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SUPPLEMENTARY MATERIAL TO

Temporal trend of perfluorinated compounds in untreated wastewater and surface water in the middle part of the Danube River belonging to the northern part of Serbia

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SAMPLING SITES AND SAMPLE COLLECTION

Sampling was carried out during November and at the beginning of December 2014. A qualified and well-trained person, from the public water management company "Vode Vojvodine" Novi Sad, collected the wastewater samples and surface water samples from the Danube River.[21] The wastewater discharge point is located on the bank of the Danube flow between Novi Sad (the second largest town in Serbia) and Belgrade (the capital of Serbia). It has been used to discharge the municipality wastewater, collected from four towns (Inđija, Stara Pazova, Nova Pazova and Batajnica, with approximately 150000 inhabitants in total) and from small villages located in that area, without any pre-treatment directly into the Danube River (Fig. S-1).

Surface water was sampled from a boat with a 1 L polyethylene (PE) bottle previously washed with methanol¸ ultra-pure water and with the sample. The bottles containing water samples were kept in an ice-box, transported to a laboratory and analyzed immediately after receiving. Field blanks were collected by filling the laboratory water (Milli-Q) from the field blank bottle into the sampling device, letting it stand for 5 min, and then transferring this water back to the field blank bottle.

CHEMICALS AND STANDARDS

In the present study the following surrogate standards (SS): perfluoro-n--[1,2,3,4,5-$^{13}C_5$] pentanoic acid (M5PFPeA), perfluoro-n-[1,2,3,4,6-$^{13}C_5$] hexanoic acid (M5PFHxA), perfluoro-n-[1,2,3,4-$^{13}C_4$]-heptanoic acid (M4PFHpA),

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perfluoro-n-[$^{13}C_8$]-octanoic acid (M8PFOA), perfluoro-n-[$^{13}C_9$]-nonanoic acid (M9PFNA), perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]-decanoic acid (M6PFDA), perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]-undecanoic acid (M7PFUnA), sodium perfluoro-1 [1,2,3-$^{13}C_3$]-hexane sulfonate (M3PFHxS); and nine internal standards (IS): perfluoro-n-[1,2,3,4-$^{13}C_4$]-butanoic acid ([$^{13}C_4$]-PFBA), perfluoro-n-[1,2-$^{13}C_2$]-hexanoic acid ([$^{13}C_2$]-PFHxA), perfluoro-n-[1,2,3,4,-$^{13}C_4$]-octanoic acid ([$^{13}C_4$]-PFOA), perfluoro-n-[1,2,3,4,5-$^{13}C_5$]-nonanoic acid ([$^{13}C_5$]-PFNA), perfluoro-n-[1,2-$^{13}C_2$]-decanoic acid ([$^{13}C_2$]-PFDA), perfluoro-n-[1,2-$^{13}C_2$]-undecanoic acid ([$^{13}C_2$]-PFUnA), perfluoro-n-[1,2,$^{13}C_2$]-dodecanoic acid ([$^{13}C_2$]-PFDoA), sodium perfluoro-1-hexane-[$^{18}O_2$]-sulfonate ([$^{18}O_2$]-PFHxS), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]-octane sulfonate ([$^{13}C_4$]-PFOS) obtained from Wellington Laboratories (Guelph, Ontario, Canada) were used.

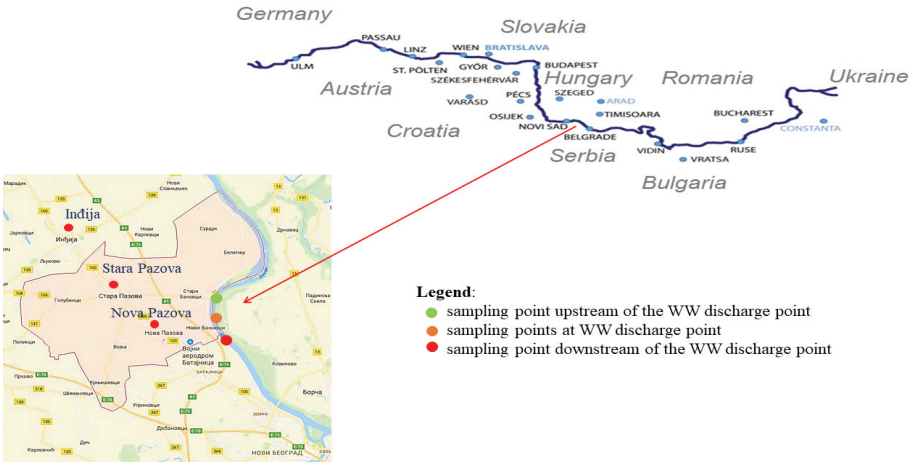


Fig. S-1. Map of sampling locations.

