Supporting information

Suspect and non-target screening of ovarian follicular fluid and serum – identification of anthropogenic chemicals and investigation of their association to fertility

Ida Hallberg,[*,†] *Merle Plassmann,*[‡] *Matts Olovsson,*[ǁ] *Jan Holte,*[ǁ,§] *Pauliina Damdimopoulou,*[⊥] *Ylva C. B. Sjunnesson,*[†] *Jonathan P. Benskin,*[‡] *Sara Persson*[†]

[†]Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences, The Centre for Reproductive Biology in Uppsala, SE-750 07 Uppsala, Sweden
[‡]Department of Environmental Science and Analytical Chemistry (ACES), Stockholm University, SE-106 91 Stockholm, Sweden
[ǁ]Department of Women´s and Children´s Health, Uppsala University, SE-751 85 Uppsala, Sweden
[§]Carl von Linnékliniken, SE-751 83 Uppsala, Sweden
[⊥]Division of Obstetrics and Gynecology, Department of Clinical Science, Intervention and Technology, Karolinska Institutet and Karolinska University Hospital, SE-141 86 Stockholm, Sweden

Contact information:

Corresponding author: I Hallberg: ida.hallberg@slu.se, +4618-671641

M Plassmann: merle.plassmann@aces.su.se,

M Olovsson: matts.olovsson@kbh.uu.se

J Holte: jan.holte@linne.se

P Damdimopoulou: pauliina.damdimopoulou@ki.se

Y Sjunnesson: ylva.sjunnesson@slu.se

J Benskin: jon.benskin@aces.su.se

S Persson: sara.persson@slu.se

Table of Contents

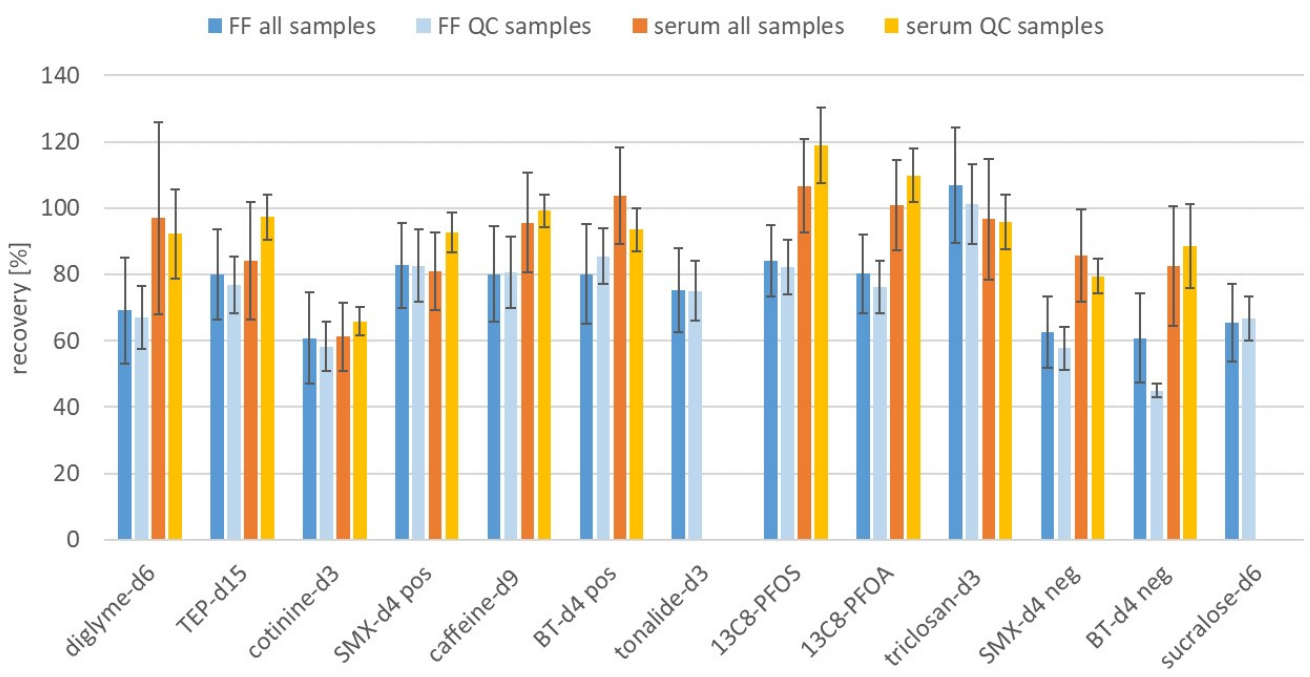

Figure S1. Internal standard recoveries in follicular fluid (FF) and serum samples.
Error bars are plus minus standard deviation (SD)

Figure S2. Chromatograms of confirmed suspects (A-R).

Exact masses (ppm = 5) of all confirmed suspects with CL1 or 2b, both reference standards and sample, plus MS2 spectra where recorded (A-R).

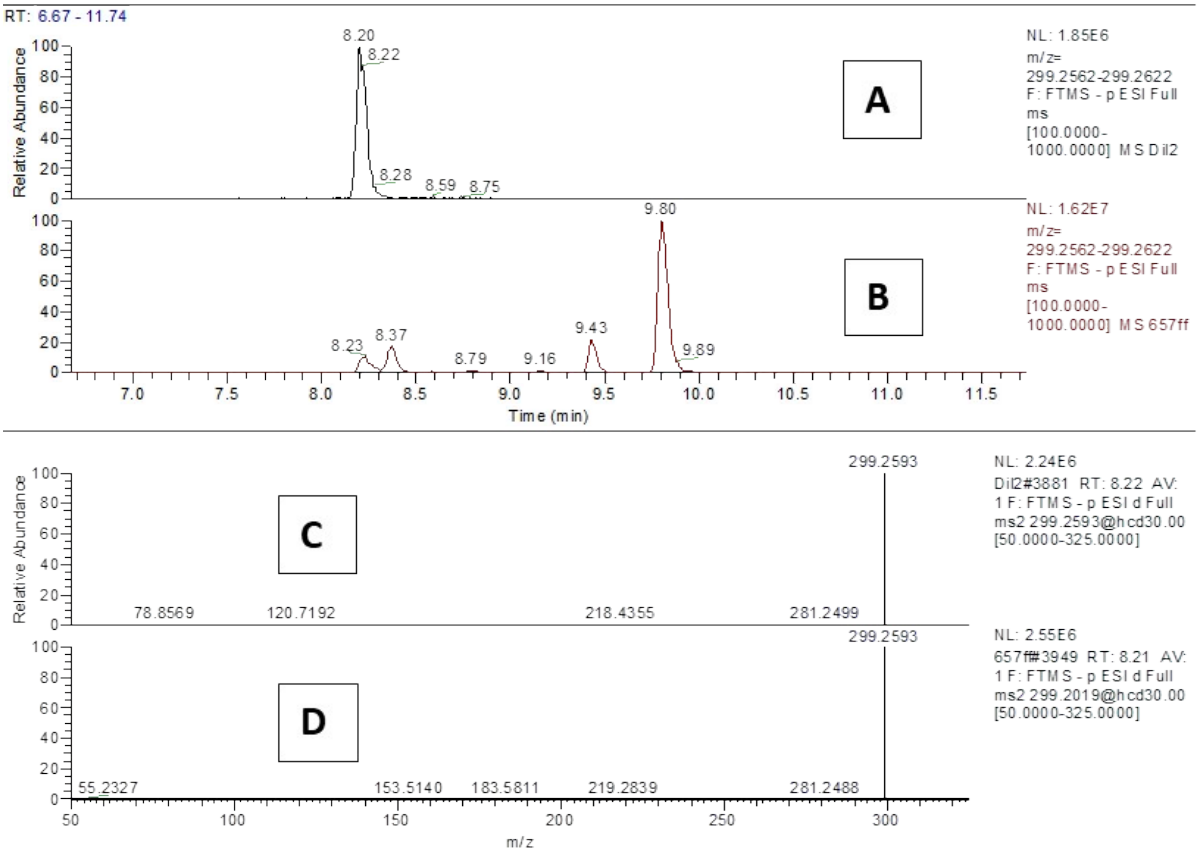

Figure S2, A: 12-hydroxyoctadecanoic acid [CASno 160-14-9],
From top and down; reference standard chromatogram; chromatogram in FF sample; MS2 spectra in reference standard; MS2 spectra in FF sample; no high intensity fragment ions are detected at this collision energy, however, the fragment at 281.2488 is also described in a spectra of the same substance on MassBank (accession ID: LU039852).

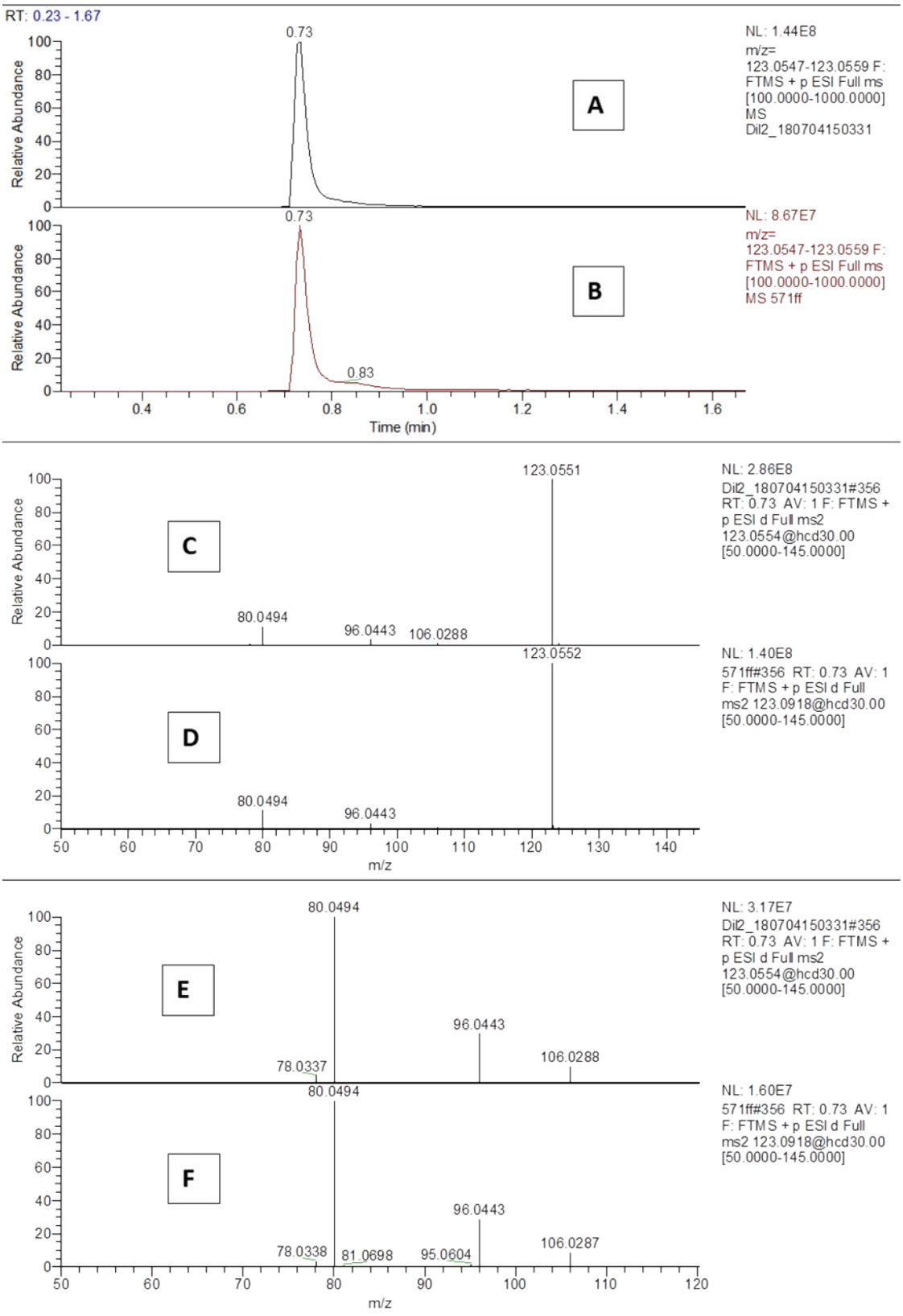

Figure S2, B: 3-pyridinecarboxamide [CASno: 98-92-0],
A; reference standard chromatogram; B chromatogram in FF sample; C MS2 spectra in reference standard; D MS2 spectra in FF sample. E zoom in M2 spectra in reference standard; F zoom in MS2 spectra in FF sample

