# Electronic Supplementary Information

## Combining targeted and untargeted screening of environmental contaminants reveals associations between PFAS exposure and vitamin D metabolism in human plasma

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## Contents of Supplementary Information

# 1. Chemical information on targeted compounds

**Table S1. Chemical formulas, monitored m/z, and retention times for targeted compounds and internal standards**

| Compound | Abbreviation | Class | Formula | m/z | rt (min) | Formula IS | m/z IS |
|---|---|---|---|---|---|---|---|
| Bisphenol A | BPA | Bisphenol | C15H16O2 | 227.1077 | 6.17 | [13C]12C3H16O2 | 239.1480 |
| rac-α-1,2,5,6,9,10-Hexabromocyclododecane | α-HBCD | HBCD | C12H18Br6 | 640.6374 | 9.59 | [13C]12H18Br6 | 652.6777 |
| rac-β-1,2,5,6,9,10-Hexabromocyclododecane | β-HBCD | HBCD | C12H18Br6 | 640.6374 | 9.71 | [13C]12H18Br6 | 652.6777 |
| rac-γ-1,2,5,6,9,10-Hexabromocyclododecane | γ-HBCD | HBCD | C12H18Br6 | 640.6374 | 9.8 | [13C]12H18Br6 | 652.6777 |
| 6-Hydroxy-2,2',4,4'-tetrabromodiphenyl ether [a] | 6-OH-BDE47 | OH-BDE | C12H6Br4O2 | 500.6987 | 9.35 | [13C]12H6Br4O2 | 512.7390 |
| 6-Hydroxy-2,2',4,4',6-pentabromodiphenyl ether [a] | 6-OH-BDE100 | OH-BDE | C12H5Br5O2 | 578.6093 | 9.64 | [13C]12H5Br5O2 | 590.6495 |
| 2, 2',3, 4',5, 5',6-Heptachloro-4-biphenylol [a] | 4-OH-PCB-187 | OH-PCB | C12H3Cl7O | 408.7901 | 9.24 | [13C]12H3Cl7O | 420.8304 |
| 2, 2',3,3', 4',5, 5'-Heptachloro-4-biphenylol a | 4-OH-PCB-172 | OH-PCB | C12H3Cl7O | 408.7901 | 9.81 | [13C]12H3Cl7O | 420.8304 |
| 2',3, 4',5, 5'-Pentachloro-4-biphenylol [a] | 4-OH-PCB-120 | OH-PCB | C12H5Cl5O | 340.8681 | 9.55 | [13C]12H5Cl5O | 352.9083 |
| 2',3', 4',5'-Tetrachloro-4-biphenylol a | 4-OH-PCB-61 | OH-PCB | C12H6Cl4O | 306.9070 | 9.21 | [13C]12H6Cl4O | 318.9473 |
| 2',3,3', 4',5, 5'-Hexachloro-4-biphenylol [a] | 4-OH-PCB-159 | OH-PCB | C12H4Cl6O | 374.8291 | 9.93 | [13C]12H4Cl6O | 386.8694 |
| 2',4',5'-Trichloro-4-biphenylol [a] | 4-OH-PCB-29 | OH-PCB | C12H7Cl3O | 270.9490 | 8.72 | [13C]12H7Cl3O | 282.9892 |
| 3',4'-Dichloro-4-biphenylol [a] | 4-OH-PCB-12 | OH-PCB | C12H8Cl2O | 236.9879 | 8.15 | [13C]12H8Cl2O | 249.0282 |
| 1H,1H,2H,2H-perfluoro-1-decanesulfonic acid (8:2) | 8:2FTS | PFAS | C10H5F17SO3 | 526.9615 | 8.2 | [13C]2C8H5F17SO3 | 528.9682 |
| 1H,1H,2H,2H-perfluoro-1-hexanesulfonic acid (4:2) | 4:2FTS | PFAS | C6H5F9SO3 | 326.9743 | 5.69 | [13C]2C4H5F9SO3 | 328.9810 |