SAMPLE EXTRACTION

Briefly, water samples (500 mL of SW and 300 mL for WW) were filtered through 0.22 μm fiberglass filters (GF/F), previously pre-conditioned with 10 mL of methanol and 10 mL ultra-pure water. Before filtration, the samples were spiked at the 10 ng/L level with surrogate standards (SSs listed in Section S-1 Chemicals and standards). 20 μL of SSs working solutions of 250 ng/mL and 150 ng/mL were used for spiking the SW and WW samples, respectively for achieving a 10 ng/L concentration of SSs in SW and WW. All samples were extracted by SPE with Oasis HLB cartridges. The SPE cartridges were first preconditioned by passing 5 mL of methanol and 10 mL of ultra-pure water at a flow rate of 10 mL/min. The samples were loaded onto SPE cartridges at a flow rate of 10 mL/min. The cartridges were rinsed with 3 mL of ultra-pure water at a flow rate 10 mL/min, and the cartridges were dried for 30 min using nitrogen at

0.6 bar. Elution was performed with 5 mL of methanol at a flow rate 3 mL/min. Evaporation of the extracts to the final volume of 500 µL was performed at a temperature of 35 °C in a gentle nitrogen stream.

INSTRUMENTAL ANALYSIS

The concentrations of PFCs were obtained by Thermo ultra-high-performance liquid chromatography (UHPLC) coupled with a Thermo TSQ Vantage triple quadrupole mass spectrometer (MS/MS). Separation of analytes was achieved using an injection volume of 10 µL of a sample on a Hypersil GOLD^{TM} (50×2.1 mm i.d. 1.9 µm) column (Thermo Fisher Scietific) at 25 °C. A flow rate of 0.35 mL/min was used and the gradient was composed of eluent A containing water/acetic acid (99:1, v/v), and eluent B containing of methanol/acetic acid (99:1, v/v). Eluent A contained 10 mM ammonium acetate, while eluent B contained 5 mM ammonium acetate. The gradient program started with 2 % B for 1 min. Then, the linear gradient was programmed up to 20 % B for 4 min.; to 50 % B for 3 min, to 98 for 2 min and maintained for 2 min. Finally, the gradient was returned to initial conditions 2 % B (0.5 min) and maintained for 2.5 min. in order to re-equilibrate the column. The MS/MS system equipped with HESI was operated in negative ionization mode. The parameters of the ion source were as follows: spray voltage: 3.4 kV, vaporizer temperature: 350 °C, sheath gas pressure: 40 arbitrary units, auxiliary gas pressure: 10 arbitrary units, and capillary temperature: 270 °C. Conditions for target Selected Reaction Monitoring (SRM) analysis are given in Table S-II. Ionization and mass spectrometric conditions were optimized by direct continuous pump infusion of 5 µg/mL standard solution of each tested compound dissolved in the initial mobile phase (98% of A and 2 % of B phase) into the mass spectrometer using a syringe pump at a flow rate of 10-20 µL/min. Data acquisition was performed initially in full scan to determine an abundant precursor ion. Next, the MS/MS fragmentation conditions were investigated and collision energies and S-lens voltage were optimized for all studied compounds and their transitions. Fragmentation reactions were done in SRM by choosing the optimum voltage of collision energies for selected compounds. Ultra high-purity argon (Ar) was used as collision gas. Two product ions were measured for precursor ion: one was used as the quantifier ion (SRM1) and the other was used as the qualifier ion (SRM2). In SRM mode, a mass resolution of 0.7 Da full width at half maximum (FWHM) was set on the first (Q1) and the third (Q3) quadrupole and scan width of 0.5 m/z were used. Instrument control and data collection were handled by computer equipped with Xcalibur 2.1.0 (Thermo Fisher Scientific, USA). For determination of SRM1/SRM2 signal transition ratios were used TraceFinder 3.1 (Thermo Fisher Scientific, USA).

Table S-I. Sampling frequency

Day	Date	Number of samples	Sample label (date+ sampling day + U/D)
WED	12-nov	3	12.11.3.-U*, 12.11.3.-D*, 12.11.14.-WW
THU	20-nov	2	20.11.5-U, 20.11.5-D
FRI	21-nov	3	21.11.6-U, 21.11.6-D, 21.11.14-WW
TUE	25-nov	2	25.11.8-U, 25.11.8-D
WED	26-nov	1	26.11.14.-WW
THU	27-nov	3	27.11.10-U, 27.11.10-D, 27.11.14-WW
FRI	28-nov	1	28.11.14.-WW
TUE	29-nov	3	29.11.12-U, 29.11.12-D, 29.11.14-WW
SAT	30-nov	1	30.11.14.-WW
MON	1-dec	1	01.12.14.-WW