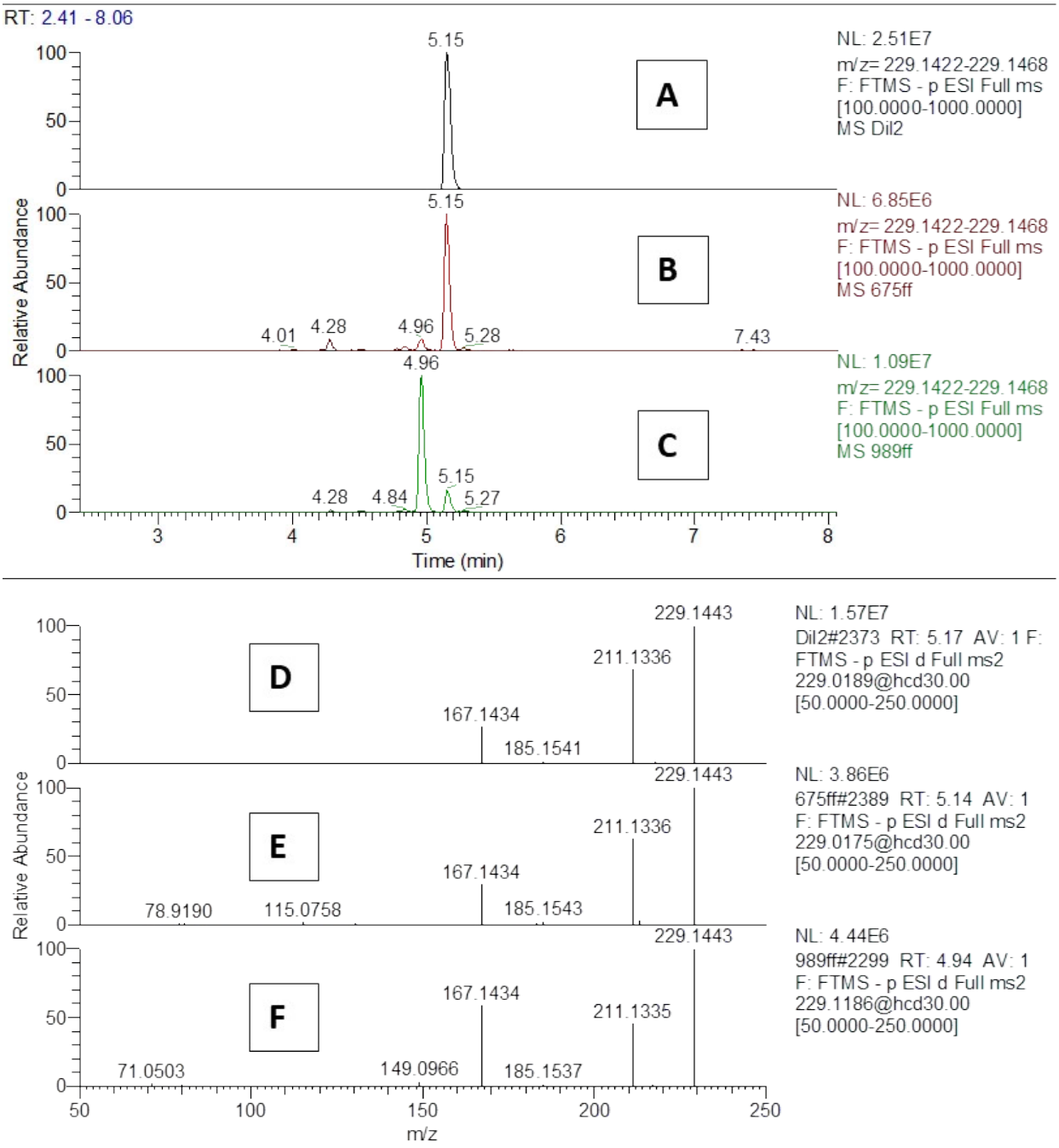

Figure S2, C: Dodecanedioic acid [CASno: 693-23-2],
A: reference standard chromatogram; B and C: chromatograms in FF samples; D: MS2 spectra in reference standard; E and F: MS2 spectra in FF samples at different RTs.

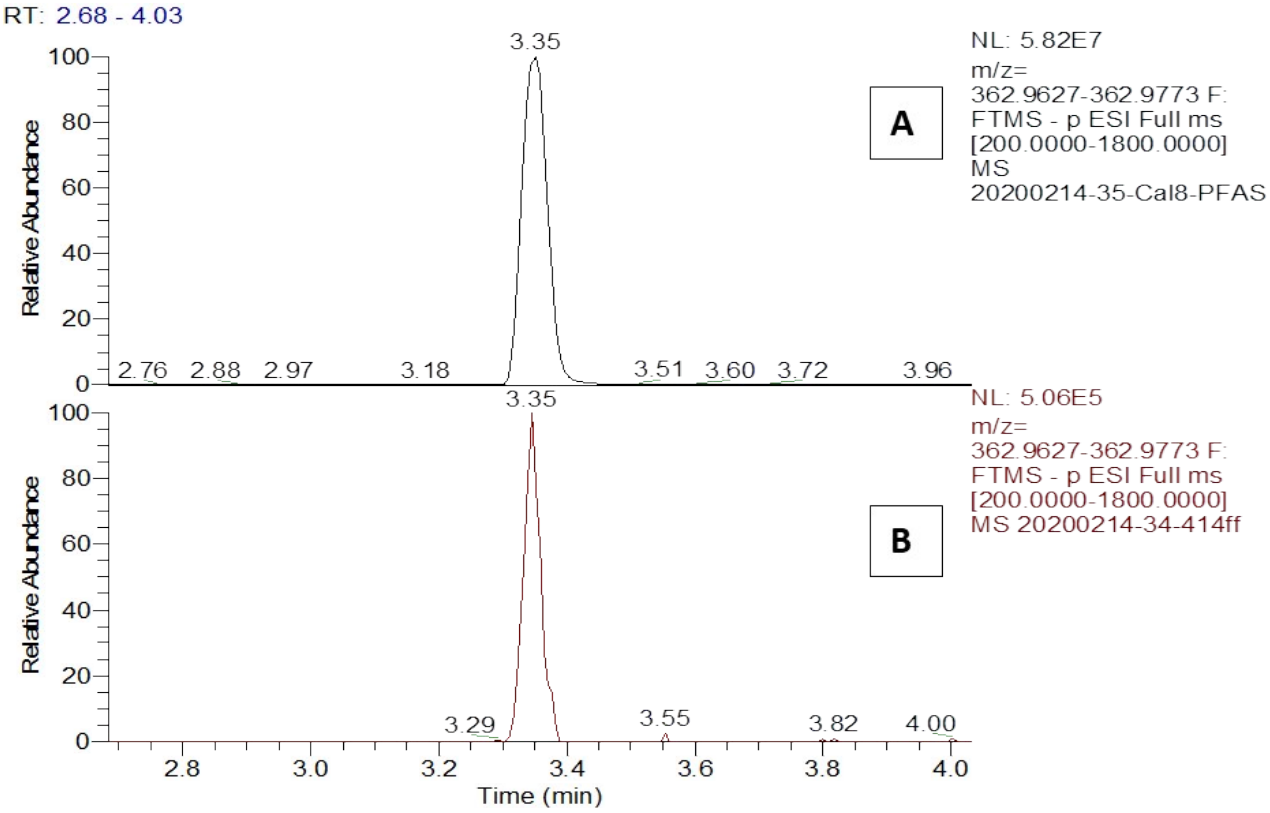

Figure S2, D: Perfluoroheptanoic acid (PFHpA) [CASno: 375-85-9],
A: reference standard chromatogram; B: chromatogram in FF sample

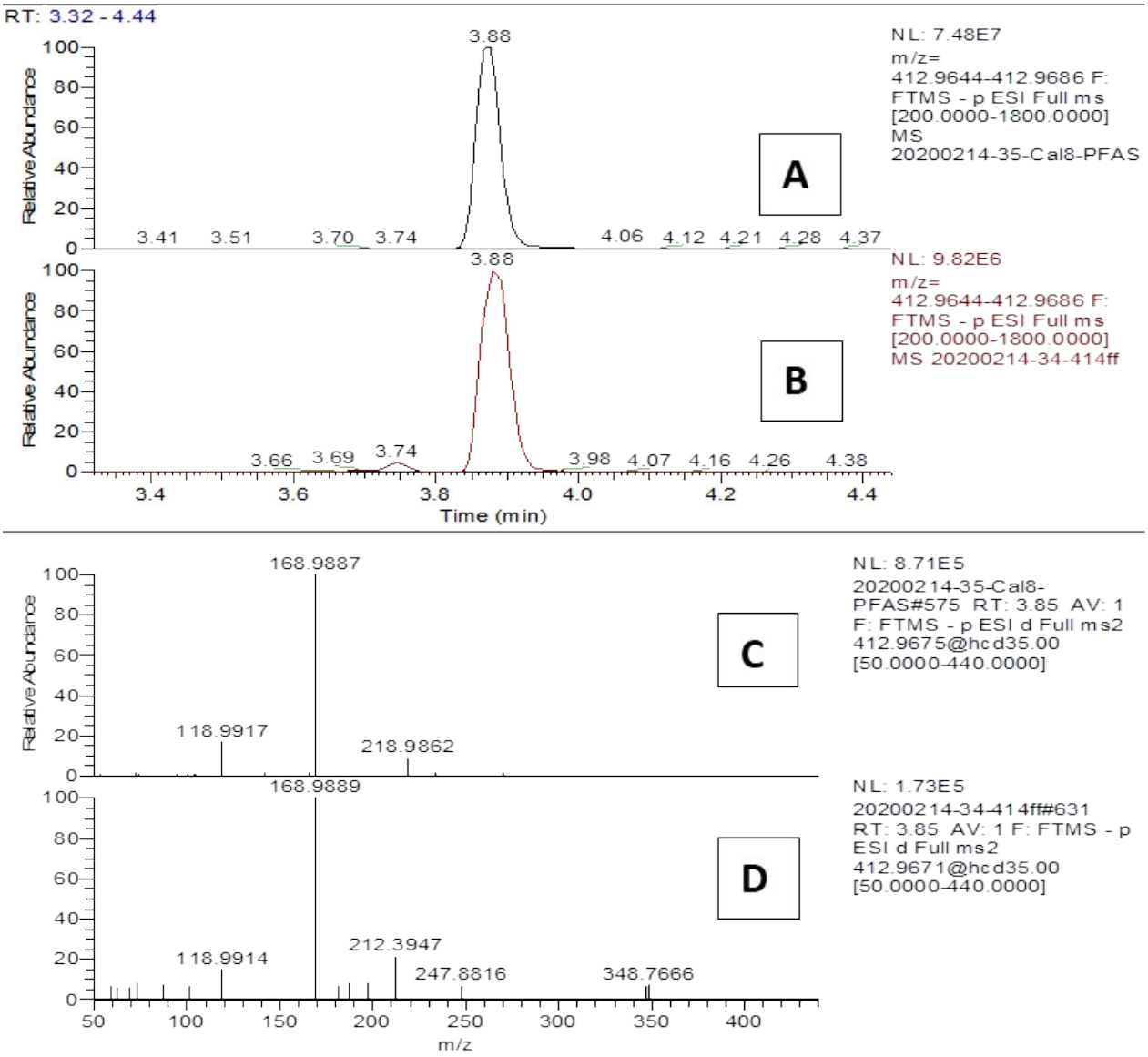

Figure S2, E: Perflourooctanoic acid (PFOA) [CASno: 335-67-1],
A: reference standard chromatogram; B: chromatogram in FF sample; C: MS2 spectra in reference standard; D: MS2 spectra in FF sample

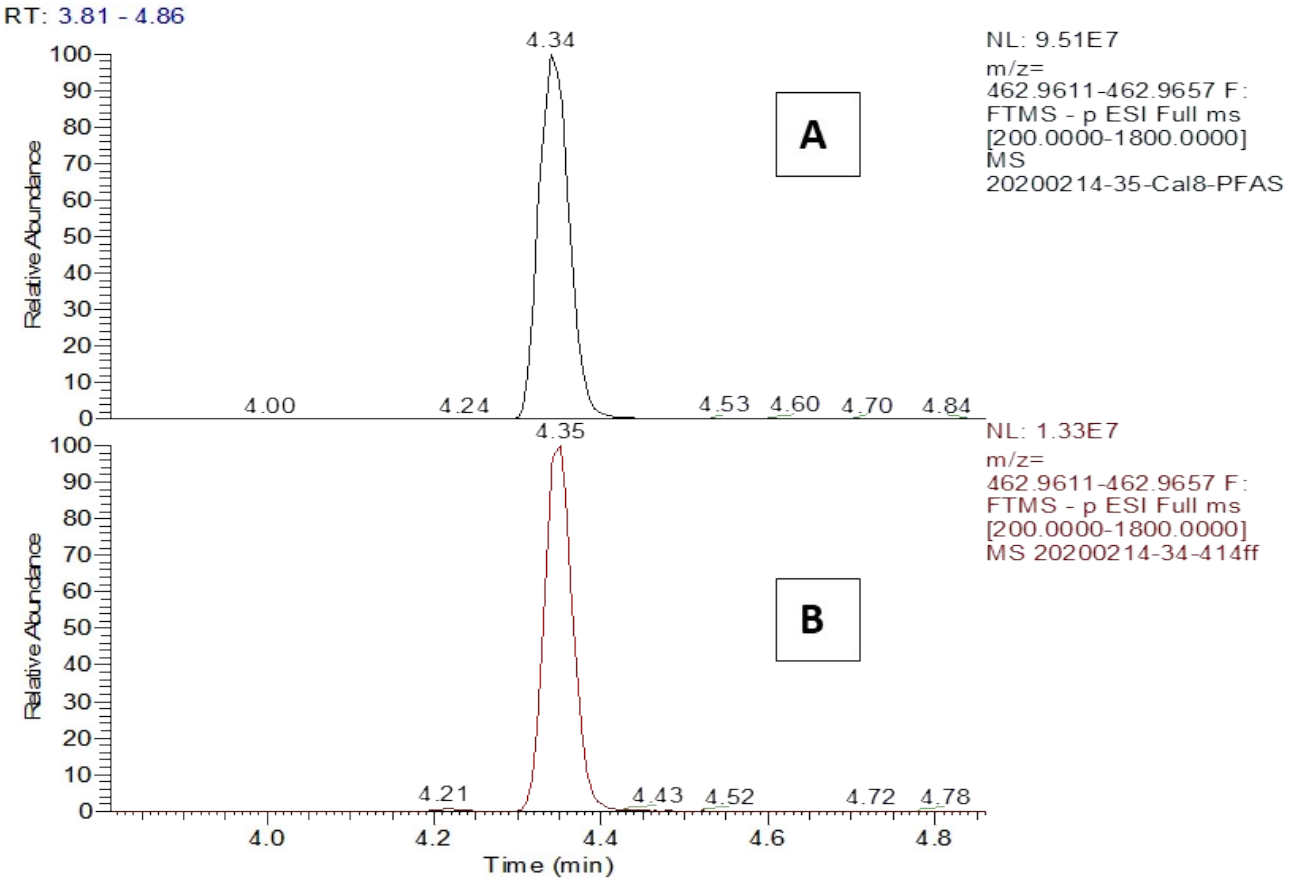

Figure S2, F: Perfluorononanoic acid (PFNA) [CASno: 375-95-1],
A: reference standard chromatogram; B: chromatogram in FF sample

