Per- and Polyfluoroalkyl Substances and Fluorinated Alternatives in Urine and Serum by On-line Solid Phase Extraction–Liquid Chromatography–Tandem Mass Spectrometry

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Supporting Information

On-line SPE-HPLC-MS/MS for the quantification of PFBS, C6-C11 PFAS, and fluorinated alternatives in serum

The SPE run of each sample starts with the conditioning of a HySphere C8-SE cartridge with HPLC-grade acetonitrile (2 mL) and 0.1 M formic acid (2 mL). Afterward, 500 µL of the sample (containing 50 µL serum) injected into the 1 mL sample loop is loaded onto the SPE column using 2 mL 0.1 M formic acid with 1 mL/min flow rate. Next, the SPE column is washed with 2 mL 90% 0.1 M formic acid/10% Acetonitrile. The time of the SPE cleanup (including injection time) is 10 min long. Before starting the clean up of the next sample, the cartridge containing the extracted analytes is transferred by a robotic gripper from the left clamp into the right clamp. Therefore, while the right clamp is used for analyte elution and HPLC-MS/MS acquisition, the left clamp could be used for the clean up of the next sample. Once, the SPE column is in the right clamp, the right clamp valve remains in by-pass (1-2) position until the HPLC-MS/MS system becomes ready to begin acquisition. At the beginning of the HPLC-MS/MS acquisition, for the first 10 min of the HPLC gradient program to transfer the analytes from the SPE column to the HPLC column. At 10 min, the SPE column is returned to the cartridge tray while the HPLC gradient program continues. The HPLC pump is operated at a 1000 µL/min flow rate with 95% of 20 mM ammonium acetate (pH 4) and 5% of acetonitrile as mobile phase A and 100% acetonitrile as mobile phase B. The analytes are separated from each other and other extracted components on two Chromolith® HighResolution RP-18e columns (4.6 × 100 mm) preceeded by a Chromolith® HighResolution RP-18e (5 X4.6 mm) guard column and a Chromolith® HighResolution RP-18e (4.6 × 25 mm)

column. To delay the elution of the PFAS contaminants leaching out from Teflon parts of the HPLC pump, a 4.6 mm x 25 mm Chromolith® HighResolution RP-18e column is inserted between the HPLC pump and the right clamp valve. Because contaminants have to go through twice the column length, their peaks elute 1 min after the main analytes bands without interfering with the measured concentration.

Table S1. HPLC Conditions

Parameters	Setting
Mobile Phase A	95% 20 mM ammonium acetate, pH = 4/5% acetonitrile
Mobile Phase B	100% acetonitrile
Flow rate	1000 µL/min

Table S2. Mobile phase gradient for the quantification of PFBS, C_6-C_{11} PFAS, and fluorinated alternatives in serum

Time (min)	0	1	2	8.5	8.51	12	12.1	13	13.2
Mobile phase B%	25	25	45	49	60	60	80	80	80
Flow rate (mL/min)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Time (min)	13.3	13.5	13.6	15.5	15.6	16.5	16.6	18	18.1
Mobile phase B%	80	95	95	95	95	95	25	25	25
Flow rate (mL/min)	1.5	1.5	1.8	1.8	2.0	2.0	1.5	1.5	1.0

Table S3. Mobile phase gradient for the quantification of PFAS and fluorinated alternatives in urine

Time (min)	0	1	8	8.1	12	16	16.1	18
Mobile phase B%	1	1	60	60	85	85	1	1
Flow rate (mL/min)	1.0	1.0	1.0	1.0	1.0	1.0	1.5	1.5

Table S4. Mass spectrometry-related parameters for the quantification of PFAS and fluorinated alternatives

	(M-H)- Precursor ion (m/z)	Product ion (m/z)	DP[1] (volts)	CE[2] (volts)
PFPrS[3]	249	99	-65	-75
PFBS	299	99	-70	-80
$^{13}C_3$-PFBS (IS)	302	99	-70	-80
PFHxS	399	99	-70	-80
$^{18}O_2$-PFHxS (IS)	403	103	-70	-80
PFHpS[4]	449	99	-35	-60
n-PFOS	499	99	-70	-90
$^{13}C_4$-PFOS_1 (IS)	503	80	-70	-85
$^{13}C_4$-PFOS_2 (IS)	503	99	-70	-85
Sm-PFOS[5-6]	499	80	-70	-90
PFBA	213	169	-10	-40
$^{13}C_4$-PFBA (IS)	217	172	-10	-40
PFPeA	263	219	-12	-22
$^{13}C_5$-PFPeA (IS)	268	223	-12	-22
PFHxA	313	269	-13	-25
$^{13}C_2$-PFHxA (IS)	315	270	-13	-25
PFHpA	363	319	-25	-13
$^{13}C_5$-PFHpA (IS)	368	323	-25	-13