*U -upstream, D - downstream, WW - wastewater

Table S-II. Instrument parameters

TSQ Vantage Triple Quadrupole UHPLC-MS/MS									
Column	Hypersil GOLDTM (50 mm x 2.1 mm i.d., 1.9 μm)								
Column temperature, °C	25								
Mobile phase	A: 10 mM annmonium accetate								
	B: Methanol 5 mM ammonium acetate								
Gradient profile	Time, min	0	1	5	8	10	12	12.5	15
	B mobile phase composition (%)	2	2	20	50	98	98	2	2
Flow rate:	500 μL/min								
Injection volume;	10 μL								
Ion source parameters ionization $ESI^{(-)}$									
Spray voltage:	3.4 kV								
Vaporizer temperature:	350 °C								
Sheath gas pressure:	40 arbitrary units								
Auxiliary gas pressure:	10 arbitrary units								
Capillary temperature:	270 °C								

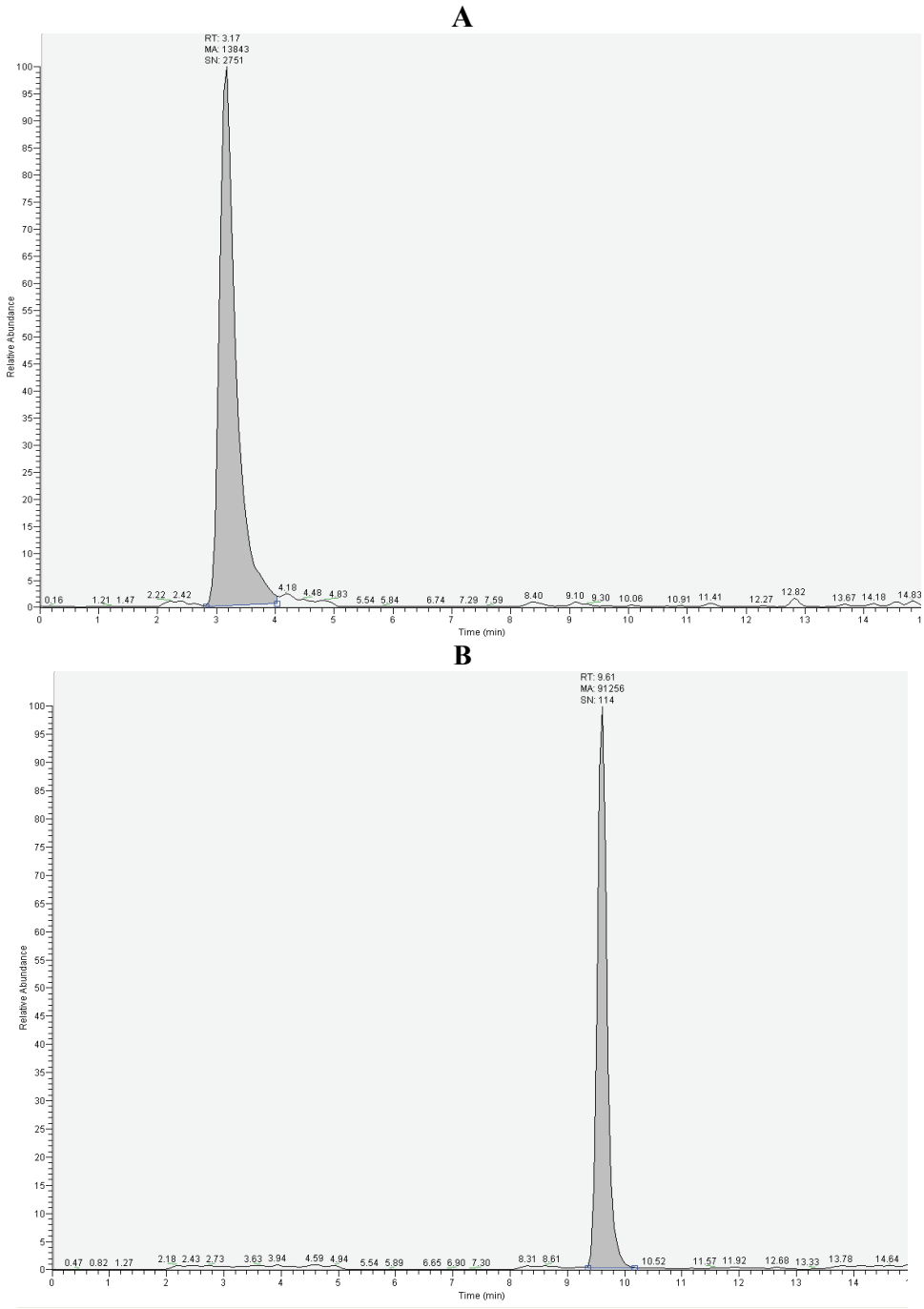


Fig. S-2. Chromatograms A: PFBA, B: PFHxA

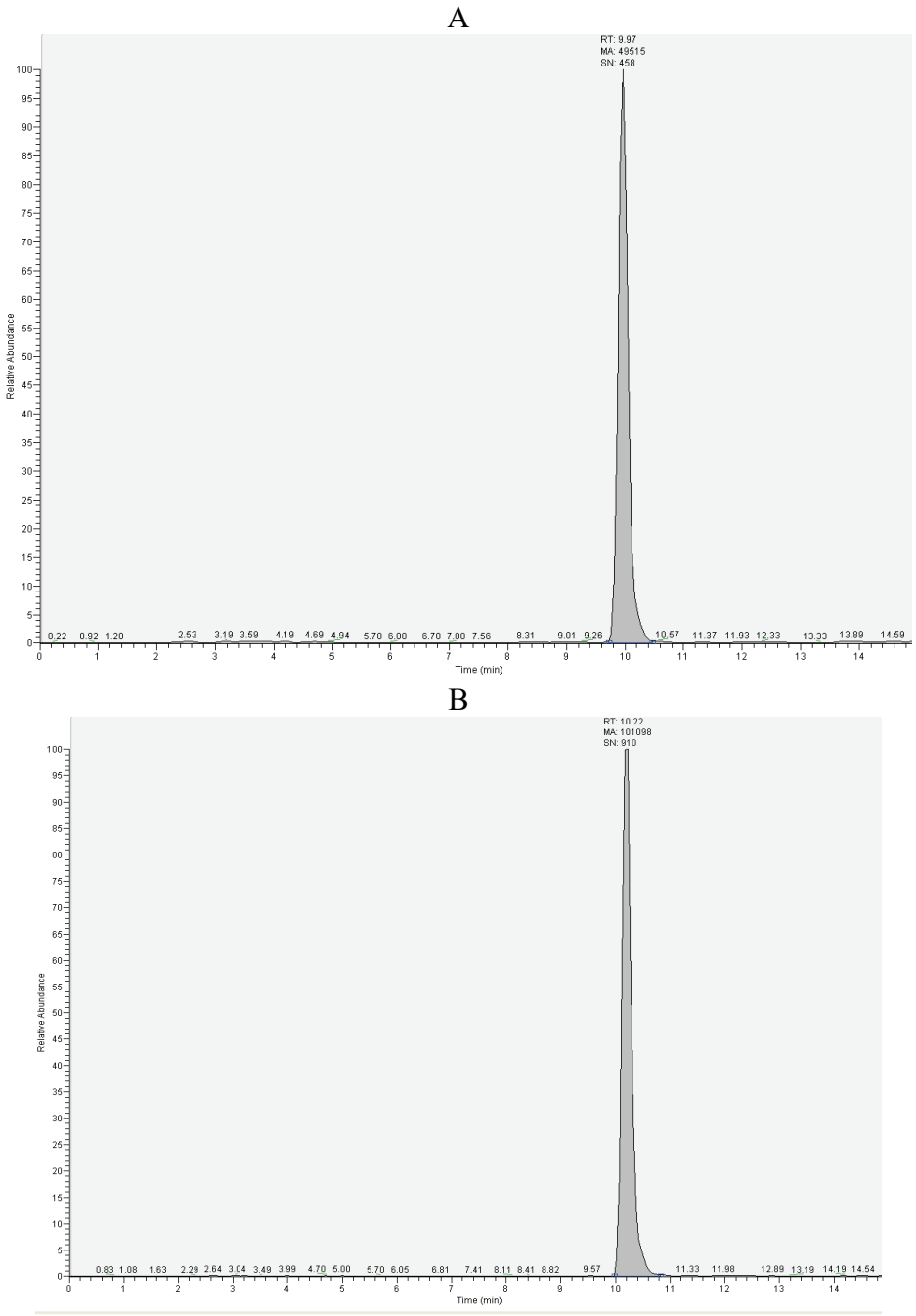
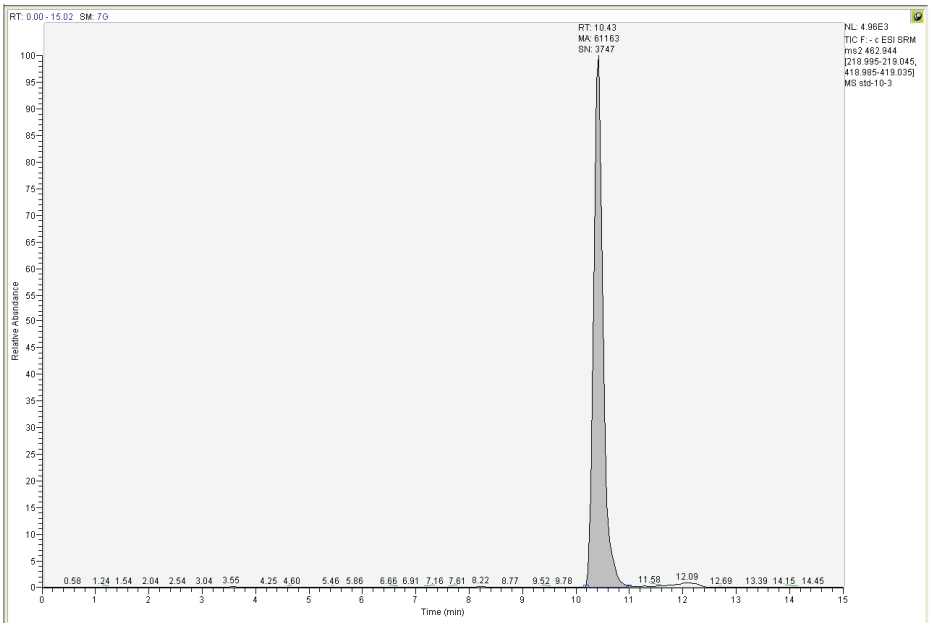