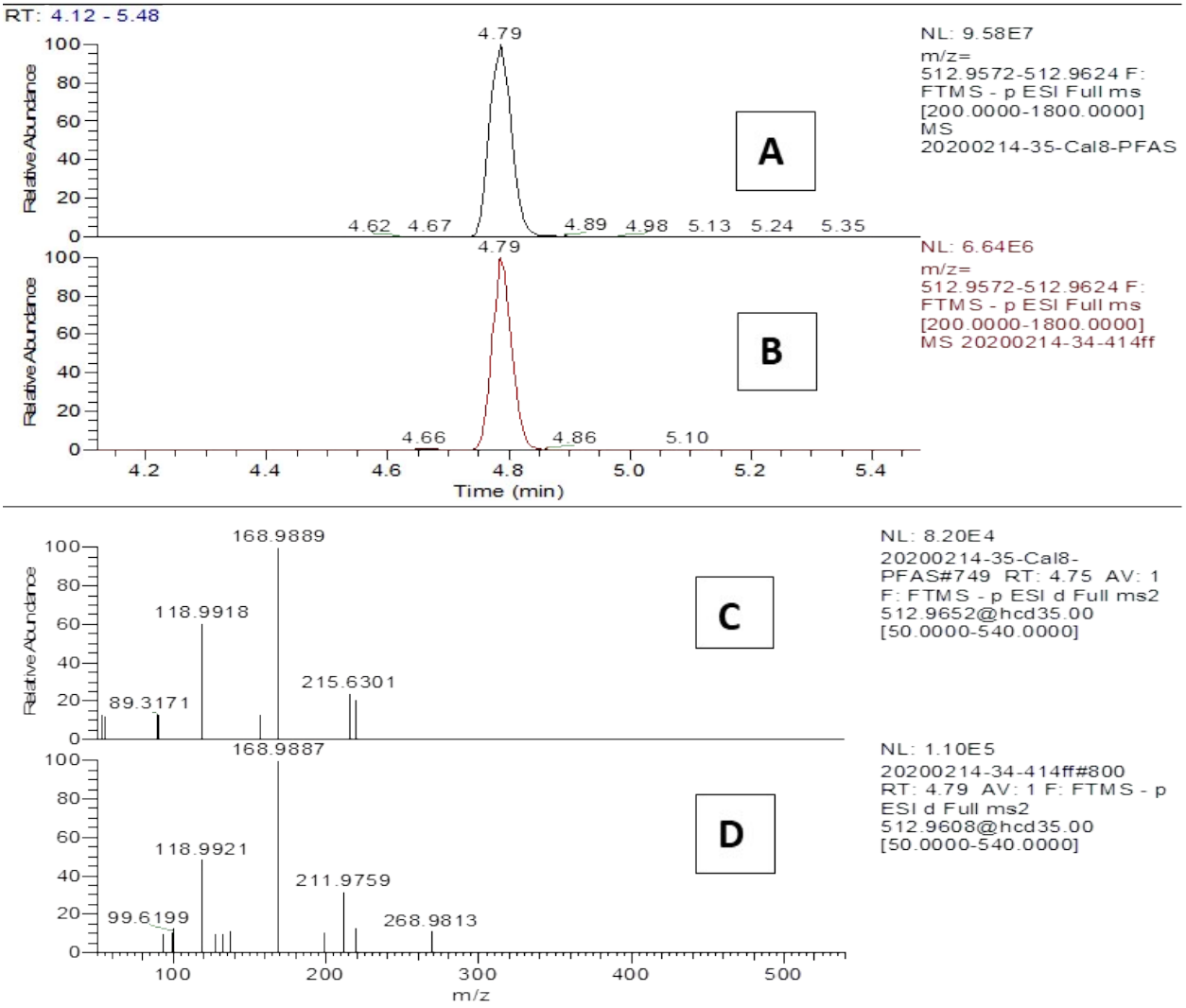

Figure S2, G: Perfluorodecanoic acid (PFDA) [CASno: 335-76-2],
A: reference standard chromatogram; B: chromatogram in FF sample; C: MS2 spectra in reference standard; D: MS2 spectra in FF sample

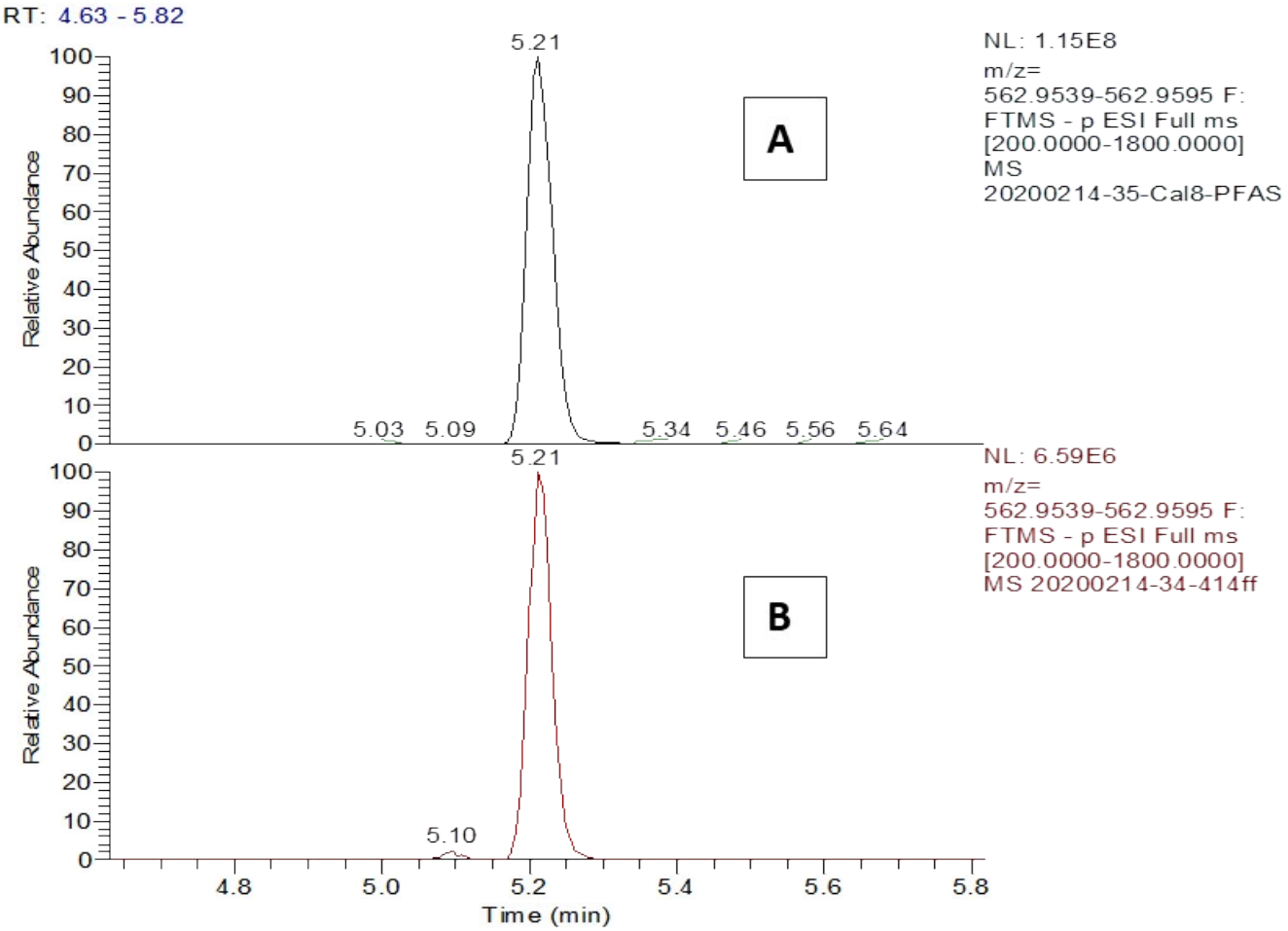

Figure S2, H: Perfluoroundecanoic acid (PFUnDA) [CASno: 2058-94-8],
A: reference standard chromatogram; B: chromatogram in FF sample

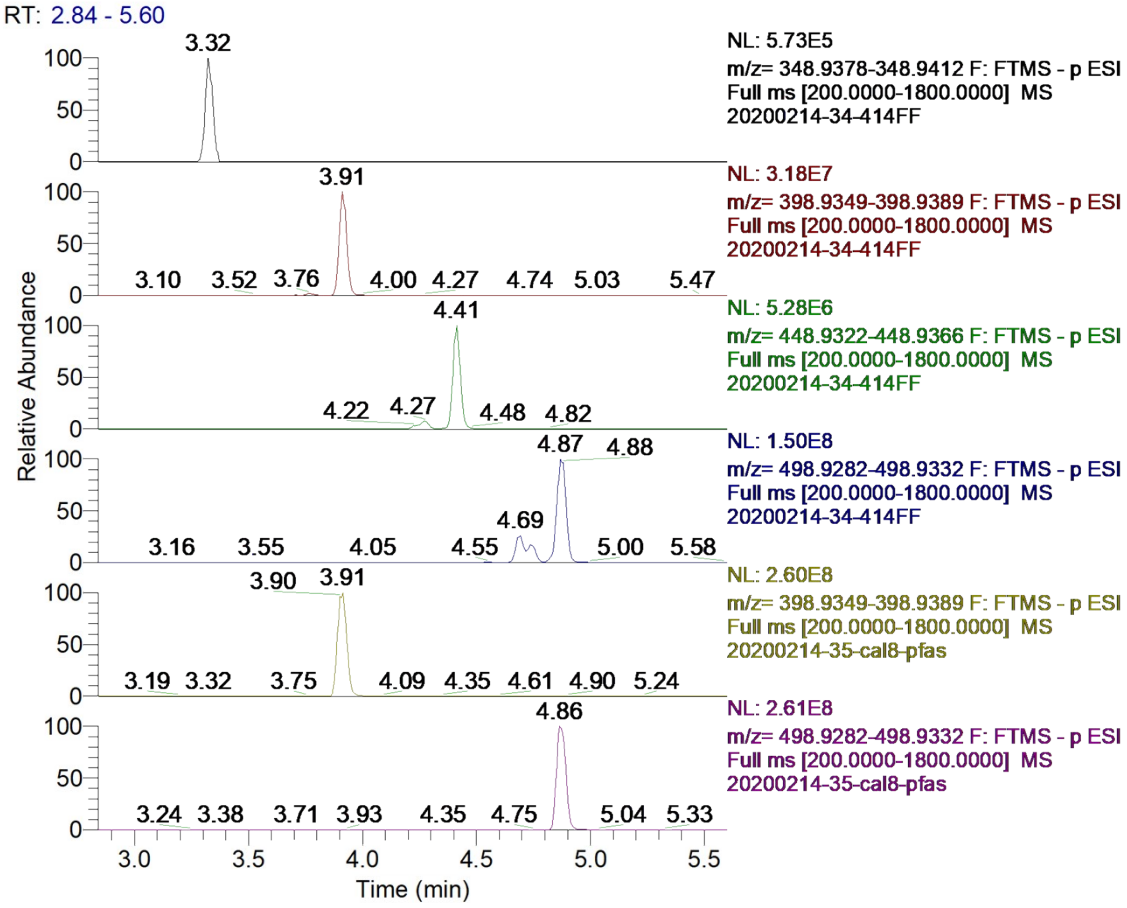

Figure S2, I: Chromatograms of exact masses in sample showing the whole homologue series of detected perfluorinated sulfonic acids: From top to bottom: Perflouropentane sulfonate (PFPeS) in sample, Perfluorohexane sulfonate (PFHxS) in sample, Perfluoroheptane sulfonate (PFHpS) in sample, Perfluorooctane sulfonate (PFOS) in sample, PFHxS in reference standard, PFOS in reference standard.

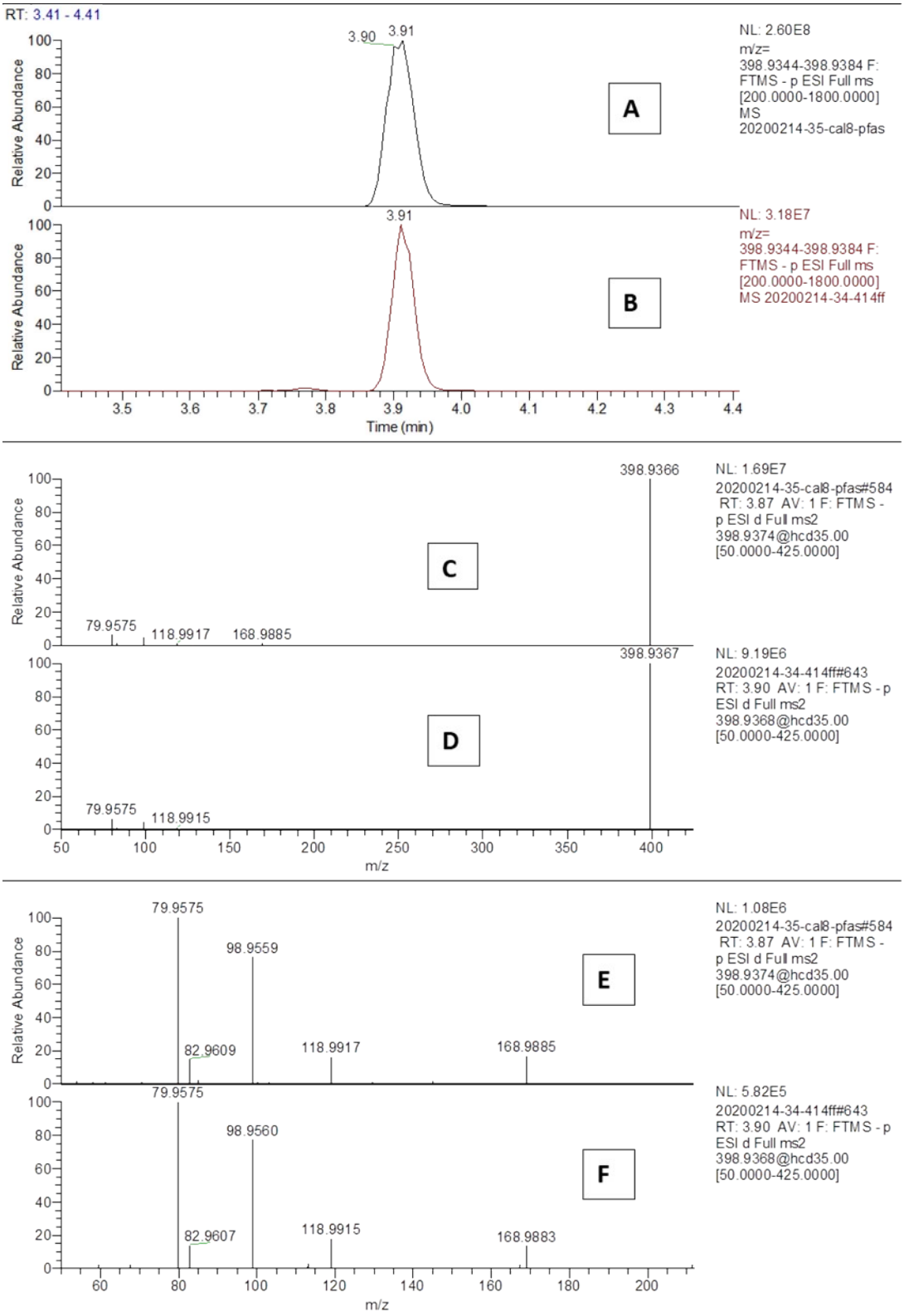

Figure S2, J: Perfluorohexane sulfonate (PFHxS) [CASno: 355-46-4], A: reference standard chromatogram; B: chromatogram in FF sample; C: MS2 spectra in reference standard; D: MS2 spectra in FF sample; E: zoom in MS2 spectra in reference standard; F: zoom in MS2 spectra in FF sample

