

# Analytical Methods

## Electronic Supplementary Information File

### **Simultaneous determination of eighteen perfluorinated compounds in dissolved and particulate phases of wastewater, and in sewage sludge by liquid chromatography - tandem mass spectrometry**

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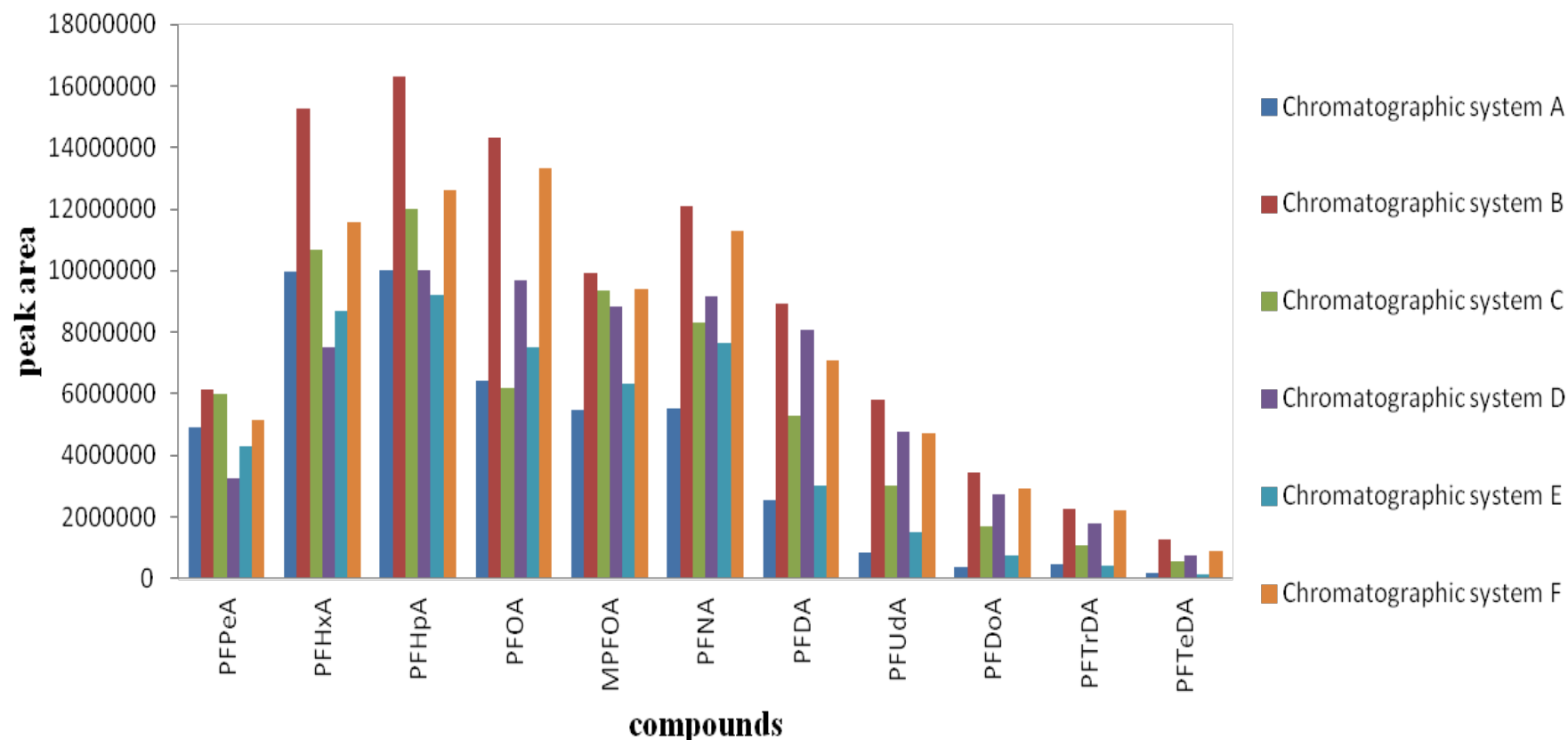
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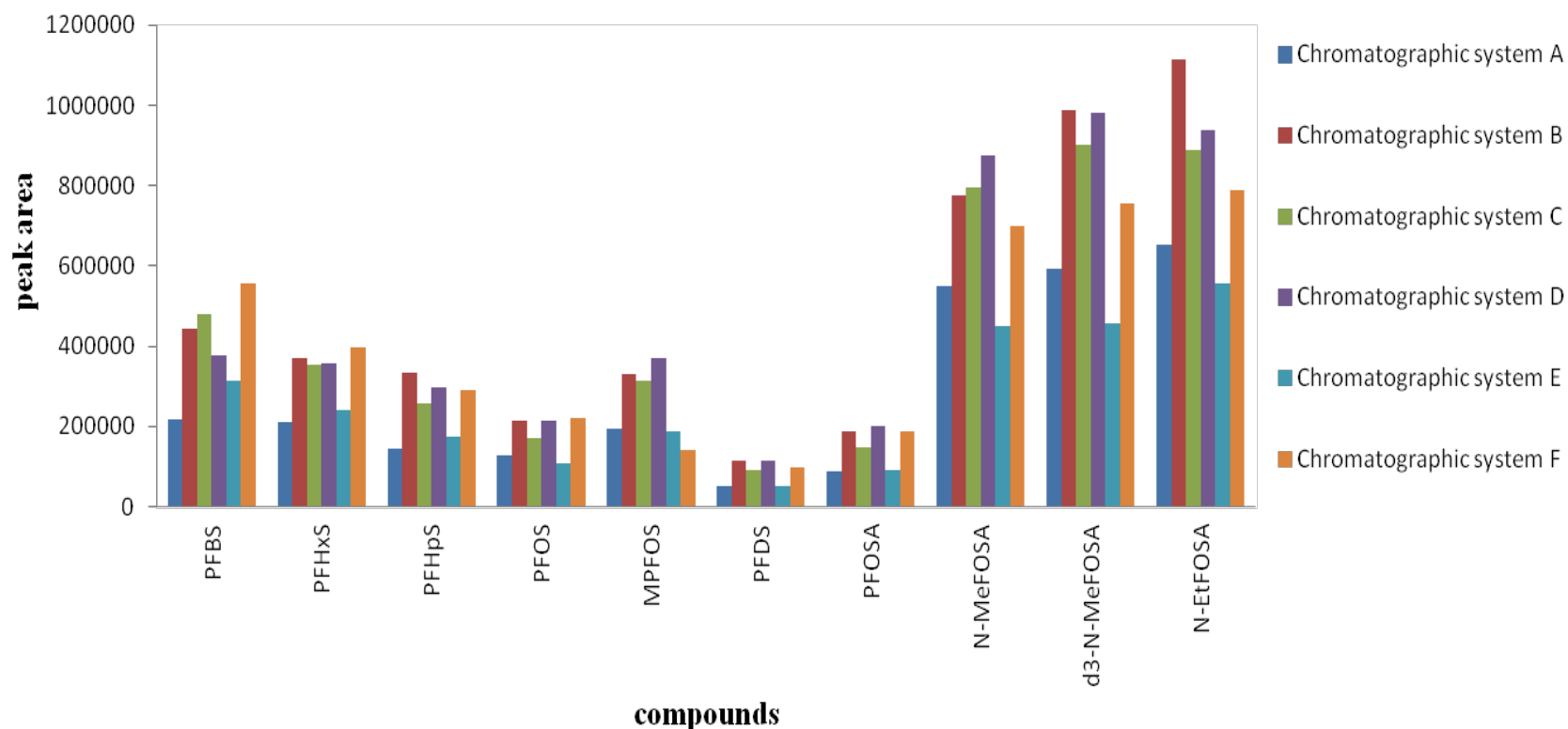
**Figures: 2**