Fig. S-3. Chromatograms of A: PFHpA, B: PFOA

A



B

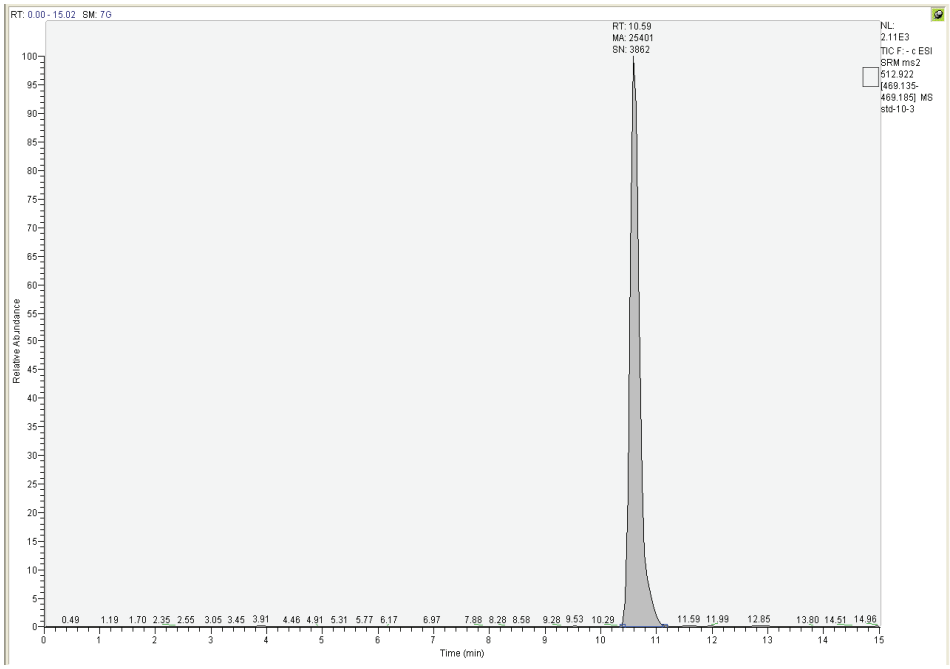
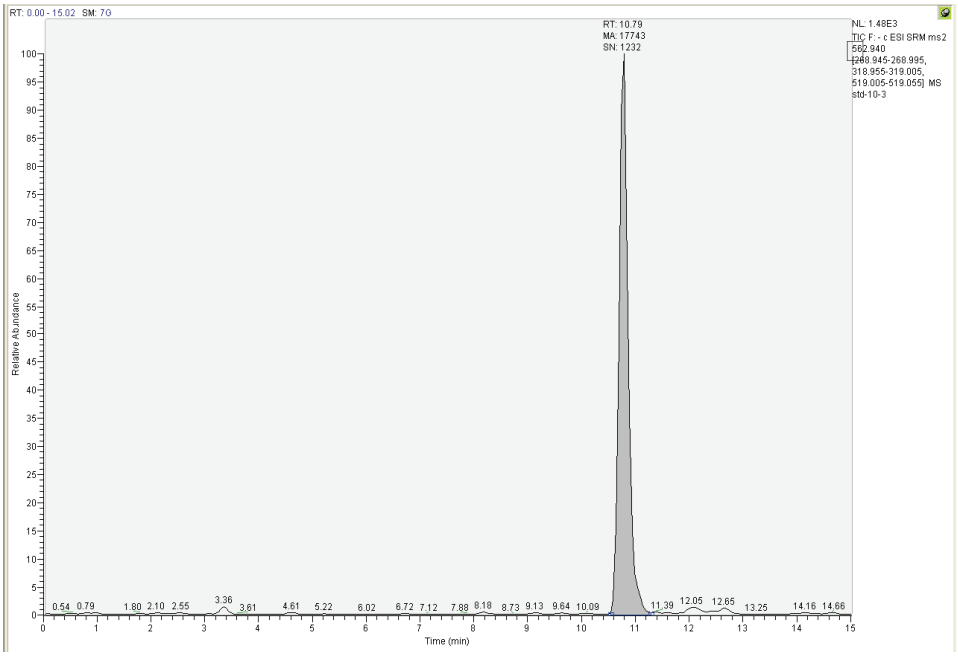