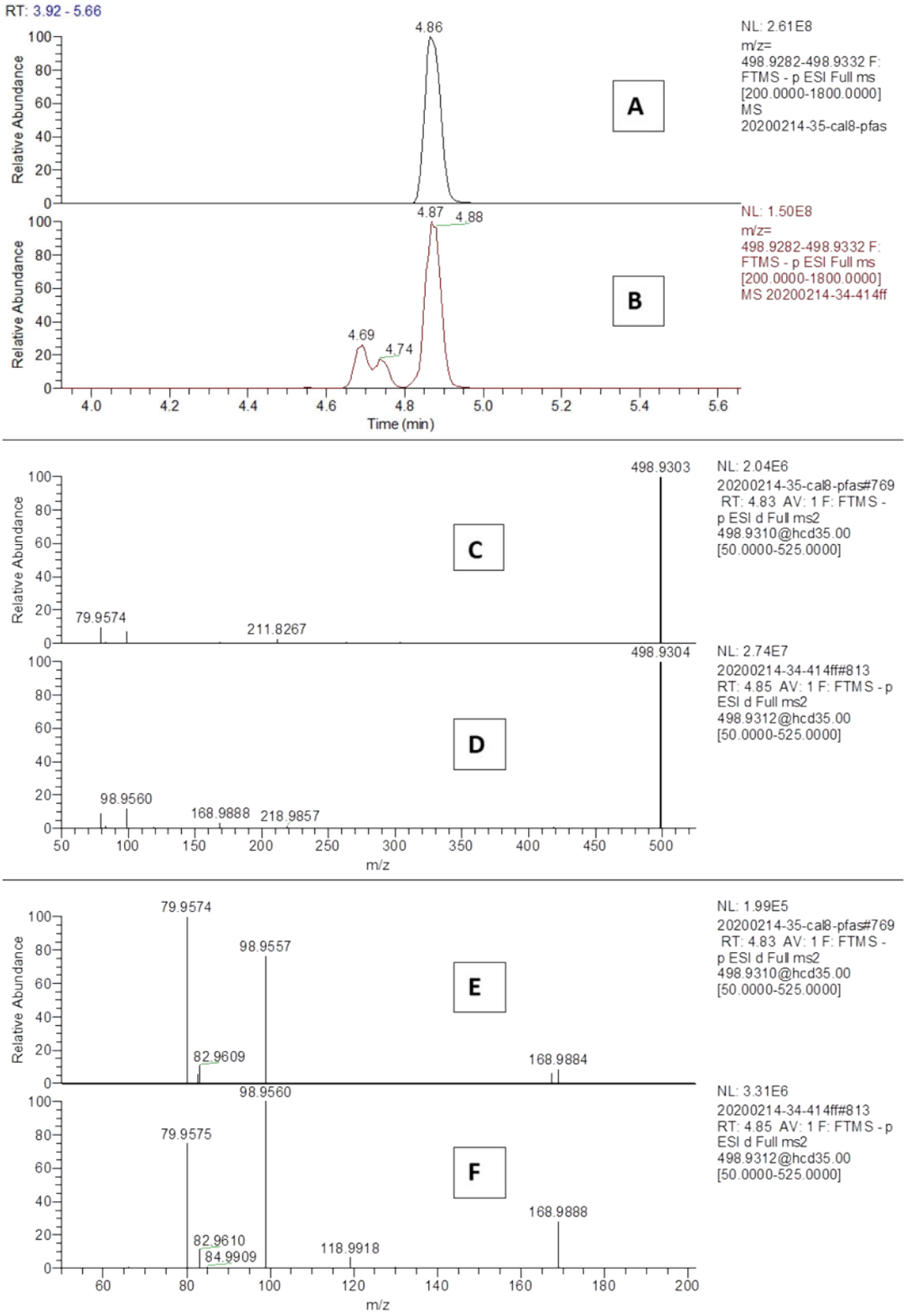

Figure S2, K: Perfluorooctane sulfonate (PFOS) [CASno: 1793-23-1],
A: reference standard chromatogram; B: chromatogram in FF sample; C: MS2 spectra in reference standard; D: MS2 spectra in FF sample; E: zoom in MS2 spectra in reference standard; F: zoom in MS2 spectra in FF sample

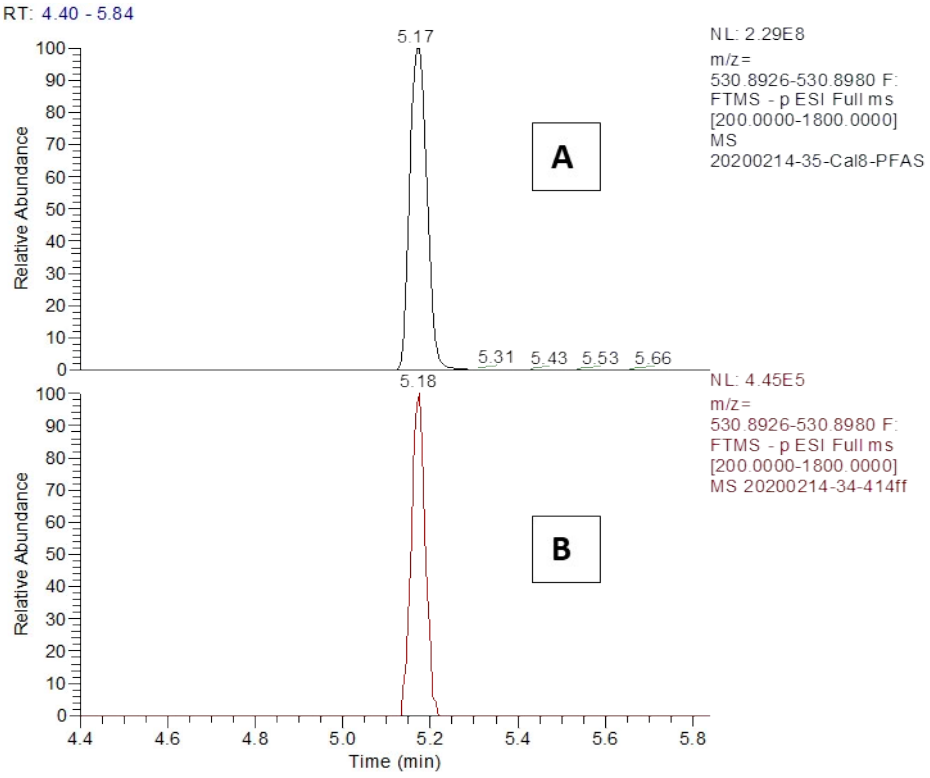

Figure S2, L: 9-Chlorohexadecafluoro-oxanonane sulfonate (9Cl-PF3ONS) [CASno: 756426-58-1], A: reference standard chromatogram; B: chromatogram in FF sample

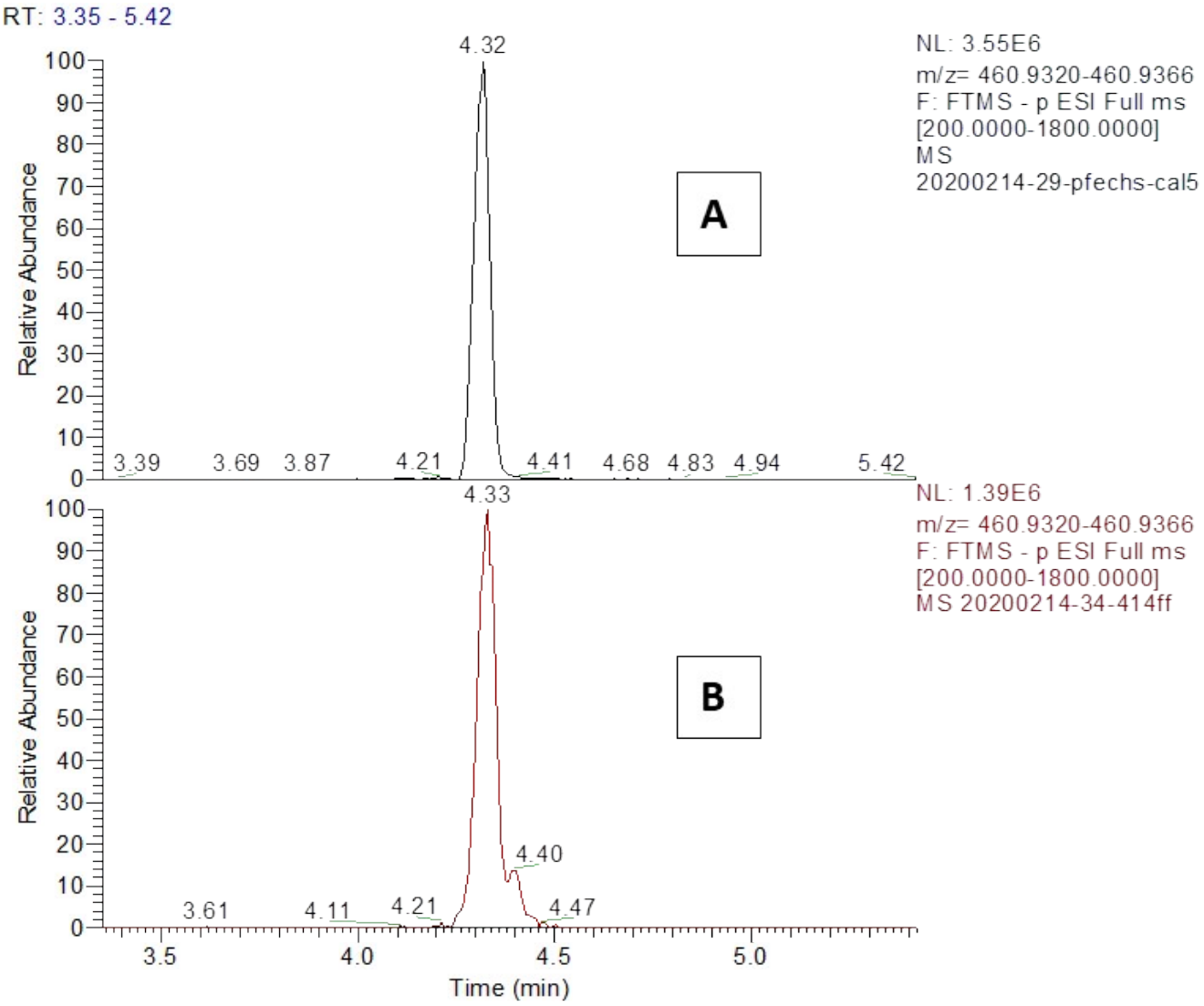

Figure S2, M: Perfluoro-ethylcyclohexane sulfonate (PFECHS) [CASno: 646-83-3], A: reference standard chromatogram; B: chromatogram in FF sample

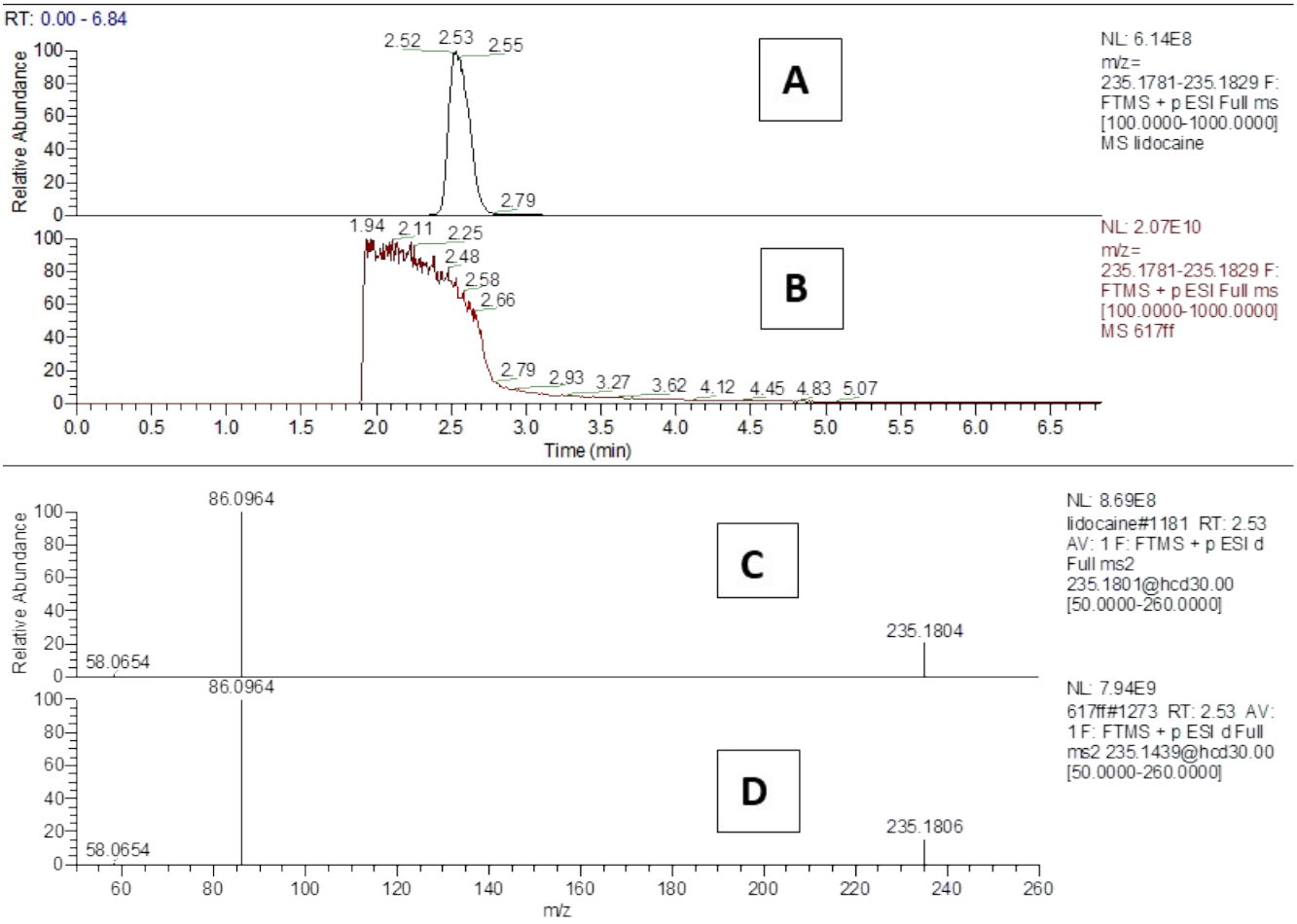

Figure S2, N: Lidocaine [CASno: 137-58-6]:
A: reference standard chromatogram; B: chromatogram in FF sample; C: MS2 spectra in reference standard; D MS2 spectra in FF sample