| 1H,1H,2H,2H-perfluoro-1-octanesulfonic acid (6:2) | 6:2FTS | PFAS | C8H5F13SO3 | 426.9679 | 7.24 | [13C]2C6H5F13SO3 | 428.9746 |
| N-ethylperfluoro-1-octanesulfonamidoacetic acid | N-EtFOSAA | PFAS | C12H8F17NO4S | 583.9830 | 8.63 | C12D5H3F17NO4S | 589.0144 |
| N-methylperfluoro-1-octanesulfonamidoacetic acid | N-MeFOSAA | PFAS | C11H6F17NO4S | 569.9673 | 8.43 | C11D3H3F17NO4S | 572.9862 |
| Perfluoro-1-octanesulfonamide | FOSA | PFAS | C8H2F17NO2S | 497.9462 | 8.66 | [13C]8H2F17NO2S | 505.9730 |
| Perfluorobutanesulfonic acid | L-PFBS | PFAS | C4HF9O3S | 298.9430 | 4.88 | [13C]3CHF9O3S | 301.9530 |
| Perfluorodecane sulfonic acid [b] | L-PFDS | PFAS | C10HF21O3S | 598.9238 | 8.56 | NA | NA |
| Perfluoroheptanesulfonic acid [b] | L-PFHpS | PFAS | C7HF15O3S | 448.9334 | 7.33 | NA | NA |
| Perfluorohexanesulfonic acid | PFHxS | PFAS | C6HF13O3S | 398.9366 | 6.75 | [13C]3C3HF13O3S | 401.9467 |
| Perfluoro-n-butanoic acid | PFBA | PFAS | C4HF7O2 | 212.9792 | 1.84 | [13C]4HF7O2 | 216.9926 |
| Perfluoro-n-decanoic acid | PFDA | PFAS | C10HF19O2 | 512.9600 | 8.21 | [13C]6C4HF19O2 | 518.9802 |
| Perfluoro-n-dodecanoic acid | PFDoA | PFAS | C12HF23O2 | 612.9537 | 8.88 | [13C]2C10HF23O2 | 614.9604 |
| Perfluoro-n-heptanoic acid | PFHpA | PFAS | C7HF13O2 | 362.9696 | 6.67 | [13C]4C3HF13O2 | 366.9830 |
| Perfluoro-n-hexanoic acid | PFHxA | PFAS | C6HF11O2 | 312.9728 | 5.8 | [13C]5CHF11O2 | 317.9896 |
| Perfluoro-n-nonanoic acid | PFNA | PFAS | C9HF17O2 | 462.9632 | 7.79 | [13C]9HF17O2 | 471.9934 |
| Perfluoro-n-octanoic acid | PFOA | PFAS | C8HF15O2 | 412.9664 | 7.29 | [13C]8HF15O2 | 420.9933 |
| Perfluorononane sulfonic acid [b] | L-PFNS | PFAS | C9HF19O3S | 548.9270 | 8.21 | NA | NA |
| Perfluoro-n-pentanoic acid | PFPeA | PFAS | C5HF9O2 | 262.9760 | 4.37 | [13C]5HF9O2 | 267.9928 |
| Perfluoro-n-tetradecanoic acid | PFTeDA | PFAS | C14HF27O2 | 712.9473 | 9.4 | [13C]2C12HF27O2 | 714.9540 |
| Perfluoro-n-tridecanoic acid [c] | PFTrDA | PFAS | C13HF25O2 | 662.9505 | 9.16 | NA | NA |
| Perfluoro-n-undecanoic acid | PFUdA | PFAS | C11HF21O2 | 562.9568 | 8.57 | [13C]7C4HF21O2 | 569.9803 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Perfluorooctanesulfonic acid | PFOS | PFAS | C8HF17O3S | 498.9302 | 7.8 | [13C]8HF17O3S | 506.9570 |
| Perfluoropentanesulfonic acid [d] | L-PFPeS | PFAS | C5HF11O3S | 348.9398 | 6 | NA | NA |