Fig. S-4. Chromatograms of A: PFNA, B: PFDA

A



B

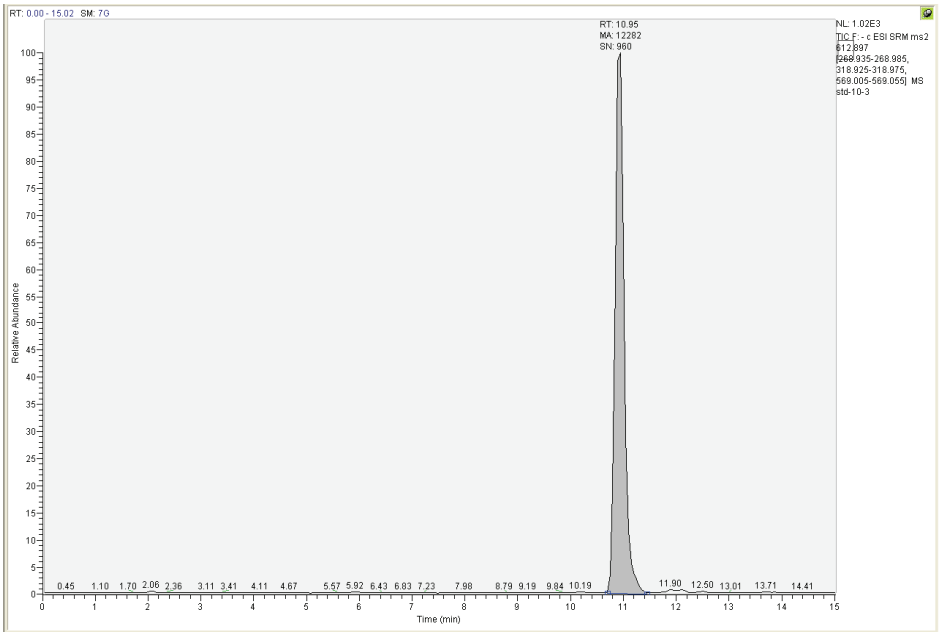


Fig. S-5. Chromatograms of A: PFUnA, B: PFDoA