n-PFOA	413	369	-27	-14
Sb-PFOA[7-8)]	413	369	-27	-14
$^{13}C_4$-PFOA (IS)	417	372	-30-	-15
PFNA	463	419	-30	-13
$^{13}C_5$-PFNA (IS)	468	423	-30	-13
PFDA	513	469	--30	-15
$^{13}C_2$-PFDA (IS)	515	470	-30	-15
PFUnDA	563	519	-30	-17
$^{13}C_2$-PFUnDA (IS)	565	520	-30	-17
HFPO-DA	329	285	-9	-5
$^{13}C_3$-HFPO-DA (IS)	332	287	-9	-5
DONA[9)]	377	251	-20	-10
9Cl-PF3ONS[10)]	531	351	-40	-25
FOSA	498	78	-60	-85
$^{18}O_2$-FOSA (IS)	503	82	-60	-85
MeFOSAA	570	512	-45	-30
D_3-MeFOSAA (IS)	573	515	-45	-30
EtFOSAA	584	526	-45	-30
D_5-EtFOSAA (IS)	589	531	-45	-30

1) DP: Declustering Potential

2) CE: Collision Energy

3) internal standard for PFPrS: $^{13}C_3$-PFBS

4) internal standard for PFHpS: $^{13}C_4$-PFOS-1

5) Sm-PFOS: perfluoro-5-methylheptane sulfonate as mixuture of branched isomers

6) internal standard for Sm-PFOS: $^{13}C_4$-PFOS-2

7) Sb-PFOA: perfluoro-5-methylheptanonate as mixuture of branched isomers

8) internal standard for Sb-PFOA: $^{13}C_4$-PFOA

9) internal standard for DONA: $^{13}C_2$-PFHxA

10) internal standard for 9Cl-PF3ONS: $^{13}C_2$-PFUnDA

Table S5. Accuracy, sensitivity, and precision data for the quantification of PFBA and PFPeA in serum using the conditions developed for the urine method.

	LOD	QCL		QCH		Accuracy (%)	
	ng/mL	ng/mL	RSD(%)	ng/mL	RSD(%)	Low	High
PFBA	0.1	2.5	8.6	6.5	5.2	98.6±3	99.7±2
PFPeA	0.1	2.6	7.3	6.4	4.8	97.4±4	98.6±1