a: Only heavy isotope labelled standards were used for these compounds, i.e. no native references

b: No IS available, use IS for perfluorooctanesulfonic acid

c: No IS available, use IS for perfluoro-n-dodecanoic acid

d: No IS available, use IS for perfluorohexanesulfonic acid

## 2. Representative chromatogram from injection of pooled sample

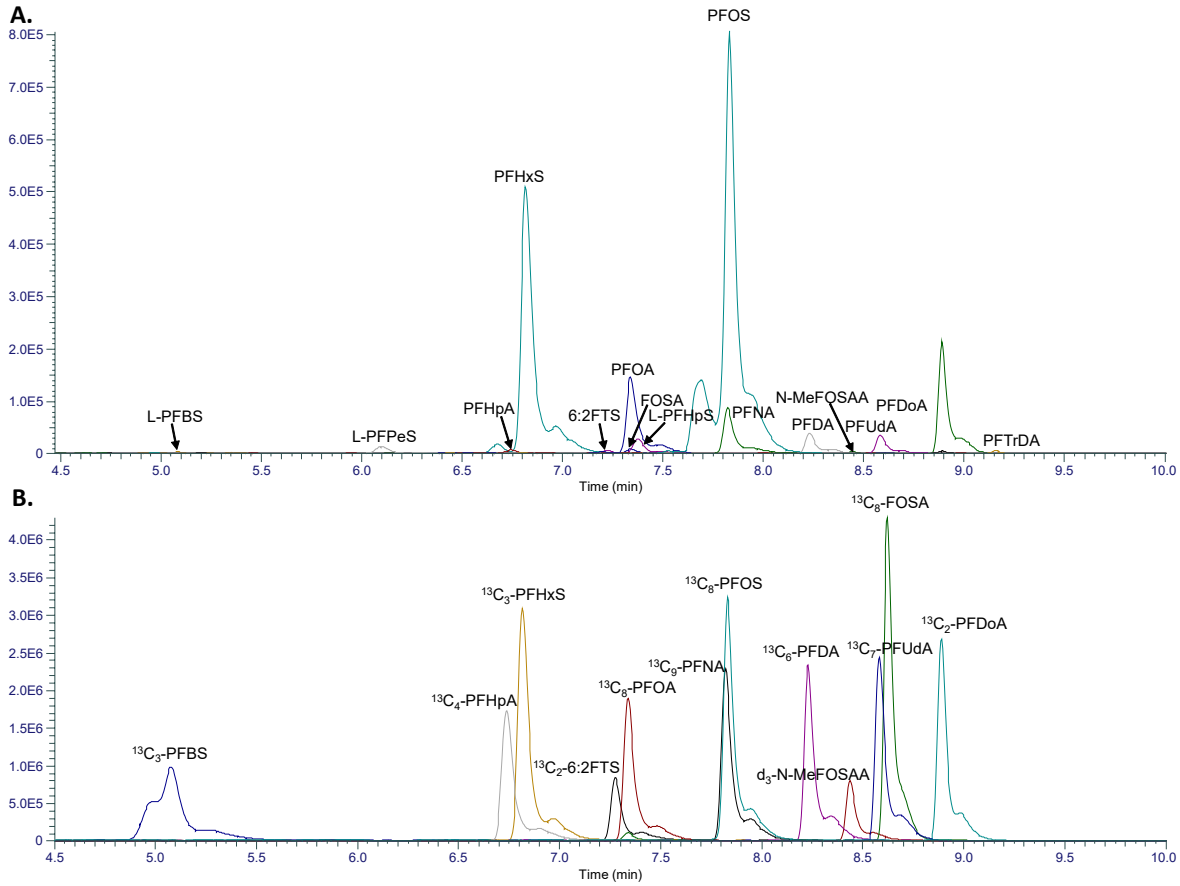



**Figure S1.** Representative chromatogram of a pooled sample containing all 100 samples under study, with the detected targeted analytes indicated (A; upper chromatogram). The corresponding internal standards (if available; not all targeted compounds had corresponding IS) are indicated in the lower chromatogram (B; concentrations of all internal standards correspond to 10 ng/mL).

# 3. Validation results

**Table S2. QC performance in terms of CV% and bias% for the validated compounds with both native standards and heavy isotope labeled standards; calibration curve slopes, the estimated limit of detection (LOD) and lowest limit of quantification (LLOQ), and estimated matrix effects for all compounds**