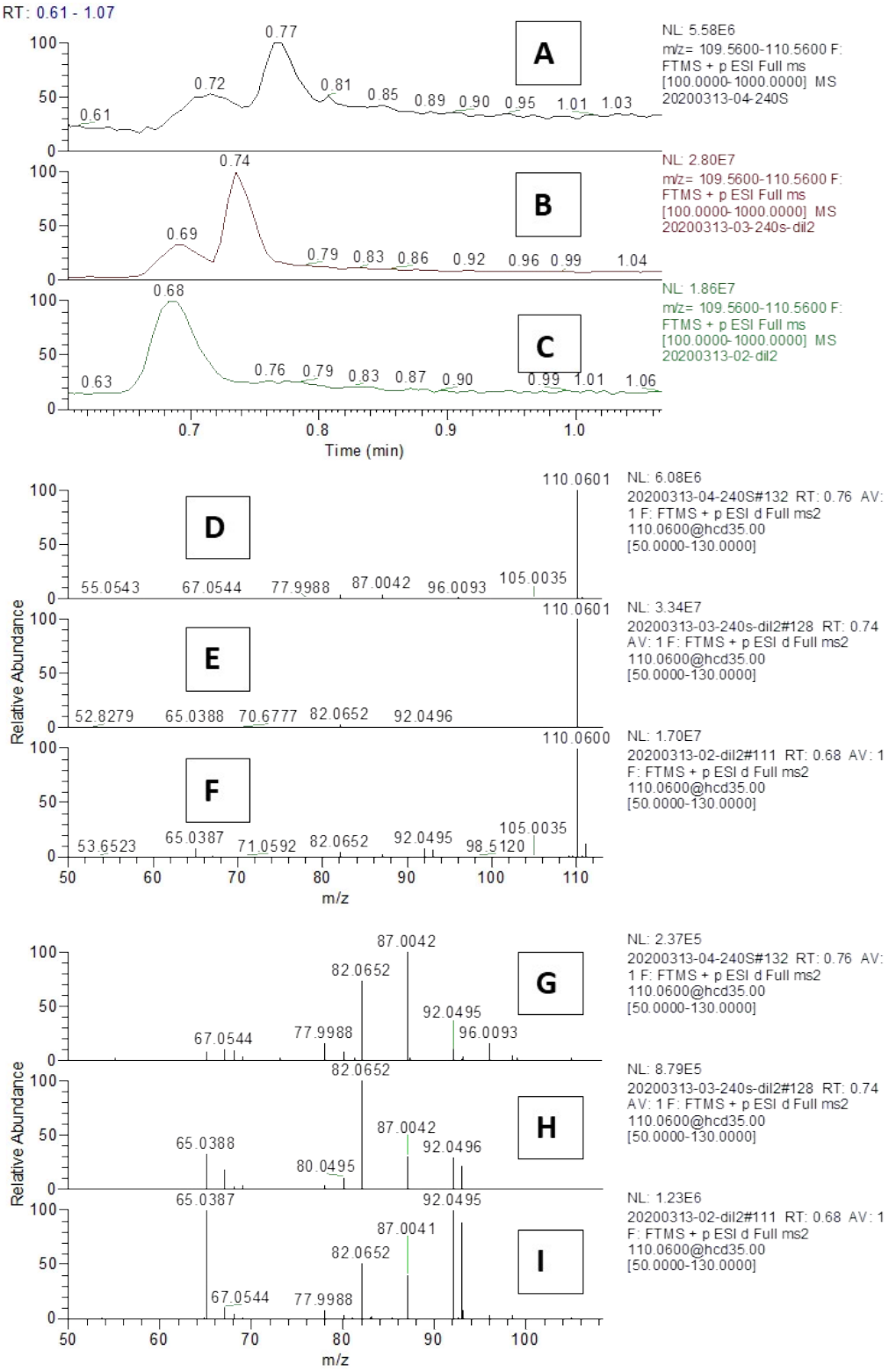

Figure S2, O: 4-aminophenol [CASno: 123-30-8]:
Chromatograms of exact masses of 4-aminophenol in A: a serum sample, B: the same serum sample spiked with 4-aminophenol and C: a reference standard; D-F: MS2 spectra of the same serum sample, spiked serum sample and reference standard, respectively; G-I: zoomed in on MS2 spectra of the same serum sample, spiked serum sample and reference standard, respectively.

The spiked sample shows a matrix effect resulting in a peak shift compared to the pure reference standard

Table S1. Internal standard mixture

	Name	Molecular formula	Mono-isotopic mass	Mixture conc. [µg/mL]	Kow (PubChem)	Manufacturer
LC+/-	1H-Benzotriazole-d4	$C_6D_4HN_3$	123.0735	10	1.44	Toronto Research Chemicals (TRC)
LC+	Caffeine-d9	$C_8HD_9N_4O_2$	203.1369	12.5	-0.07	TRC
LC-	Triclosan-d3	$C_{12}H_4Cl_3D_3O_2$	290.97	10	4.76	Dr Ehrenstorfer
LC+/-	Sulfamethoxazole-d4	$C10H_7D_4N_3O_3S$	257.0772	10	0.89	TRC
LC-	13C8-PFOA	$13C_8HF_{15}O_2$	422.0005	5	4.81 (estimated)	Wellington Laboratories
LC-	13C8-PFOS	$13C_8HF_{17}O_3S$	507.9643	5	4.49 (estimated)	Wellington Laboratories
LC+	Tri-ethyl phosphate-d15	$(C_2D_5O)3PO$	197.1649	5	0.8	TRC
LC+	Diglyme-d6	$C_6H_8D_6O_3$	140.132	10	-0.36	TRC
LC+	cotinine-d3	$C_{10}H_9D_3N_2O$	179.1138	10	0.07	Sigma Aldrich
LC-	sucralose-d6*	$C_{12}H_{13}Cl_3D_6O_8$	402.0522	9.9	-1 (estimated)	TRC
LC+	Tonalide-d3*	$C_{18}H_{23}D_3O$	261.2172	10	5.7	Dr Ehrenstorfer

Internal standard mixture was spiked to follicular fluid and serum before extraction. Used to access extraction recoveries and overall standard deviation.

*Compounds were only present in mixture spiked into follicular fluid samples. A mixture without those two was spiked into serum samples (due to them being missing at the time of mixture preparation)

Table S2. Reference standards used for confirmations of suspects

Suspected Chemical	Mass[a]	CASno	Confidence level (CL)	Manufacutrer
12-Hydroxyoctadecanoic acid	300.2664	106-14-9	1	Sigma Aldrich Sweden AB
Dodecanedioic acid	230.1518	693-23-2	1	Sigma Aldrich Sweden AB
3-Pyridinecarboxamide	122.0480	98-92-0	1	Merck
Dibutylamine	129.1518	111-92-2	1[b]	Merck
Tris(2-butoxyethyl) phosphate	398.2433	78-51-3	1[b]	Sigma Aldrich Sweden AB
4-aminophenol	109.0528	123-30-8	1	Merck
Lidocaine	234.1732	137-58-6	1	Sigma Aldrich Sweden AB
Perfluoroheptanoic acid (PFHpA)	363.9769	375-85-9	1	Wellington Laboratories
Perfluorooctanoic acid (PFOA)	413.9737	335-67-1	1	Wellington Laboratories
Perfluorononanoic acid (PFNA)	463.9705	375-95-1	1	Wellington Laboratories
Perfluorodecanoic acid (PFDA)	513.9673	335-76-2	1	Wellington Laboratories
Perfluoroundecanoic acid (PFUnDA)	563.9651	2058-94-8	1	Wellington Laboratories
Perfluoropentane sulfonate (PFPeS)	349.9470	2706-91-4	2b	Wellington Laboratories
Perfluorohexane sulfonate (PFHxS)	399.9439	355-46-4	1	Wellington Laboratories
Perfluoroheptane sulfonate (PFHpS)	449.9407	375-92-8	2b	Wellington Laboratories
Perfluorooctane sulfonate (PFOS)	499.9375	1763-23-1	1	Wellington Laboratories
9-Chlorohexadecafluoro-oxanonane sulfonate (9Cl-PF3ONS)	531.9029	756426-58-1	1	Wellington Laboratories
Perfluoro-ethylcyclohexane sulfonate (PFECHS)	461.9407	646-83-3	1	Wellington Laboratories
L-Tyrosine	181.0739	60-18-4	Not confirmed	Sigma Aldrich Sweden AB
Oleic acid	282.2559	112-80-1	Not confirmed	Merck
Triphenyl phosphate	326.0708	115-86-6	Not confirmed	Sigma Aldrich Sweden AB
aminohippuric acid	194.0691	61-78-9	Not confirmed	Sigma Aldrich Sweden AB
Benzoic acid, 4-hydroxy-, butyl ester	194.0943	94-26-8	Not confirmed	Sigma Aldrich Sweden AB
Benzoic acid, 4-hydroxy-, propyl ester	180.0786	94-13-3	Not confirmed	Sigma Aldrich Sweden AB
D-Glucose	180.0634	50-99-7	Not confirmed	Merck
Diisononyl phthalate	418.3083	68515-48-0	Not confirmed	Sigma Aldrich Sweden AB
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	226.1933	126-86-3	Not confirmed	Sigma Aldrich Sweden AB
L-Leucine	131.0946	61-90-5	Not confirmed	Sigma Aldrich Sweden AB

Reference standards for all confirmations. Tentative identifications listed as not confirmed in the table were removed from suspect list. [a]Monoisotopic mass, [b]only 2-3 samples above 3 x blank signal and interpreted as possible contamination from FF sampling procedure

Table S3. Compound discoverer 2.0 parameters

	Serum neg (pos)	**FF neg (pos)**
Select Spectra		
Lower RT Limit	0.5	0.5
Uppper RT Limit	15	15
Min Precursor Mass	100 Da	100 Da
Max Precursor Mass	1000 Da	1000 Da
Total Intensity Threshold	5000	5000
Align Retention Times		
Alignment Model	Adaptive Curve	Adaptive Curve
Mass Tolerance	5 ppm	5 ppm
Maximum Shift [min]	0.5	0.7 (0.5)
Detect Unknown Compounds		
General Settings		
Mass Tolerance	5 ppm	5 ppm
Intensity Tolerance [%]	30	30
S/N Tolerance	8 (10)	10 (8)
Min Peak Intennsity	500000 (1000000)	1000000 (500000)
Ions	[M-H]-1 ([M+H]+1; [M+K]+1; [M+Na]+1)	[M-H]-1 ([M+H]+1; [M+K]+1; [M+Na]+1)
Min Element Counts	C	C
Max Element Counts	C90 [13]C12 H190 Br10 Cl10 D15 F30 K2 N10 Na O15 P S5	C90 [13]C12 H190 Br10 Cl10 D15 F30 K2 N10 Na O15 P S5
Peak Detection		
Filter Peaks	True	True
Max Peak Width [min]	1	1
Remove Singlets	False	False
Min # Scans per Peak	5	5
Min # Isotopes	1	1
Group Unknown Compounds		
Compound Consolidation		
Mass Tolerance	5 ppm	5 ppm
RT Tolerance [min]	0.1 (0.15)	0.1 (0.15)
Best Compound Selection		
Rule #1	MS Order	MS Order
Rule #2	Ion Type	Ion Type
Preferred MS Order	MS2	MS2
Preferred Ion	[M-H]-1 ([M+H]+1)	[M-H]-1 ([M+H]+1)
Fill Gaps		
Mass Tolerance	5 ppm	5 ppm
RT Tolerance [min]	0.1	0.1
S/N Threshold	5	5
Mark Background Compounds		
Max Sample/Blank	5	5
Predict Compositions		
Prediction Settings		
Mass Tolerance	5 ppm	5 ppm
Min Element Counts	C	C
Max Element Counts	C90 H190 Br10 Cl10 F30 N10 O15 P2 S5	C90 H190 Br10 Cl10 F30 N10 O15 P2 S5
Min RDBE	-1	-1
Max RDBE	40	40
Min H/C	0.1	0.1
Max H/C	3	3
Max # Candidates	15	15
Pattern Matching		
Intensity Tolerance [%]	30	30

Intensity Threshold [%]	0.1	0.1
Min # Isotopes	2	2
Min Spectral Fit [%]	10	10
Search Mass Lists		
Consider Retention Time	False	False
Mass Tolerance	5 ppm	5 ppm
Search mzCloud		
Search Settings		
Compound Classes	All	All
Match Ion Activation Type	False	False
Match Ion Activation Energy	Match with Tolerance	Match with Tolerance
Ion Activation Energy Tolerance	40	40
Apply intensity threshold	True	True
Precursor Mass Tolerance	5 ppm	5 ppm
FT Fragment Mass Tolerance	10 ppm	10 ppm
Search Algorithm	HighChem HighRes	HighChem HighRes
Library	Reference	Reference
Post Processing	Recalibrated	Recalibrated
Match factor threshold	50	50
Max. # results per compound and spectrum	10	10

Parameters were applied to the four individual datasets resulting in four feature lists for serum and FF in negative and positive mode, respectively, parameters was used in Tier 1 and Tier 3.

