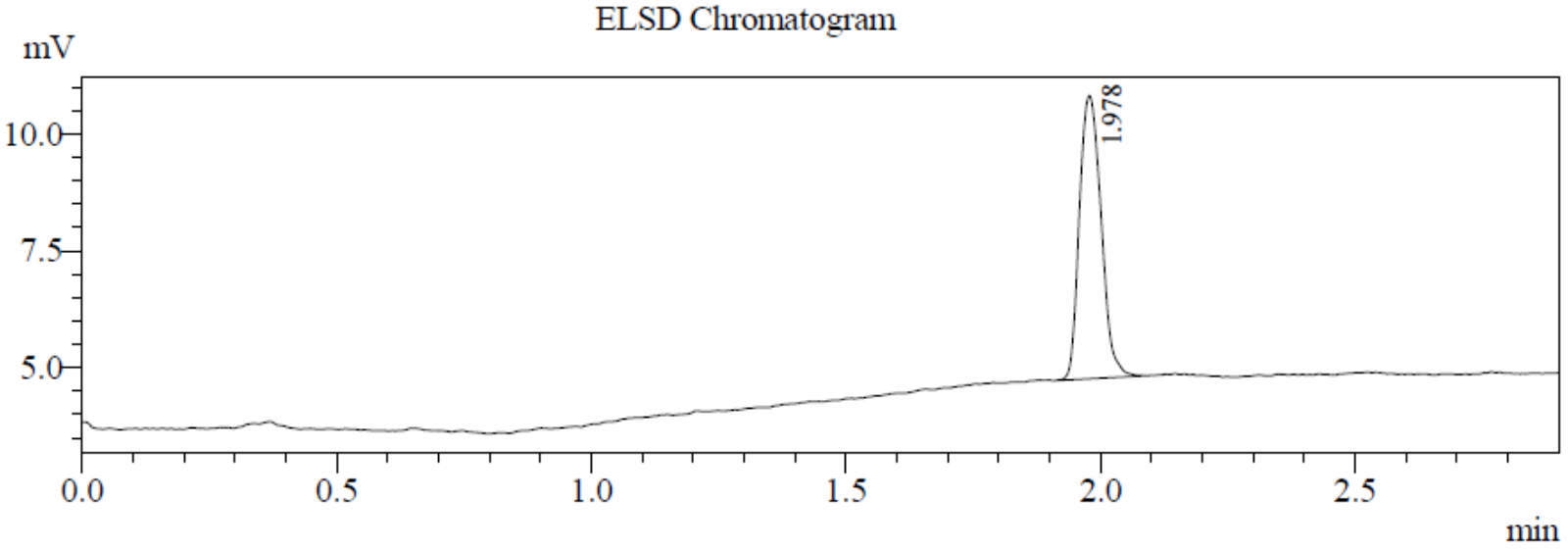
CHANNEL f2

SFO2	500.2020008	MHz
NUC2	1H	
CPDPRG[2	waltz16	
PCPD2	70.00	usec
PLW2	9.77239990	W
PLW12	0.19944000	W
PLW13	0.09772400	W

F2 - Processing parameters

SI	32768	
SF	125.7753900	MHz
WDW	EM	
SSB	0	
LB	3.00	Hz
GB	0	
PC	1.40	

Figure S11. Detailed information on the purity of the 8:2 Cl-PFAES standard based on the LC-ELSD method.



PeakTable

ELSD Ch1

Peak#	Ret. Time	Height	Height %	Area	Area %
1	1.98	5999	100.00	18003	100.00
Total		5999	100.00	18003	100.00