| | | QC performance | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **Abbreviation** | **CV% intra-batch (n=3)** | **CV% inter-batch (n=18)** | **Bias% intra-batch (3) [a]** | **Bias% inter-batch (18) [b]** | **Slope of calibration curve** | **Estimated LOD (ng/mL)** | **Estimated LLOQ (ng/mL)** | **Matrix effect % [c]** |
| Bisphenol A | BPA | 8.6 | 18.9 | 43.5 | 29.1 | 0.612 | 2.50 | 5.00 | 106 |
| Hexabromocyclododecane [d] | HBCD | 8.3 | 9.5 | -13.6 | -14.3 | 0.974 | 0.63 | 1.25 | 55 |
| 6-Hydroxy-2,2',4,4'-tetrabromodiphenyl ether [e] | 6-OH-BDE47 | NA | NA | NA | NA | 0.995 | 0.04 | 0.08 | 76 |
| 6-Hydroxy-2,2',4,4',6-pentabromodiphenyl ether [e] | 6-OH-BDE100 | NA | NA | NA | NA | 0.995 | 0.63 | 1.25 | 56 |
| 2, 2',3, 4',5, 5',6-Heptachloro-4-biphenylol [e] | 4-OH-PCB-187 | NA | NA | NA | NA | 0.995 | 0.04 | 0.08 | 55 |
| 2, 2',3,3', 4',5, 5'-Heptachloro-4-biphenylol [e] | 4-OH-PCB-172 | NA | NA | NA | NA | 0.995 | 0.16 | 0.32 | 58 |
| 2',3, 4',5, 5'-Pentachloro-4-biphenylol [e] | 4-OH-PCB-120 | NA | NA | NA | NA | 0.995 | 0.01 | 0.02 | 90 |
| 2',3', 4',5'-Tetrachloro-4-biphenylol [e] | 4-OH-PCB-61 | NA | NA | NA | NA | 0.995 | 0.04 | 0.08 | 70 |
| 2',3,3', 4',5, 5'-Hexachloro-4-biphenylol [e] | 4-OH-PCB-159 | NA | NA | NA | NA | 0.995 | 0.16 | 0.32 | 43 |
| 2',4',5'-Trichloro-4-biphenylol [e] | 4-OH-PCB-29 | NA | NA | NA | NA | 0.995 | 0.16 | 0.32 | 65 |
| 3',4'-Dichloro-4-biphenylol [e] | 4-OH-PCB-12 | NA | NA | NA | NA | 0.995 | 0.16 | 0.32 | 69 |
| 1H,1H,2H,2H-perfluoro-1-decanesulfonic acid (8:2) | 8:2FTS | 3.0 | 5.7 | -6.1 | -2.7 | 0.988 | 0.01 | 0.02 | 76 |
| 1H,1H,2H,2H-perfluoro-1-hexanesulfonic acid (4:2) | 4:2FTS | 7.1 | 9.8 | 9.5 | 3.5 | 0.983 | 0.04 | 0.08 | 118 |
| 1H,1H,2H,2H-perfluoro-1-octanesulfonic acid (6:2) | 6:2FTS | 3.3 | 5.6 | 12.1 | 15.6 | 0.594 | 0.01 | 0.02 | 168 |
| N-ethylperfluoro-1-octanesulfonamidoacetic acid | N-EtFOSAA | 2.8 | 5.6 | -7.8 | -4.9 | 0.995 | 0.04 | 0.08 | 100 |
| N-methylperflu | N-MeFO | 2.9 | 4.8 | -6.5 | -3.1 | 0.987 | 0.04 | 0.08 | 92 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| oro-1-octanesulfonamidoacetic acid | SAA | | | | | | | | |
| Perfluoro-1-octanesulfonamide | FOSA | 2.6 | 5.1 | -3.7 | 0.0 | 0.985 | 0.01 | 0.02 | 85 |
| Perfluorobutanesulfonic acid | L-PFBS | 1.4 | 6.3 | 5.5 | 0.9 | 0.988 | 0.01 | 0.02 | 136 |
| Perfluorodecane sulfonic acid [f] | L-PFDS | 1.5 | 6.7 | -10.1 | -1.4 | 0.980 | 0.01 | 0.02 | 78 |
| Perfluoroheptanesulfonic acid [f] | L-PFHpS | 3.7 | 6.7 | -4.1 | 4.0 | 0.977 | 0.01 | 0.02 | 92 |
| Perfluorohexanesulfonic acid | PFHxS | 3.0 | 5.1 | -4.1 | -1.0 | 0.991 | 0.01 | 0.02 | 93 |
| Perfluoro-n-butanoic acid | PFBA | 3.3 | 6.3 | 33.1 | 35.0 | 0.866 | 0.63 | 1.25 | 129 |
| Perfluoro-n-decanoic acid | PFDA | 2.5 | 5.5 | -7.1 | -2.1 | 0.995 | 0.01 | 0.02 | 69 |
| Perfluoro-n-dodecanoic acid | PFDoA | 3.2 | 5.2 | -1.4 | 2.0 | 0.987 | 0.01 | 0.02 | 92 |
| Perfluoro-n-heptanoic acid | PFHpA | 2.5 | 5.3 | -0.9 | 2.5 | 0.975 | 0.01 | 0.02 | 101 |
| Perfluoro-n-hexanoic acid | PFHxA | 2.7 | 7.4 | -8.0 | -2.7 | 0.977 | 0.01 | 0.02 | 93 |
| Perfluoro-n-nonanoic acid | PFNA | 2.3 | 6.9 | 21.2 | 33.7 | 0.982 | 0.01 | 0.02 | 92 |
| Perfluoro-n-octanoic acid | PFOA | 9.0 | 6.3 | -4.0 | 0.7 | 0.982 | 0.01 | 0.02 | 99 |
| Perfluorononane sulfonic acid [f] | L-PFNS | 3.2 | 5.8 | -1.9 | -0.1 | 0.981 | 0.01 | 0.02 | 66 |
| Perfluoro-n-pentanoic acid | PFPeA | 16.8 | 18.7 | -6.9 | 4.6 | 0.981 | 0.01 | 0.02 | 56 |
| Perfluoro-n-tetradecanoic acid | PFTeDA | 2.8 | 5.3 | -1.1 | 0.3 | 0.995 | 0.01 | 0.02 | 98 |
| Perfluoro-n-tridecanoic acid [g] | PFTrDA | 4.8 | 6.9 | 12.8 | 20.4 | 0.990 | 0.01 | 0.02 | 107 |
| Perfluoro-n-undecanoic acid | PFUdA | 2.2 | 7.9 | 33.6 | 53.7 | 0.881 | 0.01 | 0.02 | 89 |
| Perfluorooctanesulfonic acid | PFOS | 3.3 | 5.5 | -1.6 | 2.7 | 0.984 | 0.01 | 0.02 | 93 |
| Perfluoropentanesulfonic acid [h] | L-PFPeS | 3.2 | 6.2 | -6.9 | -0.5 | 0.980 | 0.01 | 0.02 | 90 |

a: Matrix effects (%) were calculated for each compound by dividing the mean peak area of the calibrators prepared with newborn bovine serum by the mean peak area of calibrators prepared without matrix (prepared in 3:1 MeOH:H2O), and then multiplied with 100.