**Table S1.** Chromatographic systems assessed.

	Chromatographic system A		Chromatographic system B		Chromatographic system C		Chromatographic system D		Chromatographic system E		Chromatographic system F	
Mobile Phase	A: MeOH		A: MeOH		A: MeOH		A: MeOH		A: MeOH		A: MeOH	
	B: 5 mM ammonium acetate		B: 5 mM ammonium formate		B: 5 mM ammonium acetate		B: 5 mM ammonium formate		B: 5 mM ammonium acetate		B: 5 mM ammonium formate	
Isocratic/ Gradient Program	<i>Time</i> ( <i>min</i> )	<i>A</i> (%)	<i>Time</i> ( <i>min</i> )	<i>A</i> (%)			<i>Time</i> ( <i>min</i> )	<i>A</i> (%)	<i>Time</i> ( <i>min</i> )	<i>A</i> (%)	<i>Time</i> ( <i>min</i> )	<i>A</i> (%)
	0	30	0	30			0	80	0	35	0	35
	1.5	75	1.5	75	<i>Time</i> ( <i>min</i> )	<i>A</i> (%)	10	80	4	75	4	75
	12	100	12	100	0	80			9	100	9	100
	17	100	17	100	10	80			12	100	12	100
	17.1	30	17.1	30					12.1	35	12.1	35
Flow Rate	100 $\mu\text{L min}^{-1}$						200 $\mu\text{L min}^{-1}$					



**Fig. S1.** Response of PFCAs ( $100 \text{ ng mL}^{-1}$ ) using different gradient and isocratic programs (where Chromatographic system A: *gradient elution program with MeOH/5 mM ammonium acetate, flow rate:  $100 \mu\text{L min}^{-1}$* ; B: *gradient elution program with MeOH/5 mM ammonium formate, flow rate:  $100 \mu\text{L min}^{-1}$* ; C: *isocratic elution program with MeOH/5 mM ammonium acetate, flow rate:  $100 \mu\text{L min}^{-1}$* ; D: *isocratic elution program with MeOH/5 mM ammonium formate, flow rate:  $100 \mu\text{L min}^{-1}$* ; E: *gradient elution program with MeOH/5 mM ammonium acetate, flow rate:  $200 \mu\text{L min}^{-1}$* ; F: *gradient elution program with MeOH/5 mM ammonium formate, flow rate:  $200 \mu\text{L min}^{-1}$* ).



**Fig. S2.** Response of PFASs and PFSAIs (100 ng mL<sup>-1</sup>) using different gradient and isocratic programs (where Chromatographic system A: gradient elution program with MeOH/5 mM ammonium acetate, flow rate: 100  $\mu$ L min<sup>-1</sup>; B: gradient elution program with MeOH/5 mM ammonium formate, flow rate: 100  $\mu$ L min<sup>-1</sup>; C: isocratic elution program with MeOH/5 mM ammonium acetate, flow rate: 100  $\mu$ L min<sup>-1</sup>; D: isocratic elution program with MeOH/5 mM ammonium formate, flow rate: 100  $\mu$ L min<sup>-1</sup>; E: gradient elution program with MeOH/5 mM ammonium acetate, flow rate: 200  $\mu$ L min<sup>-1</sup>; F: gradient elution program with MeOH/5 mM ammonium formate, flow rate: 200  $\mu$ L min<sup>-1</sup>).

**Table S2.** Repeatability ( $RSD_r$  %;  $N=9$ ,  $k=1$ ) and reproducibility ( $RSD_R$  %;  $N=3$ ,  $k=3$ ) of the methods.

Chemicals	Wastewater		Sewage sludge	
	$RSD_r$ %	$RSD_R$ %	$RSD_r$ %	$RSD_R$ %
PFPeA	3.9	4.1	1.0	27
PFHxA	4.5	13	4.7	4.2
PFHpA	4.3	7.3	2.9	3.3
PFOA	4.8	6.1	1.4	8.4
PFNA	5.1	9.2	5.6	6.9
PFDA	10	11	6.6	5.3
PFUdA	8.4	9.6	11	12
PFDoA	10	12	8.2	11
PFTTrDA	13	18	4.7	10
PFTeDA	12	17	4.3	7.9
PFBS	6.0	6.0	0.93	13
PFHxS	4.8	4.8	2.8	3.3
PFHpS	6.1	6.0	3.7	3.5
PFOS	5.0	10	1.5	2.6
PFDS	11	12	6.8	9.2
PFOSA	17	23	12	26
<i>N</i> -MeFOSA	8.2	9.0	3.9	9.6
<i>N</i> -EtFOSA	9.0	12	8.1	22

**Table S3.** Repeatability (RSD %;  $N=6$ ) of retention times and relative retention times of a standard solvent solution containing the target analytes at 0.25 ng and three internal standards at 1.25 ng.