Table S4. Compound discoverer 3.1 parameters

	neg (pos if different from neg)
Select Spectra	
Lower RT Limit	0.5
Uppper RT Limit	15
Min Precursor Mass	100 Da
Max Precursor Mass	1000 Da
Total Intensity Threshold	5000
Align Retention Times	
Alignment Model	Adaptive Curve
Mass Tolerance	5 ppm
Maximum Shift [min]	1.5
Detect Unknown Compounds	
General Settings	
Mass Tolerance	5 ppm
Intensity Tolerance [%]	30
S/N Tolerance	15 (30)
Min Peak Intesntiy	1500000 (5000000)
Ions	$[M-H]^-$ ($[M+H]^+$)
Min Element Counts	C
Max Element Counts	C90 [13]C12 H190 Br10 Cl10 D15 F30 K2 N10 Na O15 P S5
Peak Detection	
Filter Peaks	True
Max Peak Width [min]	1
Remove Singlets	False
Min # Scans per Peak	5
Min # Isotopes	1
Group Unknown Compounds	
Compound Consolidation	
Mass Tolerance	5 ppm
RT Tolerance [min]	0.4 (0.6)
Fragment Data Selection	
Preferred Ions	[M-H]-1 ([M+H]+1)
Fill Gaps	
Mass Tolerance	5 ppm
S/N Threshold	5 (20)
Use Real Peak Detection	True
Mark Background Compounds	
Max Sample/Blank	5
Search mzCloud	
Search Settings	
Compound Classes	All
Precursor Mass Tolerance	5 ppm
FT Fragment Mass Tolerance	10 ppm
DDA Search	
Identity Search	HigChem HighRes
Match Ion Activation Type	True
Match Ion Activation Energy	Match with Tolerance
Ion Activation Energy Tolerance	20
Apply intensity threshold	True
Max. # results per compound and spectrum	10

Compound discoverer parameters applied to two combined datasets for negative and positive mode, respectively, including both FF and serum data. Resulting data was used in tier 2.

Table S5. Level of confidence of identification of features

Confidence level [CL]			Description
Structure identification	1		**Confirmed substance** via appropriate measurement of a reference standard with MS, MS/MS and retention time matching
	2	a	**Probable structure** – library match. Unambiguous spectrum-structure match
		b	**Probable structure –** diagnostic. No other structure fit the experimental information, but without reference match
Substance class, formula	3		**Tentative candidate** with evidence for possible structure(s) but insufficient for one exact structure
	4		**Unequivocal molecular formula** with formula unambiguously assigned using spectral information but insufficient for possible structures
	5		**Exact mass** but with no equivocal information about the formula or structure exist

Confidence levels from Schymanski et al., 2014.[1]

Table S6. Features before and after blank subtraction

	No. features before blank subtraction	No. features after blank subtraction
FF pos	19126	14735
FF neg	9777	6244
Serum pos	9342	8020
Serum neg	19316	8849
Combined pos	3483	3034
Combined neg	21098	9128

Table S7. Median ratios of internal standard peak areas between follicular fluid (FF) and serum (n=116).

	Mode	Median Ratio (range)	RSD
diglyme-d6	Pos	0.39 (0.13-107.44) 0.38 (0.13-0.87)*	482.6 30.2*
Sulfamethoxazole-d4	Pos	0.22 (0.13-0.39)	17.0
caffeine-d9	Pos	0.21 (0.13-0.34)	19.3
tri-ethyl-phosphate-d15	Pos	0.22 (0.002-2.31)	101.1
13C8-PFOS	Neg	0.86 (0.45-1,22)	16.5
Triclosan-d3	Neg	0.89 (0.002-1.71)	35.6
13C8-PFOA	Neg	0.60 (0.29-0.93)	15.8
Sulfamethoxazole-d4	Neg	0.015 (0.006-0.24)	123.7

A value of one indicate equivalent matrix effect in serum as in follicular fluid. Ratios shown for all samples (including the relative standard deviation (RSD) in samples).

* Four samples showed a very low sample area in serum (ca 100 times lower), resulting in very high ratios and thus a high RSD of 482.6 %. These numbers are presenting the median, range and RSD without these outliers (thus n=112).

Table S8. Relative standard deviations (RSDs) of peak areas of internal standards

	Follicular fluid					Serum				
	Average RT after alignment	Average Δppm mass deviation	Peak area RSD of IS runs (n=14)	Peak area RSD of controls (n=10)	Peak area RSD of all samples (n=160)	Average RT after alignment	Average Δppm mass deviation	Peak area RSD of IS runs (n=10)	Peak area RSD of controls (n=6)	Peak area RSD of all samples (n=116)
Positive mode										
Diglyme-d6	1.93	0.26	5.3	14.2	23.2	2.04	0.72	12.4	15.9	29.9
Tri-ethyl phosphate-d15	3.66	0.8	4.6	11.1	16.9	3.83	-0.18	4.1	7.8	21.0
cotinine-d3	0.74	1.0	7.7	12.8	22.7	0.79	0.97	6.2	7.2	16.8
Tonalide-D3	8.91	0.54	16.3	12.0	16.8	-	-	-	-	-
Sulfamethoxazole-d4	3.51	1.51	4.1	13.2	15.3	3.65	1.08	2.7	7.1	14.4
Caffeine-d9	2.29	1.18	5.2	13.3	18.1	2.4	0.94	4.0	5.4	15.7
1H-benzotriazole	2.6	0.69	4.2	9.9	18.8	2.73	0.86	1.8	7.7	14.0
Negative mode										
13C8-PFOS	6.6	-2.02	19.7	10.1	12.8	6.47	-2.06	11.1	9.6	13.2
13C8-PFOA	5.79	-0.81	12.8	10.3	14.9	5.63	-0.68	6.9	7.3	13.6
Triclosan-d3	7.79	0.32	21.0	11.8	16.3	7.67	0.49	5.1	8.6	18.7
Sulfamethoxazole-d4	3.85	-0.004	15.1	11.1	17.0	3.64	0.43	13.5	6.5	16.3
1H-Benzotriazole-d4	2.93	-8.48	10.1	4.5	22.1	2.74	-7.88	12.8	14.2	21.9
Sucralose-d6	2.87	0.46	8.9	10.1	17.9	-	-	-	-	-

Relative standard deviations of peak areas for pure internal standard-mix solved in acetonitrile and water (IS), spiked in control and all samples, for both follicular fluid and serum

Table S9: Tentative identifications from tier 1-3 including confidence level (CL) of identification

Tier	Molecular weight	Suspected compound	Database	Charge	CL
1	300.26643	12-Hydroxyoctadecanoic acid	KemI Market List	neg	1
1	230.15146	Dodecanedioic acid	KemI Market List	neg	1
1	122.04816	3-Pyridinecarboxamide[a]	KemI Market List	pos	1
1	129.15185	N-Butyl-1-butanamine[a]	KemI Market List	pos	1
1	398.24403	Tris(2-butoxyethyl) phosphate	KemI Market List	pos	1
1	363.97712	Perfluoroheptanoic acid (PFHpA)	In-house PFAS database	neg	1
1	413.97402	Perfluorooctanoic acid (PFOA)	In-house PFAS database	neg	1
1	463.97109	Perfluorononanoic acid (PFNA)	In-house PFAS database	neg	1
1	513.96773	Perfluorodecanoic acid (PFDA)	In-house PFAS database	neg	1
1	563.96479	Perfluoroundecanoic acid (PFUnDA)	In-house PFAS database	neg	1
1	399.94404	Perfluorohexane sulfonate (PFHxS)	In-house PFAS database	neg	1
1	499.93789	Perfluorooctane sulfonate (PFOS)	In-house PFAS database	neg	1
1	531.90322	9-Chlorohexadecafluoro-oxanonane sulfonate (9Cl-PF3ONS)	In-house PFAS database	neg	1
1	461.94127	Perfluoro-ethylcyclohexane sulfonate (PFECHS)	In-house PFAS database	neg	1
1	349.94736	Perfluoropentane sulfonate (PFPeS)	In-house PFAS database	neg	2b
1	449.94121	Perfluoroheptane sulfonate (PFHpS)	In-house PFAS database	neg	2b
1	181.07405	L-Tyrosine	KemI Market List	pos	3
1	362.24591	1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7-rich	KemI Market List	neg	5
1	424.35553	1,2-cyclohexane dicarboxylic acid dinonyl ester branched and linear	KemI Market List	neg	5
1	216.17211	2,2,4-Trimethyl-1,3-pentanediol monoisobutyrate	KemI Market List	neg	5
1	172.14554	2-Ethylhexyl acetate	KemI Market List	neg	5
1	172.14554	2H-Pyran-4-ol, tetrahydro-4-methyl-2-(2-methylpropyl)-	KemI Market List	neg	5
1	326.1915	4-Dodecylbenzenesulfonic acid	KemI Market List	neg	5
1	282.25584	9-Octadecenoic acid (9Z)-, compd. with morpholine (1:1)	KemI Market List	neg	5
1	282.25584	9-Octadecenoic acid (9Z)-, potassium salt (1:1)	KemI Market List	neg	5
1	282.25584	9-Octadecenoic acid (Z)-, sodium salt	KemI Market List	neg	5
1	298.25066	9-Octadecenoic acid, 12-hydroxy-, zinc salt (2:1), (9Z,12R)-	KemI Market List	neg	5
1	326.1915	Benzenesulfonic acid, C10-16-alkyl derivs.	KemI Market List	neg	5
1	194.09367	Benzoic acid, 4-hydroxy-, 2-methylpropyl ester	KemI Market List	neg	5
1	180.07785	Benzoic acid, 4-hydroxy-, propyl ester	KemI Market List	neg	5
1	390.27738	Bis(2-ethylhexyl) terephthalate	KemI Market List	neg	5
1	282.25584	Carnauba wax, reaction products with borax, morpholine and oleic acid	KemI Market List	neg	5

1	172.14554	Cyclohexanemethanol, 2-hydroxy-.alpha.,.alpha.,4-trimethyl-	KemI Market List	neg	5
1	172.14554	Decanoic acid	KemI Market List	neg	5
1	172.14554	Decanoic acid, mixed diesters with octanoic acid and propylene glycol	KemI Market List	neg	5
1	172.14554	Decanoic acid, mixed esters with neopentyl glycol and octanoic acid	KemI Market List	neg	5
1	390.27738	Di(2-ethylhexyl) phthalate	KemI Market List	neg	5
1	426.37122	Diisodecyl hexanedioate	KemI Market List	neg	5
1	230.15146	Diisopropyl adipate	KemI Market List	neg	5
1	326.1915	Dodecylbenzene sulfonate triethanolamine (1:1)	KemI Market List	neg	5
1	499.30505	Ethanol, 2-[2-(2-methoxyethoxy)ethoxy]-, triester with boric acid	KemI Market List	neg	5
1	470.36079	Heptanoic acid, 1,1'-[2-ethyl-2-[[(1-oxoheptyl)oxy]methyl]-1,3-propanediyl] ester	KemI Market List	neg	5
1	180.0639	Maltodextrin	KemI Market List	neg	5
1	300.26643	Octadecanoic acid, 12-hydroxy-, calcium salt (2:1)	KemI Market List	neg	5
1	428.31528	Octadecanoic acid, 2-(1-carboxyethoxy)-1-methyl-2-oxoethyl ester, sodium salt (1:1)	KemI Market List	neg	5
1	172.14554	Octanal, 7-hydroxy-3,7-dimethyl-	KemI Market List	neg	5
1	474.37106	Phthalic acid, diundecyl ester, branched and linear	KemI Market List	neg	5
1	102.06773	Propyl acetate	KemI Market List	neg	5
1	326.1915	Sodium dodecylbenzenesulfonate	KemI Market List	neg	5
1	428.31528	Sorbitan, mono-9-octadecenoate, (Z)-	KemI Market List	neg	5
1	308.03557	Sulisobenzone	KemI Market List	neg	5
1	198.07463	Syrups, hydrolyzed starch	KemI Market List	neg	5
1	234.1101	Triethylene glycol, diacetate	KemI Market List	neg	5
1	404.29293	1,2-Benzenedicarboxylic acid, di-C8-10-alkyl esters	KemI Market List	pos	5
1	418.30849	1,2-Benzenedicarboxylic acid, di-C9-11-alkyl esters	KemI Market List	pos	5
1	271.25147	1-Dodecanaminium, N-(carboxymethyl)-N,N-dimethyl-, inner salt	KemI Market List	pos	5
1	300.20886	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, (1R,4aS,10aR)-	KemI Market List	pos	5
1	164.08379	2-Phenylethyl acetate	KemI Market List	pos	5
1	134.07313	3-Phenyl-2-propen-1-ol	KemI Market List	pos	5
1	108.05746	4-Methylphenol	KemI Market List	pos	5
1	234.17303	Acetamide, 2-(diethylamino)-N-(2,6-dimethylphenyl)-, monohydrochloride	KemI Market List	pos	5
1	312.14776	Acetic acid, (4-chloro-2-methylphenoxy)-, isooctyl ester	KemI Market List	pos	5
1	232.21916	Benzene, C10-13-alkyl derivs.	KemI Market List	pos	5
1	164.08379	Benzenemethanol, alpha-methyl-, acetate	KemI Market List	pos	5
1	142.09935	Butyl methacrylate	KemI Market List	pos	5
1	289.06635	Cobalt, borate neodecanoate complexes	KemI Market List	pos	5
1	222.08942	Diethyl phthalate	KemI Market List	pos	5
1	103.09971	Dimepranol	KemI Market List	pos	5
1	215.15225	Ethyl 3-(N-butylacetamido)propionate	KemI Market List	pos	5