b: Bias% inter-batch was calculated within the first batch (three QCs per concentration level per batch) comparing the calculated concentration for QCs with the nominal concentrations. Equation: ((“mean calculated concentration for QC” - ”nominal concentration”)/”nominal concentration”)*100

c: Bias% intra-batch was calculated for all batches (including the total 18 QCs at each concentration level) comparing the calculated concentration for QCs with the nominal concentrations. Equation: ((“mean calculated concentration for QC” - ”nominal concentration”)/”nominal concentration”)*100

d: Combined validation results for the three evaluated HBCD isomers (α, β, and γ)

e: Only heavy isotope labelled standards were used for these compounds, i.e. no native references. No spiked QCs were prepared for these compounds.

f: No IS available, use IS for perfluorooctanesulfonic acid

g: No IS available, use IS for perfluoro-n-dodecanoic acid

h: No IS available, use IS for perfluorohexanesulfonic acid

# 4. Detailed correlation heatmaps including p-values

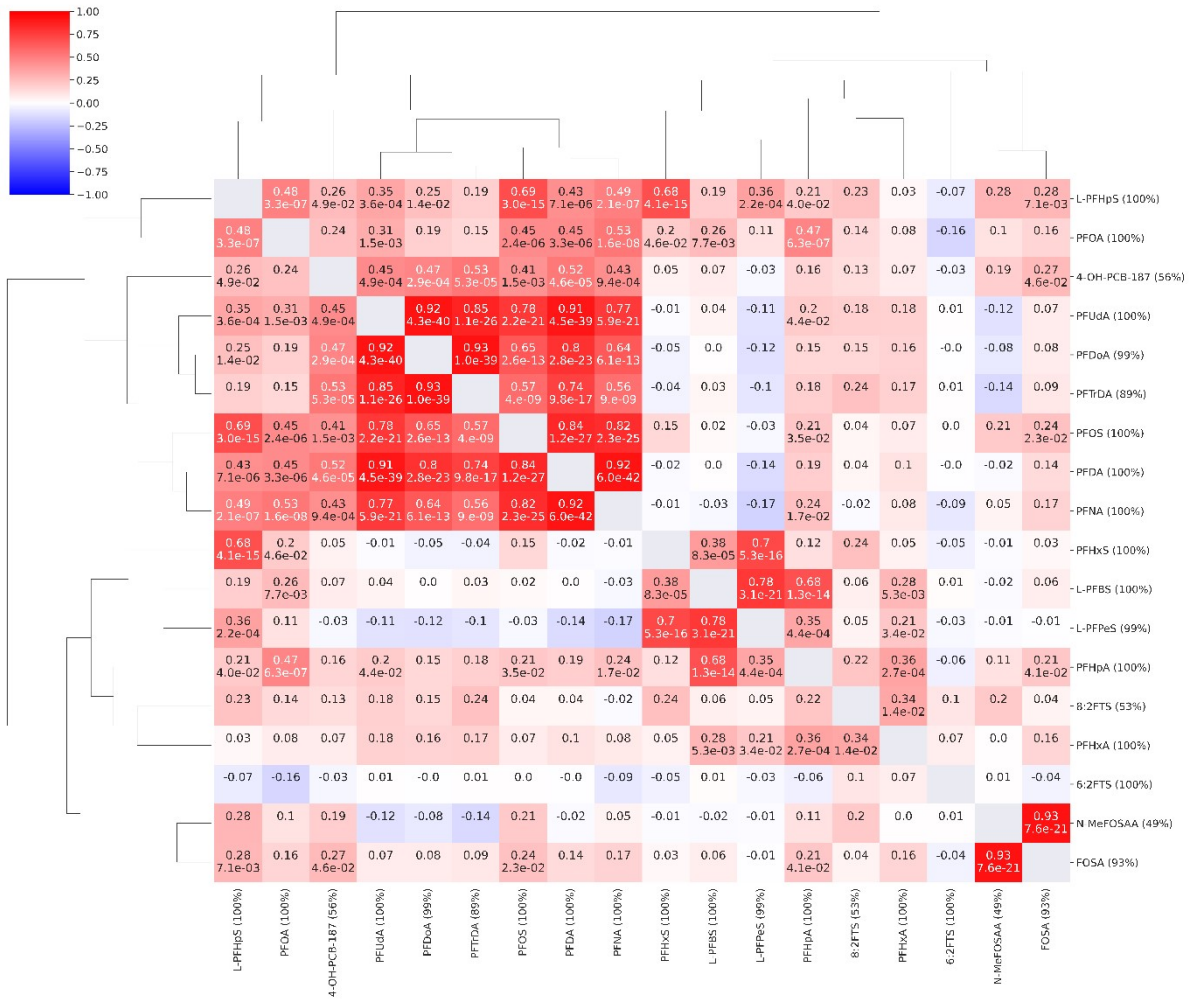



**Figure S2.** Detailed correlation heatmap including p-values (below correlation coefficients) corresponding to Figure 2 in manuscript: Correlation heatmap for the compounds observed in the targeted analysis (n=18, coverage cutoff ≥15%). The percentage values within parentheses next to compound names indicate compound coverage.