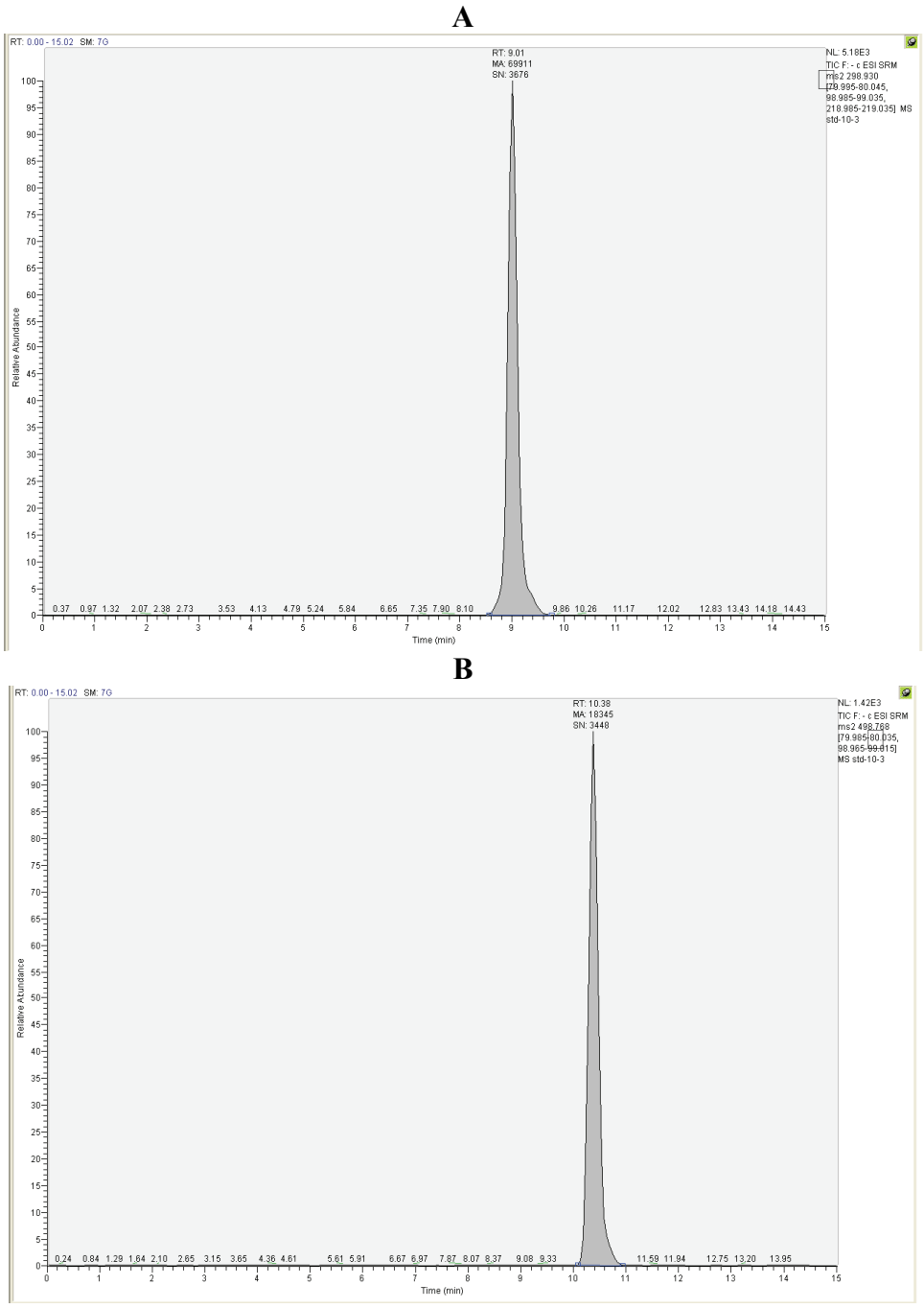


Fig. S-6. Chromatograms of A: PFBS B: PFOS

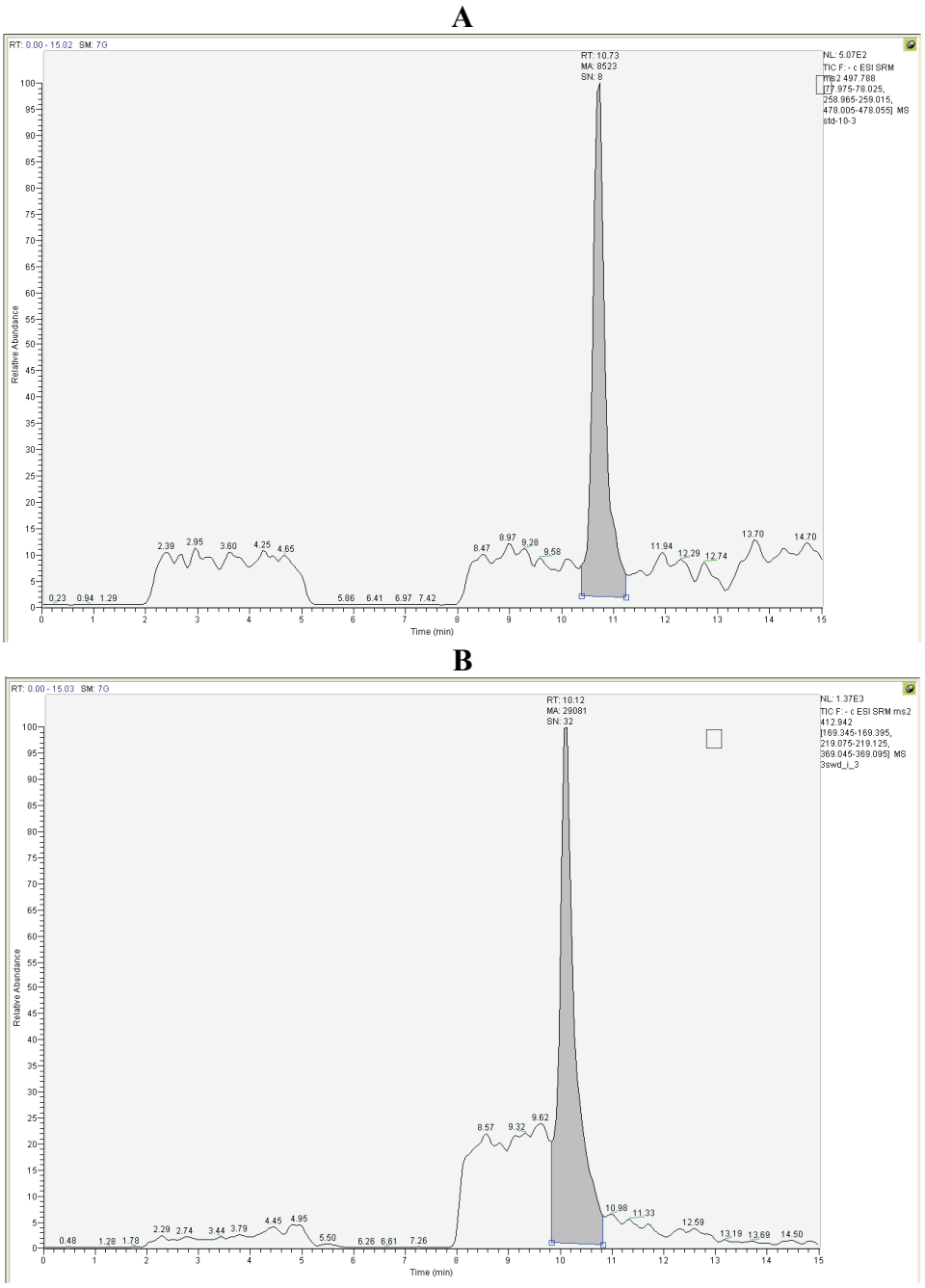