Accuracy: n=5

Limit of detection (LOD): n=5

Intraday precision: n=20

Figure S1. The Symbiosis system for concurrent SPE/HPLC mode

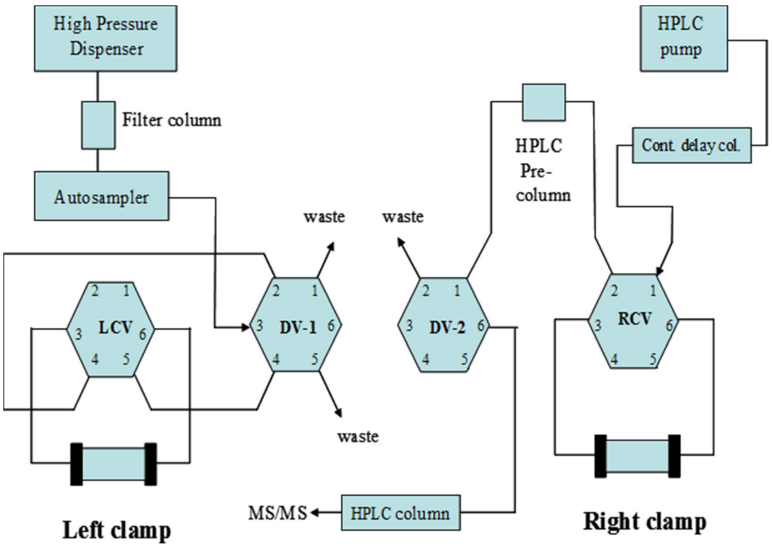
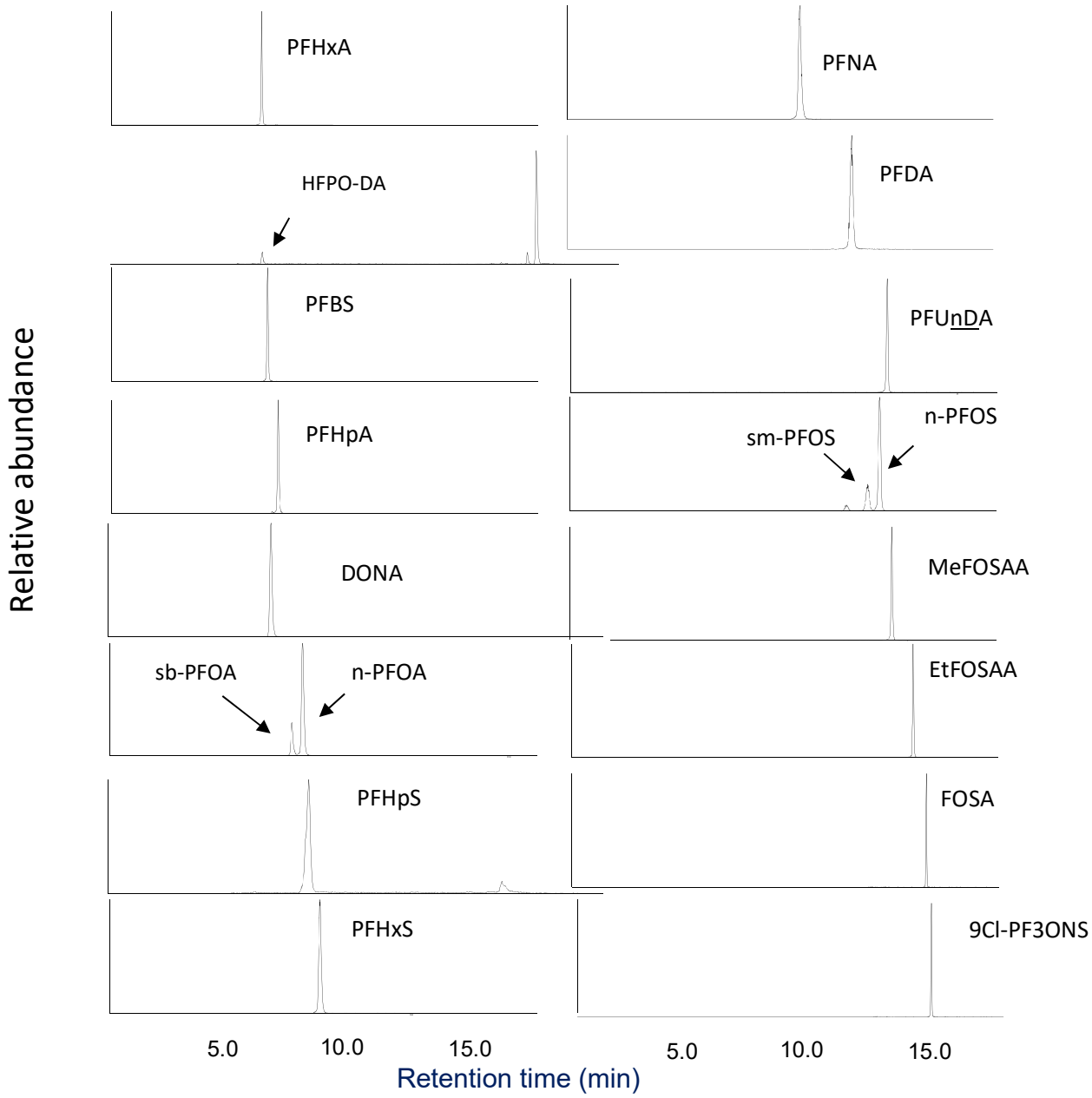
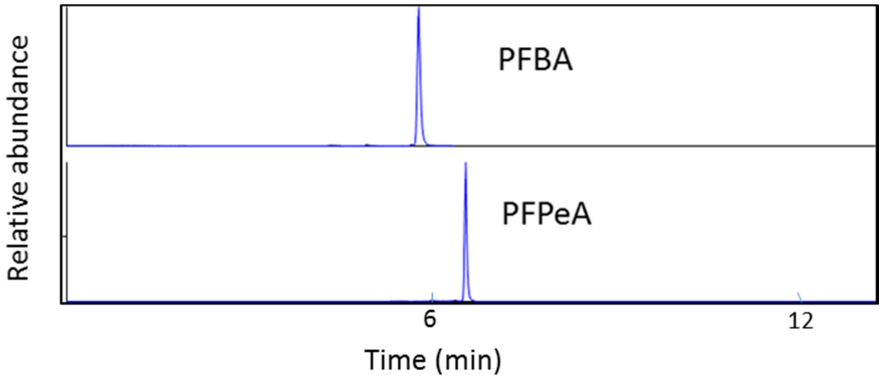


Figure S2. Chromatograms of a serum QC material with concentrations ~5 – 10 ng/mL, depending on the analyte.[a]



[a]Quantification obtained using the analytical approach described for the analyses of NHANES 2013–2014 serum samples (https://www.cdc.gov/nchs/data/nhanes/nhanes_13_14/PFAS_H_MET.pdf)

Figure S3. Chromatogram for select PFBA and PFPeA in a serum QC material (~6.5 ng/mL) using the conditions of the developed urine method.



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