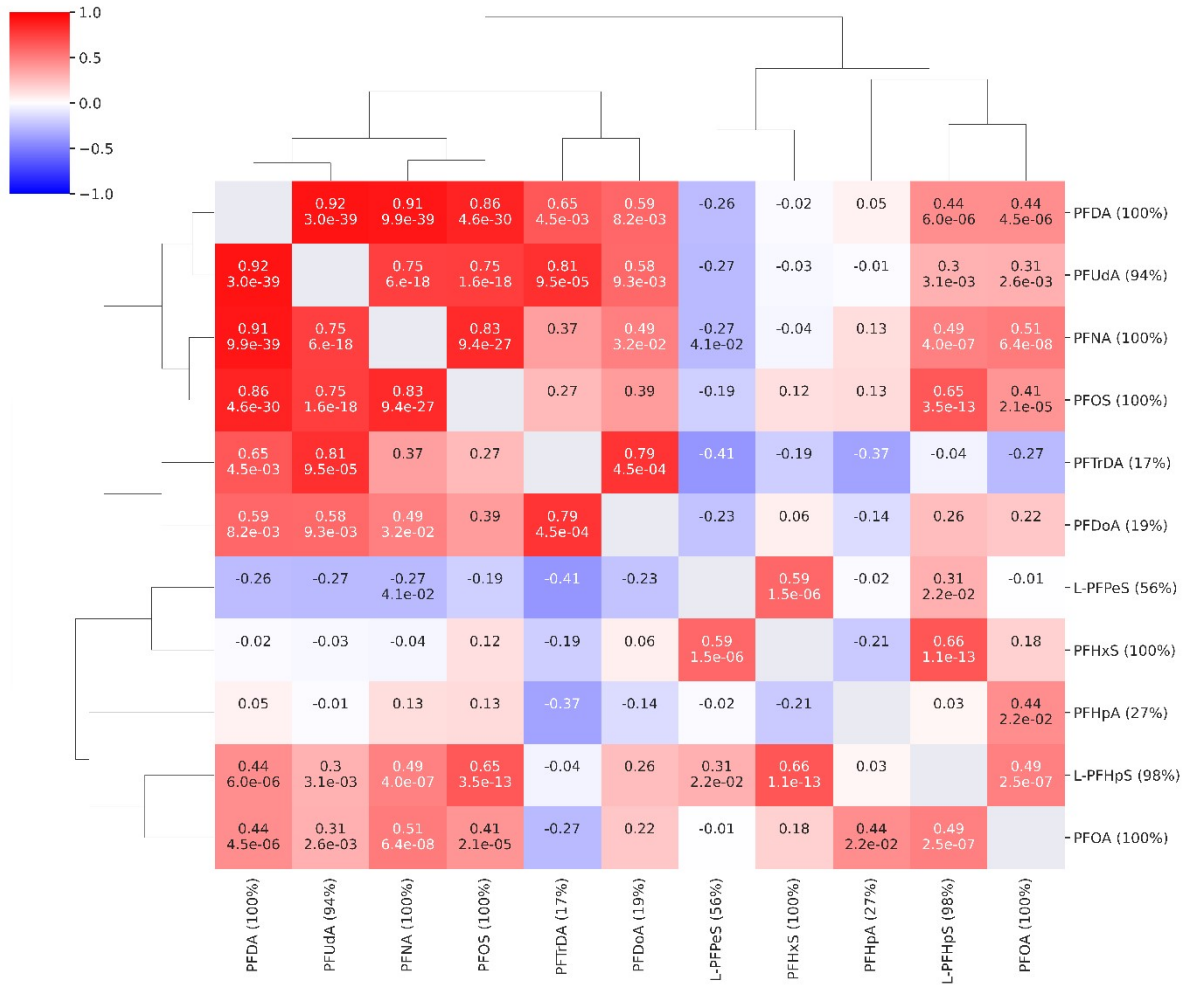



**Figure S3.** Detailed correlation heatmap including p-values (below correlation coefficients) corresponding to Figure 3 in manuscript: Correlation heatmap for the compounds from the target list observed in the untargeted analysis (n=11, coverage cutoff ≥15%). The percentage values within parentheses next to compound names indicate compound coverage.