1	106.07829	Ethylbenzene	KemI Market List	pos	5
1	330.27702	Glycerides, C16-22	KemI Market List	pos	5
1	142.09935	Isobutyl methacrylate	KemI Market List	pos	5
1	234.17303	Lidocaine	KemI Market List	pos	5
1	199.19369	N,N-Dimethyldecanamide	KemI Market List	pos	5
1	188.10487	Nonanedioic acid	KemI Market List	pos	5
1	188.10487	Nonanedioic acid, compd. with 2,2'-iminobis[ethanol] (1:?)	KemI Market List	pos	5
1	283.28769	Octadecanamide	KemI Market List	pos	5
1	356.29274	Octanoic acid, 1,1'-(2,2-dimethyl-1,3-propanediyl) ester	KemI Market List	pos	5
1	164.08379	Phenol, 2-methoxy-4-(1-propenyl)-	KemI Market List	pos	5
1	129.15185	Phosphorothioic acid, O,O-dibutyl ester, compd. with 1-octanamine (1:1)	KemI Market List	pos	5
1	302.22455	Rosin	KemI Market List	pos	5
1	604.41671	Sorbitan, mono-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs., (Z)-	KemI Market List	pos	5
2	234.17312	Lidocaine	mzCloud	pos	1
2	314.22439	Progesterone	mzCloud	pos	2a
2	288.20927	Epitestosterone	mzCloud	pos	2a
2	330.21934	17α-Hydroxyprogesterone	mzCloud	pos	2a
2	330.21968	17α-Hydroxyprogesterone	mzCloud	pos	2a
2	316.24042	17-(1-Hydroxyethyl)-10,13-dimethyl-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-one	mzCloud	pos	2a
2	350.28237	2,5-Furandione, dihydro-3-(octadecenyl)-	KemI Market List	neg	5
2	332.23522	Androstan-3-one, 17-(acetyloxy)-, (5?,17?)-	KemI Market List	neg	5
2	314.12697	Benzoic acid, 3,3'-methylenebis[6-amino-, 1,1'-dimethyl ester	KemI Market List	neg	5
2	314.12683	Benzoic acid, 3,3'-methylenebis[6-amino-, 1,1'-dimethyl ester	KemI Market List	neg	5
2	334.1241	N-[4-(Dimethylamino)-3,5-difluorobenzoyl]-N'-(pyridin-3-ylmethyl)urea	mzCloud	neg	5
2	324.26656	Labdanolic acid	mzCloud	neg	5
2	368.12954	Estriol 17-sulfate	mzCloud	neg	5
2	346.21472	Corticosterone	mzCloud	neg	5
2	138.04072	4-Nitroaniline	mzCloud	neg	5
2	406.19955	20β-Dihydroprednisone	mzCloud	neg	5
2	200.01988	1-([(Ethylsulfonyl)methyl]sulfonyl)ethane	mzCloud	neg	5
2	145.06151	1,3-Diiminoisoindoline	mzCloud	neg	5
2	244.2051	(R)-3-Hydroxy myristic acid	mzCloud	neg	5
2	376.2981	(+)-CP 55,940	mzCloud	neg	5
2	674.41636	PPG Acrylate n10	mzCloud	pos	5
2	396.30322	Diosgenin	mzCloud	pos	5
2	174.0271	2-Anisic acid	mzCloud	pos	5
2	552.24946	(5ξ,6α,7α,9ξ,16ξ)-16-(β-D-Glucopyranosyloxy)-6,7,17-trihydroxykauran-19-oic acid	mzCloud	pos	5
3	300.26645	12-Hydroxyoctadecanoic acid	KemI Market List	neg	1
3	300.26654	12-Hydroxyoctadecanoic acid	KemI Market List	neg	1

3	109.05291	4-Aminophenol	KemI Market List / mzCloud	pos	1
3	133.05289	5-Hydroxyindole	mzCloud	pos	2a
3	218.02451	Benzenemethanesulfonic acid, .alpha.-hydroxy-4-methoxy-, sodium salt (1:1)	KemI Market List	neg	4
3	232.04031	Ethenylsilanetriyl triacetate	KemI Market List	neg	4
3	278.22456	Linolenic acid	KemI Market List	neg	4
3	226.19297	2,4,7,9-Tetramethyl-5-decyne-4,7-diol	KemI Market List	neg	4
3	169.07306	3,4-Pyridinedimethanol, 5-hydroxy-6-methyl-, hydrochloride	KemI Market List	neg	4
3	516.0119	Benzeneacetic acid, ?,?'-(1,2-ethanediyldiimino)bis[2-hydroxy-5-sulfo-	KemI Market List	neg	4
3	362.10607	Benzoxazole, 2,2'-(1,4-naphthalenediyl)bis-	KemI Market List	pos	4
3	289.06667	Cobalt, borate neodecanoate complexes	KemI Market List	pos	4
3	219.03818	1H-Imidazole-2-carbonitrile, 5-chloro-4-(4-methylphenyl)-	KemI Market List	pos	4
3	364.30895	7-Oxa-3,20-diazadispiro[5.1.11.2]heneicosan-21-one, 2,2,4,4-tetramethyl-	KemI Market List	pos	4
3	528.07512	1-Octanesulfonamide, N-[3-(dimethylamino)propyl]-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-, N-oxide	KemI Market List	neg	5
3	386.15681	Butanoic acid, 3-oxo-, 1,1'-[2-[(1,3-dioxobutoxy)methyl]-2-ethyl-1,3-propanediyl] ester	KemI Market List	neg	5
3	312.23019	9-Octadecenedioic acid, (9Z)-	KemI Market List	neg	5
3	249.99678	4-(2,4-Dichlorophenoxy)butyric acid	KemI Market List	neg	5
3	338.24579	Naphthenic acids, zinc salts	KemI Market List	neg	5
3	242.18801	Acetic acid, 2-ethoxy-, (1R,2S,5R)-5-methyl-2-(1-methylethyl)cyclohexyl ester, rel-	KemI Market List	neg	5
3	414.16305	1,5-Benzothiazepin-4(5H)-one, 3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride, (2S-cis)-	KemI Market List	neg	5
3	199.19369	N,N-Dimethyldecanamide	KemI Market List	pos	5
3	392.27187	Phenol, 2,2'-methylenebis[6-cyclohexyl-4-methyl-	KemI Market List	pos	5
3	165.07927	Benzoic acid, 4-amino-, ethyl ester	KemI Market List	pos	5
3	239.09489	9H-Carbazole, 4-(2-oxiranylmethoxy)-	KemI Market List	pos	5
3	197.14158	Kinase (enzyme-activating), strepto- (no match in chemspider)	KemI Market List	pos	5
3	165.07908	Benzoic acid, 4-amino-, ethyl ester	KemI Market List	pos	5
3	283.28709	Octadecanamide	KemI Market List	pos	5
3	244.12137	1,3-Bis(2-isocyanatopropan-2-yl)benzene	KemI Market List	pos	5
3	136.0888	3-Phenyl-1-propanol	KemI Market List	pos	5
3	108.09395	4-Vinylcyclohexene	KemI Market List	pos	5

Features detected in follicular fluid and serum within the tiers 1-3 with tentative identifications from databases presented in the table. [a]only 2-3 samples above 3 x blank-signal and interpreted as contamination from FF sampling

Table S10: Tentative identifications from tier 1-3 of including confidence level (CL) of identifications, features without suspected compound

Tier	Molecular weight	Retention time	Charge	CL
2	188.02031	2.88	neg	5
2	188.0203	2.907	neg	5
2	230.06365	4.323	neg	5
2	188.0204	3.031	neg	5
2	174.0052	2.13	neg	5
2	214.03471	4.014	neg	5
2	174.00517	2.065	neg	5
2	202.0356	3.633	neg	5
2	231.02268	0.867	neg	5
2	130.07289	2.357	neg	5
2	130.07288	2.422	neg	5
2	130.07294	2.279	neg	5
2	213.01419	2.688	neg	5
2	189.9999	1.942	neg	5
2	189.99996	1.861	neg	5
2	382.14509	4.323	neg	5
2	204.01494	2.745	neg	5
2	352.13458	4.396	neg	5
2	205.99308	1.815	neg	5
2	130.07289	2.109	neg	5
2	231.06679	4.313	neg	5
2	247.0166	1.567	neg	5
2	130.073	2.176	neg	5
2	245.91689	5.047	neg	5
2	130.0729	2.561	neg	5
2	214.03473	3.894	neg	5
2	130.0729	2.03	neg	5
2	633.32663	8.066	neg	5
2	108.06223	2.88	neg	5
2	247.91392	5.052	neg	5
2	189.99985	2.067	neg	5
2	256.00321	2.908	neg	5
2	215.03789	4.009	neg	5
2	175.0086	2.133	neg	5
2	282.10799	2.357	neg	5
2	496.34065	7.112	neg	5
2	383.14801	4.317	neg	5
2	175.00853	2.065	neg	5
2	353.13746	4.394	neg	5
2	232.02594	1.686	neg	5
2	108.06223	2.908	neg	5
2	203.03869	3.628	neg	5
2	214.01748	2.456	neg	5
2	173.97184	10.201	neg	5
2	228.04956	4.187	neg	5
2	229.00846	2.273	neg	5
2	326.28233	9.996	neg	5
2	230.87093	0.861	neg	5
2	180.08561	6.138	neg	5
2	216.03047	4.009	neg	5

2	139.95709	0.851	neg	5
2	562.31213	7.387	neg	5
2	234.16459	7.819	neg	5
2	134.08264	4.01	neg	5
2	450.13273	4.322	neg	5
2	233.01849	1.677	neg	5
2	233.01847	1.701	neg	5
2	204.03136	3.631	neg	5
2	504.14065	4.787	neg	5
2	215.01007	2.458	neg	5
2	416.29304	7.362	neg	5
2	372.26655	8.995	neg	5
2	249.91093	5.04	neg	5
2	328.00856	2.361	neg	5
2	500.31158	8.214	neg	5
2	420.12225	4.396	neg	5
2	506.1564	4.994	neg	5
2	191.00319	1.934	neg	5
2	241.98888	2.14	neg	5
2	205.017	2.734	neg	5
2	218.02856	3.508	neg	5
2	266.0379	2.358	neg	5
2	414.2385	8.666	neg	5
2	390.23856	8.832	neg	5
2	282.10802	2.126	neg	5
2	108.06222	3.078	neg	5
2	151.07154	1.991	neg	5
2	260.0209	2.129	neg	5
2	348.98466	2.458	neg	5
2	392.25424	9.312	neg	5
2	325.26989	9.314	neg	5
2	224.01816	3.808	neg	5
2	402.1116	6.212	neg	5
2	151.07154	1.697	neg	5
2	215.04539	2.35	neg	5
2	247.08365	3.346	neg	5
2	227.02914	3.792	neg	5
2	334.02552	2.356	neg	5
2	165.05081	1.991	neg	5
2	178.06983	5.909	neg	5
2	254.15338	5.855	neg	5
2	458.21295	10.297	neg	5
2	188.11105	3.834	neg	5
2	432.19726	10.107	neg	5
2	233.93464	10.327	neg	5
2	206.00953	2.733	neg	5
2	147.9822	0.761	neg	5
2	328.00858	2.124	neg	5
2	395.99596	2.355	neg	5
2	532.1721	5.518	neg	5
2	179.06518	2.617	neg	5
2	184.04078	1.401	neg	5
2	362.20721	8.195	neg	5

2	534.18775	5.171	neg	5
2	230.0275	3.438	neg	5
2	240.09543	3.921	neg	5
2	205.01707	2.296	neg	5
2	350.09555	2.358	neg	5
2	384.14109	4.321	neg	5
2	110.04402	1.934	neg	5
2	354.13042	4.395	neg	5
2	568.12697	4.993	neg	5
2	484.31685	10.091	neg	5
2	395.99596	2.127	neg	5
2	266.03793	2.129	neg	5
2	155.03846	2.769	neg	5
2	344.07856	2.358	neg	5
2	334.02549	2.131	neg	5
2	516.04554	2.801	neg	5
2	215.03795	3.894	neg	5
2	574.14394	4.994	neg	5
2	232.00677	2.286	neg	5
2	463.98362	2.125	neg	5
2	412.06611	2.358	neg	5
2	400.29778	7.389	neg	5
2	463.98363	2.348	neg	5
2	228.03244	3.79	neg	5
2	167.06549	1.56	neg	5
2	173.97253	2.714	neg	5
2	402.01295	2.347	neg	5
2	508.30402	5.719	neg	5
2	418.08309	2.357	neg	5
2	506.15664	4.921	neg	5
2	456.19729	9.851	neg	5
2	374.28242	9.457	neg	5
2	518.12005	4.322	neg	5
2	374.28242	9.271	neg	5
2	402.013	2.134	neg	5
2	414.27741	7.322	neg	5
2	199.05923	2.356	neg	5
2	370.12542	4.59	neg	5
2	244.04464	2.357	neg	5
2	480.05349	2.358	neg	5
2	304.02833	2.357	neg	5
2	210.16669	5.855	neg	5
2	312.03091	2.357	neg	5
2	568.29917	8.213	neg	5
2	187.12712	3.043	neg	5
2	600.15982	5.518	neg	5
2	508.304	5.662	neg	5
2	247.04715	2.617	neg	5
2	213.0144	3.169	neg	5
2	202.16256	6.077	neg	5
2	484.24149	9.135	neg	5
2	548.04099	2.357	neg	5
2	222.16567	7.812	neg	5

2	240.17537	6.935	neg	5
2	488.10964	4.396	neg	5
2	380.0184	2.372	neg	5
2	594.14283	5.518	neg	5
2	222.96702	5.326	neg	5
2	188.14747	5.712	neg	5
2	574.14407	4.92	neg	5
2	531.97089	2.365	neg	5
2	460.2417	9.313	neg	5
2	389.9791	2.357	neg	5
2	195.91864	0.635	neg	5
2	486.07046	2.357	neg	5
2	181.1173	5.736	neg	5
2	525.95395	2.356	neg	5
2	457.9666	2.355	neg	5
2	201.03055	1.397	neg	5
2	196.15166	6.516	neg	5
2	662.13047	5.518	neg	5
2	562.28225	8.212	neg	5
2	508.30399	5.646	neg	5
2	215.15702	4.197	neg	5
2	313.9928	1.39	neg	5
2	255.1567	5.855	neg	5
2	730.11804	5.517	neg	5
2	252.02377	1.396	neg	5
2	320.00975	1.387	neg	5
2	668.14739	5.517	neg	5
2	165.0508	3.48	neg	5
2	144.0146	0.809	neg	5
2	506.15663	4.397	neg	5
2	160.11783	4.298	neg	5
2	194.09959	5.799	neg	5
2	228.10127	4.297	neg	5
2	452.09787	5.859	neg	5
2	241.92148	0.676	neg	5
2	196.99396	0.716	pos	5
2	204.01745	0.819	pos	5
2	208.03456	2.407	pos	5
2	235.1763	3.053	pos	5
2	256.15534	3.056	pos	5
2	276.04273	1.983	pos	5
2	277.0244	2.354	pos	5
2	278.65221	7.75	pos	5
2	287.20996	4.953	pos	5
2	289.63217	7.181	pos	5
2	296.67554	8.14	pos	5
2	296.68418	7.319	pos	5
2	298.22996	7.406	pos	5
2	298.23002	8.222	pos	5
2	299.23327	8.221	pos	5
2	308.01476	2.857	pos	5
2	315.22789	7.756	pos	5
2	316.23069	7.751	pos	5