Chemicals	Repeatability of Retention Time (RSD %)	Repeatability of Relative Retention Time (RSD %)
PFPeA	0.63	0.42
PFHxA	0.43	0.30
PFHpA	0.58	0.46
PFOA	0.58	0.21
PFNA	0.52	0.20
PFDA	0.48	0.24
PFUdA	0.56	0.21
PFDoA	0.69	0.70
PFTTrDA	1.1	0.28
PFTeDA	1.3	0.26
PFBS	0.39	0.30
PFHxS	0.54	0.19
PFHpS	0.45	0.33
PFOS	0.56	0.25
PFDS	0.65	0.18
PFOSA	0.58	0.55
<i>N</i> -MeFOSA	0.95	0.14
<i>N</i> -EtFOSA	1.1	0.05

**Table S4.** Matrix effects (MEs %; SD, n=3) for all chemicals in dissolved and particulate phase.

Chemicals	Dissolved phase	Particulate matter
	% ME Mean (SD)	% ME Mean (SD)
PFPeA	+ 77.9 (15)	+ 111 (23)
PFHxA	+ 90.3 (19)	+ 112 (11)
PFHpA	+ 68.6 (18)	+ 167 (22)
PFOA	+ 84.9 (11)	+ 81.5 (11)
MPFOA	+ 71.8 (11)	+ 68.4 (11)
PFNA	+ 155 (22)	+ 127 (17)
PFDA	+ 127.5 (10)	+ 189 (16)
PFUdA	+ 101 (2.4)	+ 213 (25)
PFDoA	+ 86.9 (8.9)	+ 222 (35)
PFTTrDA	+ 140 (16)	+ 126 (26)
PFTeDA	+ 95.6 (16)	+ 249 (25)
PFBS	+ 72.9 (10)	+ 130 (6.4)
PFHxS	+ 103 (8.2)	+ 145 (4.2)
PFHpS	+ 99.8 (8.9)	+ 83.5 (15)
PFOS	+ 120 (9.6)	+ 85.1 (13)
MPFOS	+ 110 (11)	+ 74 (12)
PFDS	+ 118 (10)	+ 124 (29)
PFOSA	+ 50.3 (13)	+ 129 (23)
<sup>2</sup> D <sub>3</sub> -N-MeFOSA	+ 67.1 (13)	+ 76.8 (13)
N-MeFOSA	+ 48.8 (12)	+ 69.4 (16)
N-EtFOSA	+ 52.9 (6.9)	+ 71.3 (15)

**Table S5.** PFCAs Ion Ratios (%) at the fortification level of 10 ng.

Analytes	(C/Q)	Standard solvent solution		Dissolved phase of wastewater		Particulate matter of sewage sludge		Acceptable RSD % (2002/657/EE)
		(C/Q) × 100%	RSD % (N = 9)	(C/Q) × 100%	RSD % (N = 9)	(C/Q) × 100%	RSD % (N = 9)	
PFHxA	119 / 269	1.9	1.4	2.2	15.6	1.9	4.6	50
PFHpA	169 / 319	4.5	6.2	4.7	2.9	4.6	7.0	50
PFOA	169 / 369	15.4	5.3	15.9	6.4	15.4	5.2	30
PFNA	169 / 419	4.9	1.8	5.1	4.0	5.2	2.9	50
PFDA	169 / 469	1.5	11	1.3	7.0	1.2	15.1	50
PFUdA	169 / 519	4.9	4.0	5.0	2.3	4.9	5.2	50
PFDoA	169 / 569	6.8	4.3	6.9	2.5	6.8	7.1	50
PFTTrDA	169 / 619	7.9	3.4	7.6	4.5	7.4	6.1	50
PFTeDA	419 / 669	6.3	7.1	6.3	4.8	6.5	6.8	50



**Table S6.** Levels of PFCs in wastewater (dissolved phase and particulate matter) and sewage sludge samples from various sampling points in Athens WWTP (Greece).