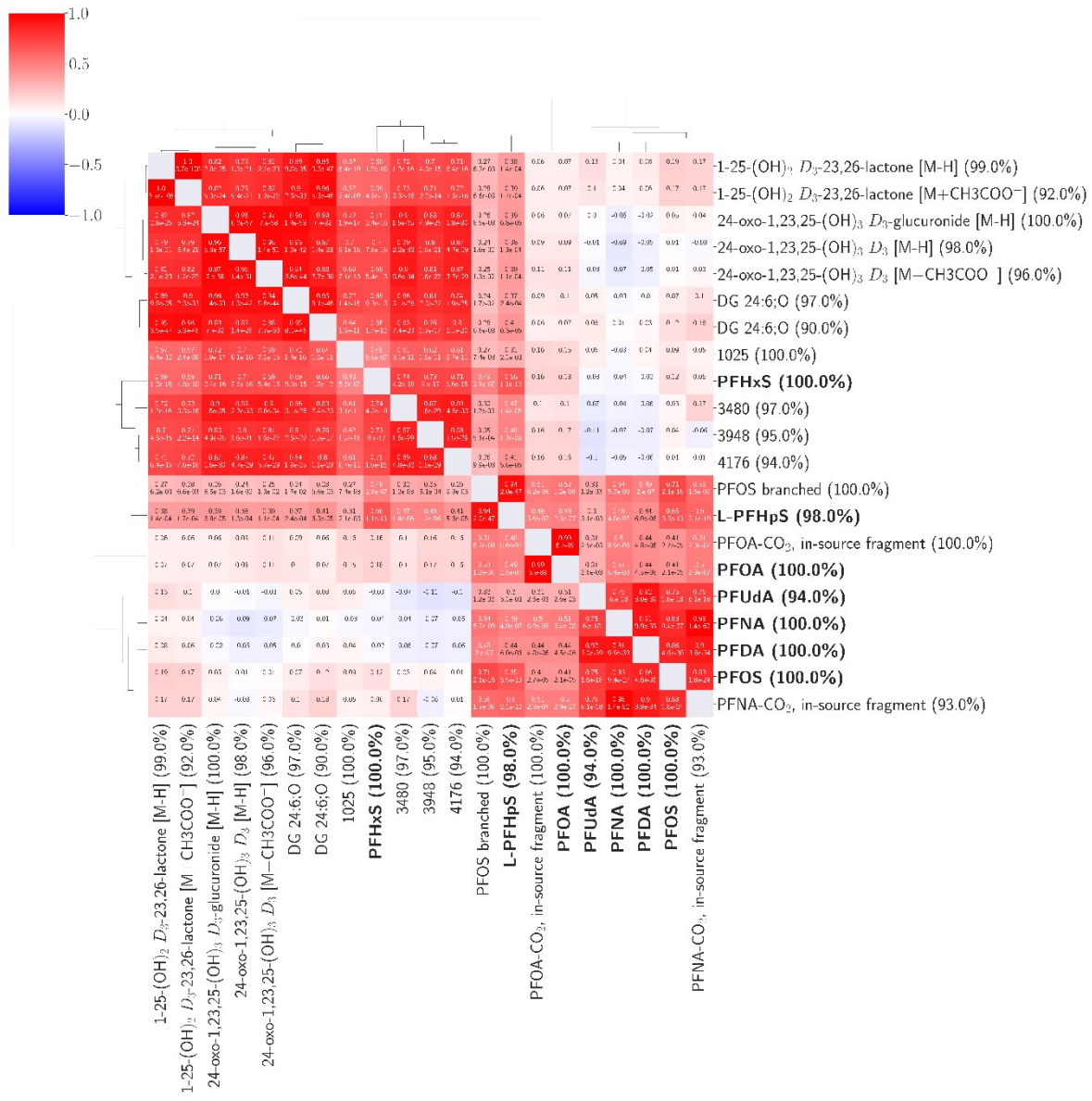
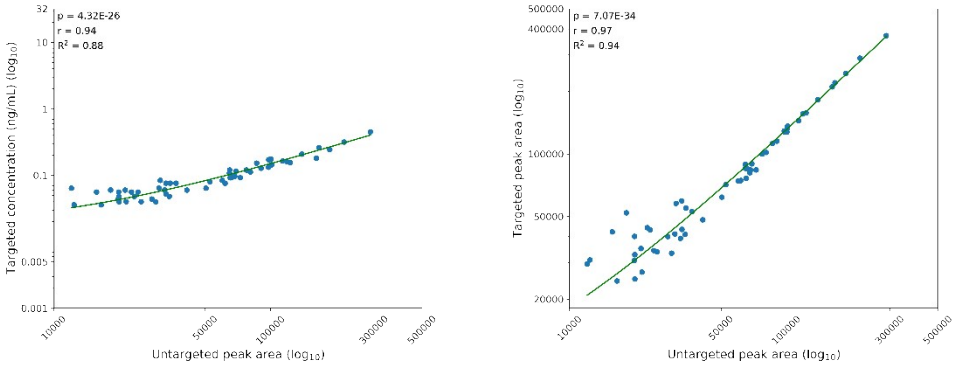