Fig. S-7. Chromatograms of A: PFOSA B: PFOA-12.11.3-D

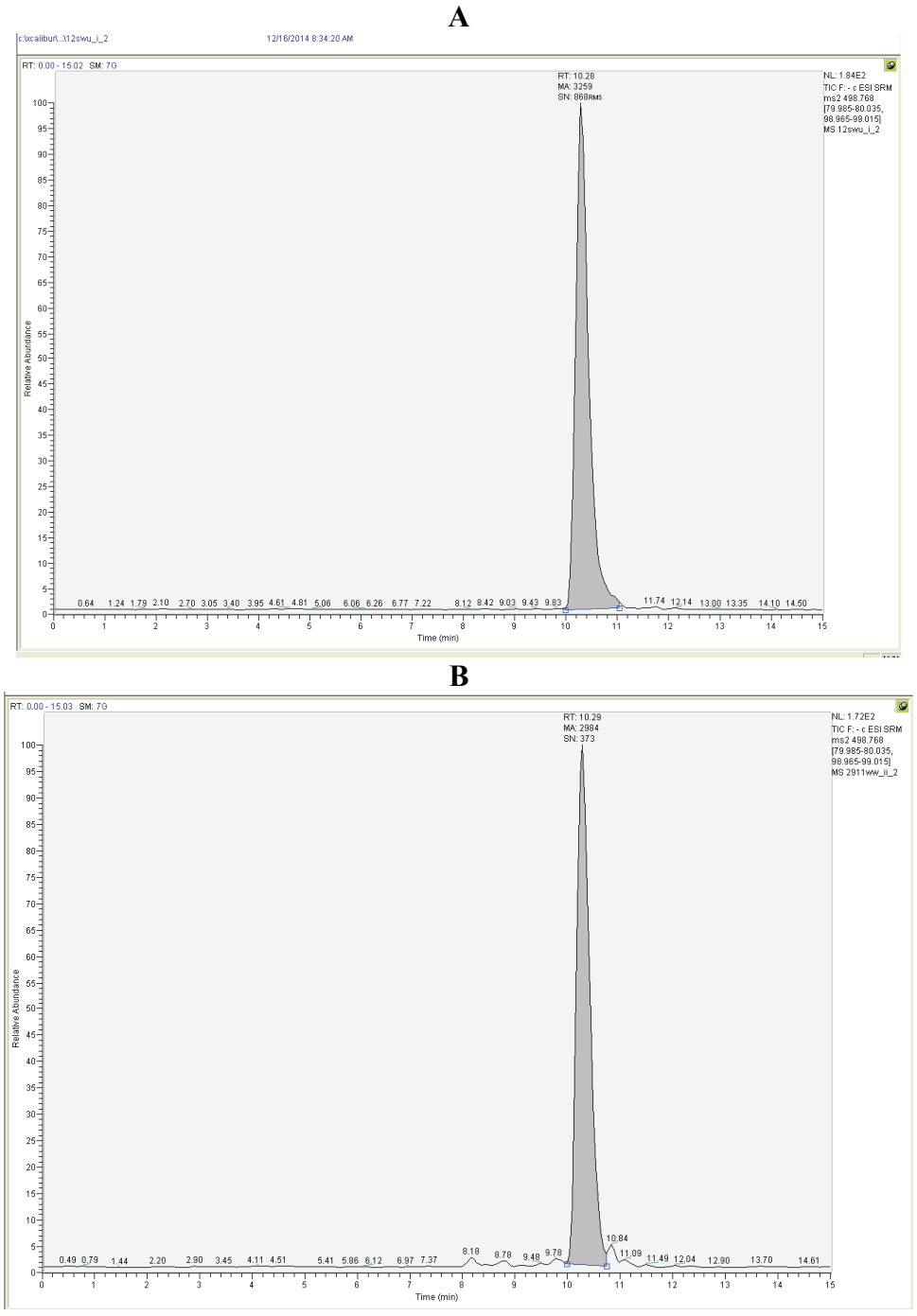


Fig. S-8. A: PFOS-29.11.12-U, B: PFOS-29.11.14.WW.