Target analytes	Influent wastewater (24-h composite samples)				Primary sludge (grab samples)		Secondary sludge (grab samples)		Effluent wastewater (24-h composite samples)				Dewatered sludge
	D.P. <sup>1</sup> (ng/L)	P.M. <sup>2</sup> (ng/L)	Total <sup>3</sup> (ng/L)	Distribution in P.M. <sup>2</sup> (%)	D.P. <sup>1</sup> (ng/L)	P.M. <sup>2</sup> (ng/g)	D.P. <sup>1</sup> (ng/L)	P.M. <sup>2</sup> (ng/g)	D.P. <sup>1</sup> (ng/L)	P.M. <sup>2</sup> (ng/L)	Total <sup>3</sup> (ng/L)	Distribution in P.M. <sup>2</sup> (%)	(ng/g)
	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample1/ Sample2	Sample 1/ Sample2
PFPeA	n.d./n.d.	2.6/4.4	2.6/4.4	100/100	18/n.d.	18/27	n.d./n.d.	n.d./2.7	n.d./n.d.	2.9/2.7	2.9/2.7	100/100	n.d./n.d.
PFHxA	n.d./n.d.	3.7/6.4	3.7/6.4	100/100	n.d./n.d.	5.3/14	5.3/n.d.	3.2/3.8	4.4/<LLOQ	0.60/2.4	5.0/2.4	12/100	1.2/1.7
PFHpA	<LLOQ/ <LLOQ	<LLOQ/ <LLOQ	<LLOQ/ <LLOQ	-/-	65/53	<LLOQ/ <LLOQ	2.8/3.0	n.d./n.d.	2.6/2.7	0.28/0.90	2.9/3.6	9/25	n.d./n.d.
PFOA	14/22	12/7.7	26/30	46/26	15/13	3.1/28	18/19	3.8/16	25/19	7.9/7.1	33/27	24/26	15/7.3
PFNA	<LLOQ/4.2	n.d./n.d.	<LLOQ/4.2	-/0	<LLOQ/ <LLOQ	3.0/8.1	<LLOQ/ <LLOQ	<LLOQ/4.7	<LLOQ/ <LLOQ	n.d./n.d.	<LLOQ/ <LLOQ	-/-	3.8/<LLOQ
PFDA	<LLOQ/11	2.7/2.6	2.7/14	100/19	2.4/n.d.	21/80	2.7/<LLOQ	12/50	3.2/n.d.	13/5.4	16/5.4	81/100	7.3/6.3
PFUDa	17/1.1	n.d./112	17/113	0/99	3.3/n.d.	14/22	2.8/n.d.	6.5/17	n.d./0.50	14/n.d.	14/0.50	100/0	1042/47
PFDoA	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.	447/177	n.d./n.d.	224/154	n.d./n.d.	15/0.50	15/0.50	100/100	2.9/<LLOQ
PFTTrDA	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.
PFTeDA	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.
PFBS	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	169/153	80/n.d.	6.3/2.7	39.9/n.d.	n.d./1.0	n.d./n.d.	n.d./1.0	-/0	n.d./n.d.
PFHxS	<LLOQ/1.3	n.d./n.d.	<LLOQ/1.3	-/0	23/12	2.5/n.d.	3.7/1.1	n.d./n.d.	2.9/2.2	n.d./n.d.	2.9/2.2	0/0	n.d./n.d.
PFHpS	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.
PFOS	18/17	2.5/1.6	20/19	13/8	18/5.0	7.3/4.7	8.9/4.8	28/35	15/17	10/2.8	25/20	40/14	8.2/5.3
PFDS	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.
PFOSA	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./0.80	n.d./n.d.	n.d./n.d.	n.d./n.d.	<LLOQ/n.d.	n.d./n.d.	<LLOQ/n.d.	-/-	n.d./n.d.
N-MeFOSA	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.
N-EtFOSA	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	n.d./n.d.	-/-	n.d./n.d.

\*Abbreviations: <sup>1</sup>D.P., Dissolved Phase; <sup>2</sup>P.M., Particulate Matter; <sup>3</sup>Total, Sum of D.P. and P.M; n.d., not detected; LLOQ, Lower Limit of Quantification