2	317.18917	8.167	pos	5
2	317.24367	7.408	pos	5
2	328.20428	7.273	pos	5
2	331.22299	6.43	pos	5
2	331.2232	5.391	pos	5
2	332.22589	6.43	pos	5
2	349.0413	2.845	pos	5
2	355.25113	7.752	pos	5
2	356.25443	7.751	pos	5
2	371.24628	6.43	pos	5
2	386.74923	7.745	pos	5
2	397.30648	10.54	pos	5
2	398.31866	9.998	pos	5
2	398.31867	9.882	pos	5
2	454.30657	7.438	pos	5
2	454.32768	8.285	pos	5
2	480.35841	9.58	pos	5
2	481.3617	9.582	pos	5
2	513.21566	4.721	pos	5
2	529.19301	4.722	pos	5
2	530.19583	4.719	pos	5
2	531.20879	4.863	pos	5
2	531.20884	4.928	pos	5
2	543.26286	5.103	pos	5
2	557.22461	5.453	pos	5
2	559.24026	5.103	pos	5
2	573.29876	7.318	pos	5
3	413.76914	7.181	pos	5
3	497.28082	4.913	neg	5
3	440.08356	1.775	pos	5
3	515.29155	4.658	neg	5
3	497.18444	4.108	pos	5
3	660.9652	6.23	pos	5
3	380.3081	9.402	pos	5
3	313.74227	8.945	pos	5
3	335.12694	6.318	neg	5
3	313.2406	8.946	pos	5
3	388.19234	3.807	neg	5
3	419.31905	9.272	pos	5
3	396.30322	7.671	pos	5
3	194.10343	0.737	pos	5
3	279.55606	1.771	pos	5
3	190.99603	1.966	neg	5
3	562.27297	6.624	pos	5
3	566.40879	11.707	pos	5
3	295.16141	7.414	pos	5
3	584.26445	7.196	pos	5
3	805.54779	7.857	neg	5
3	479.17374	5.922	pos	5
3	648.27607	8.681	pos	5
3	415.3171	9.098	pos	5
3	836.2567	4.893	neg	5
3	298.14534	7.134	pos	5

3	312.2455	7.668	pos	5
3	577.47946	10.177	pos	5
3	518.0613	1.773	pos	5
3	516.27706	6.918	pos	5
3	401.61291	7.442	pos	5
3	268.67478	9.357	pos	5
3	444.28827	6.809	pos	5
3	288.68647	8.597	pos	5
3	268.10068	4.142	pos	5
3	313.23342	7.709	neg	5
3	399.32203	9.9	pos	5
3	433.31933	7.331	neg	5
3	529.15704	4.299	pos	5
3	453.75928	0.616	pos	5
3	179.04209	1.764	pos	5
3	185.12926	7.818	pos	5
3	378.29274	6.056	pos	5
3	325.98917	0.738	pos	5
3	481.31749	7.805	pos	5
3	388.1923	4.054	neg	5
3	260.12339	9.966	pos	5
3	450.13228	4.451	neg	5
3	440.2564	7.825	neg	5
3	513.3065	7.634	neg	5
3	417.32209	10.26	pos	5
3	182.0606	3.525	neg	5
3	280.68908	8.767	pos	5
3	187.12023	4.002	neg	5
3	365.88506	0.6	neg	5
3	367.01572	1.382	pos	5
3	279.17498	8.598	pos	5
3	225.89824	0.747	neg	5
3	412.29799	9.049	pos	5
3	386.60663	7.747	pos	5
3	378.29262	7.198	pos	5
3	334.1238	6.318	neg	5
3	221.09165	0.742	pos	5
3	380.30827	8.24	pos	5
3	127.88722	7.767	neg	5
3	397.30643	7.668	pos	5
3	288.18034	7.638	pos	5
3	287.67026	6.921	pos	5
3	789.26487	8.699	pos	5
3	135.05471	0.722	pos	5
3	630.44778	9.571	pos	5
3	482.24636	7.159	pos	5
3	382.03613	4.107	neg	5
3	393.32468	9.43	pos	5
3	207.13363	9.218	pos	5
3	385.00813	1.76	pos	5
3	380.30809	8.766	pos	5
3	389.16298	7.693	neg	5
3	188.94838	10.347	neg	5

3	205.01195	3.025	neg	5
3	276.67216	7.073	pos	5
3	301.13172	4.655	pos	5
3	415.31699	9.266	pos	5
3	287.66995	8.841	pos	5
3	255.02007	4.163	neg	5
3	229.02914	2.628	pos	5
3	389.19557	4.054	neg	5
3	448.31918	7.402	neg	5
3	380.30821	10.841	pos	5
3	287.54474	1.77	pos	5
3	294.83011	0.655	neg	5
3	508.23087	6.679	neg	5
3	372.27814	8.308	pos	5
3	488.13026	4.3	pos	5
3	447.33526	9.838	pos	5
3	258.59461	1.77	pos	5
3	430.30884	7.329	pos	5
3	346.21482	4.992	pos	5
3	400.22805	5.46	neg	5
3	357.12495	4.278	neg	5
3	467.13622	4.036	pos	5
3	356.2717	9.709	pos	5
3	240.17217	8.444	neg	5
3	397.30656	7.898	pos	5
3	262.03391	1.936	neg	5
3	300.24537	7.883	pos	5
3	162.55697	1.65	pos	5
3	233.03538	1.651	pos	5
3	541.15706	4.297	pos	5
3	179.5512	0.739	pos	5
3	313.20203	6.655	pos	5
3	274.05122	3.711	neg	5
3	191.05876	1.89	pos	5
3	770.41053	5.482	neg	5
3	229.14693	7.253	pos	5
3	425.04526	1.969	neg	5
3	398.23028	6.601	neg	5
3	760.38628	11.699	pos	5
3	369.08899	6.371	pos	5
3	542.31806	7.731	pos	5
3	588.58049	0.629	neg	5
3	222.13338	9.146	pos	5
3	171.05028	2.03	pos	5
3	319.24853	7.269	pos	5
3	234.00176	3.326	neg	5
3	313.2249	5.672	pos	5
3	471.33453	7.771	pos	5
3	538.42365	9.965	neg	5
3	447.04934	1.872	neg	5
3	188.8676	5.302	neg	5
3	270.19835	8.06	pos	5
3	328.22513	6.939	neg	5

3	748.45088	3.345	pos	5
3	305.00176	1.846	neg	5
3	382.18156	10.27	neg	5
3	526.38724	9.004	neg	5
3	366.28848	10.309	pos	5
3	124.97915	0.595	pos	5
3	367.27215	7.62	pos	5
3	332.18359	4.853	neg	5
3	515.99096	0.759	neg	5
3	240.17239	6.753	neg	5
3	246.01939	3.463	neg	5
3	321.26671	9.655	pos	5
3	186.64418	2.808	neg	5
3	329.01824	1.777	neg	5
3	411.17205	5.389	neg	5
3	365.05473	2.367	neg	5
3	492.0508	0.758	neg	5
3	244.04043	3.736	neg	5
3	338.26077	6.96	pos	5
3	882.59605	3.882	pos	5
3	272.03556	4.449	neg	5
3	318.12155	3.934	neg	5
3	277.02535	0.754	neg	5
3	131.03725	2.21	pos	5
3	430.30834	8.671	pos	5
3	451.71604	0.605	neg	5
3	397.78452	5.119	neg	5
3	274.05121	3.319	neg	5
3	362.25729	9.614	neg	5
3	415.06642	9.446	neg	5
3	562.54362	10.143	pos	5
3	359.96037	3.932	neg	5
3	347.28227	8.63	pos	5
3	470.33987	8.338	neg	5
3	151.015	0.679	pos	5
3	151.06233	1.603	neg	5
3	261.03054	0.757	neg	5
3	151.06233	1.86	neg	5
3	591.34717	3.366	pos	5
3	335.3188	10.293	pos	5
3	440.07802	3.125	neg	5
3	402.27698	8.852	pos	5
3	272.06852	5.19	neg	5
3	385.28215	6.54	pos	5
3	124.05133	2.853	neg	5
3	207.03241	0.59	pos	5
3	837.45793	9.648	pos	5
3	645.44091	10.271	neg	5
3	226.21237	7.958	pos	5
3	237.20917	9.284	pos	5
3	434.33972	9.878	neg	5
3	265.24865	10.312	pos	5
3	300.03024	4.74	neg	5

3	441.08201	9.615	neg	5
3	808.50855	2.902	pos	5
3	258.01995	4.052	neg	5
3	520.41323	8.762	pos	5
3	239.93893	0.589	neg	5
3	529.31678	7.784	neg	5
3	190.01717	2.808	neg	5
3	208.08427	1.868	neg	5
3	280.00411	4.392	neg	5
3	449.37164	8.172	pos	5
3	497.2816	4.499	neg	5
3	284.0354	4.418	neg	5
3	496.36327	2.762	pos	5
3	228.92879	5.296	neg	5
3	426.31337	8.099	pos	5
3	301.09514	4.109	pos	5
3	401.69956	0.613	neg	5
3	136.03621	0.652	neg	5
3	450.13877	3.645	neg	5
3	739.40427	9.238	neg	5
3	205.0121	2.201	neg	5
3	587.60175	0.605	neg	5
3	399.22951	2.792	pos	5
3	319.2486	8.241	pos	5
3	272.99198	2.787	neg	5
3	230.01193	0.754	neg	5
3	544.16548	4.42	neg	5
3	220.99892	0.746	neg	5
3	433.16838	9.413	neg	5
3	608.33885	3.91	pos	5
3	472.24968	5.177	neg	5
3	392.22026	5.117	neg	5
3	415.21133	4.831	neg	5

Additional features detected in criteria's for Tier 2-3 but without matches in databases, confidence level of identification, CL = 5.

Table S11. Comparison of literature detecting PFAS in follicular fluid

Suspected compound	Confidence level (CL)	Detection frequency, % (Kang et al., [2])		Ratio FF:Blood (Kang et al.,[2], McCoy et al.,[3], Heffernan et al., [4])
		Serum	Follicular fluid	
Perfluoroheptanoic acid (PFHpA)	1	86.2 (75)	87.7 (79)	0.65 (0.96 [2])
Perfluorooctanoic acid (PFOA)	1	99.1	99.1 (100)	0.72 (0.76-0.79 [2-4])
Perfluorononanoic acid (PFNA)	1	66.4	66.7 (100)	0.79 (0.71-0.81 [2-4])
Perfluorodecanoic acid (PFDA)	1	14.7	14.0 (100)	0.69 (0.65-0.79[2, 3])
Perfluoroundecanoic acid (PFUnDA)	1	7.8	7.0 (100)	0.71 (0.59-0.68[2, 3])
Perfluoropentane sulfonate (PFPeS)	2b	8.6	10.5	0.88
Perfluorohexane sulfonate (PFHxS)	1	94.8	98.2 (100)	1.04 (0.78-0.86 [2-4])
Perfluoroheptane sulfonate (PFHpS)	2b	24.1	42.1	0.79
Perfluorooctane sulfonate (PFOS)	1	91.4	59.6 (100)	0.64 (0.59-0.81 [2-4])
9-Chlorohexadecafluoro-oxanonane sulfonate (9Cl-PF3ONS)	1	3.4	3.5	0.94
Perfluoro-ethylcyclohexane sulfonate (PFECHS)	1	0.0	0.9	0.88

Kang et al., 2020 used a high-resolution mass-spectrometry for identification of legacy as well as emerging PFAS in follicular fluid from 28 women in China undergoing ovum pick-up for *in vitro* fertilization during 2018-2019. They reported transfer efficiencies to serum as well as detection frequencies in follicular fluid. McCoy et al. 2017, studied the association between PFAS in plasma and follicular fluid related to ovarian function in 50 women from the USA recruited during 2013-2014. PFAS was analyzed by liquid chromatography tandem mass spectrometry, the ratios are calculated by dividing reported concentration in follicular fluid with plasma. Heffernan et al. 2018, analyzed PFAS in 29 women with polycystic ovarian syndrome (PCOS) and 30 controls from the UK using a high performance liquid chromatography tandem mass spectrometry in women with PCOS and controls, ratio was calculated by reported levels in blood compared to follicular fluid.

Table S12. Results from logistic regression comparing serum mean intensity of 6-hydroxyindole in women with high and low embryo quality

Model	Estimate	Std error	p-value
4-aminophenol			
- Log-4-aminophenol	0.25	0.21	0.24
- Log-AMH	-0.15	0.32	0.45
- Age	-0.17	0.23	0.46
- BMI	0.01	0.21	0.98
6-hydroxyindole			
- Log-6-hydroxyindole	-0.59	0.25	0.02
- Age	-0.01	0.06	0.93
- BMI	-0.00	0.07	0.99
- Log-AMH	0.02	0.27	0.95

References

1. Schymanski, E.L., et al., *Identifying small molecules via high resolution mass spectrometry: communicating confidence.* Environ Sci Technol, 2014. **48**(4): p. 2097-8.
2. Kang, Q., et al., *Nontargeted identification of per- and polyfluoroalkyl substances in human follicular fluid and their blood-follicle transfer.* Environ Int, 2020. **139**: p. 105686.
3. McCoy, J.A., et al., *Associations between perfluorinated alkyl acids in blood and ovarian follicular fluid and ovarian function in women undergoing assisted reproductive treatment.* Sci Total Environ, 2017. **605-606**: p. 9-17.
4. Heffernan, A.L., et al., *Perfluorinated alkyl acids in the serum and follicular fluid of UK women with and without polycystic ovarian syndrome undergoing fertility treatment and associations with hormonal and metabolic parameters.* Int J Hyg Environ Health, 2018. **221**(7): p. 1068-1075.