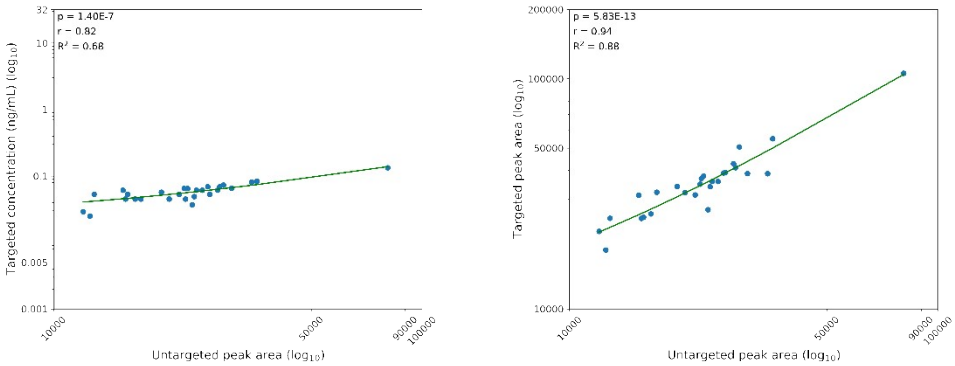

**Figure S4.** Detailed correlation heatmap including p-values (below correlation coefficients) corresponding to Figure 4 in manuscript: Fourteen unknown features were found to be associated with seven confirmed environmental contaminants. The percentage values within parentheses next to compound names indicate compound coverage.

# 5. Correlation plots targeted vs untargeted methods

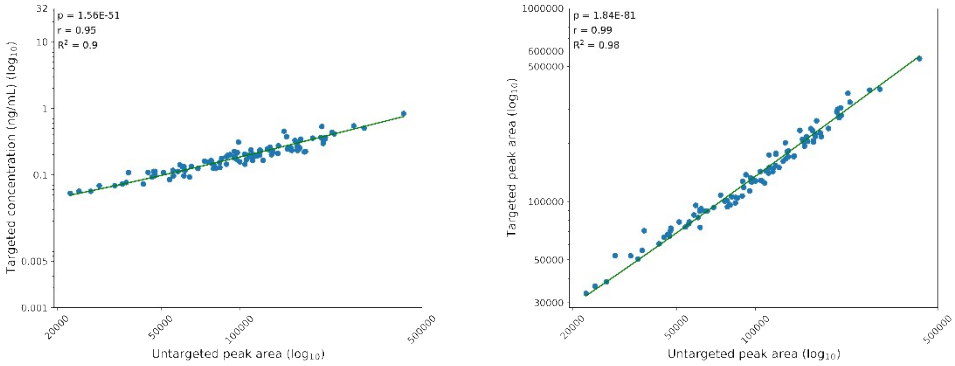
L-PFPeS



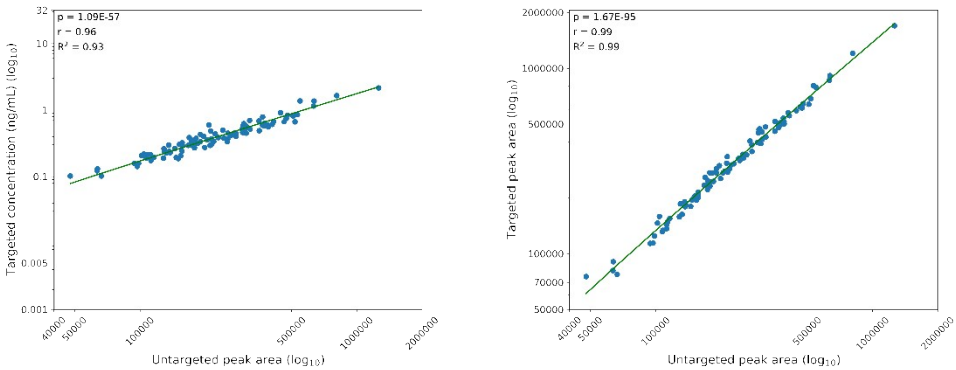

PFHpA



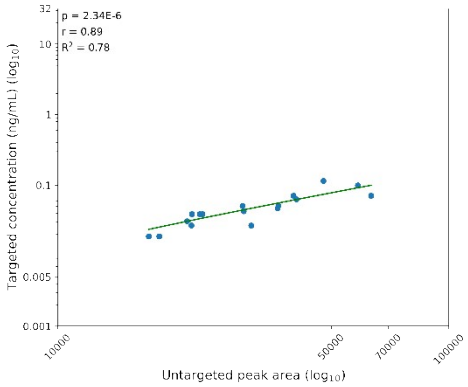

PFDA

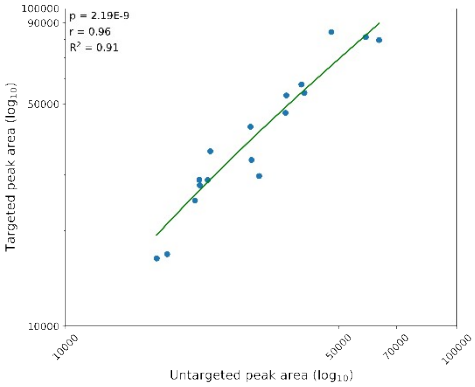



PFNA

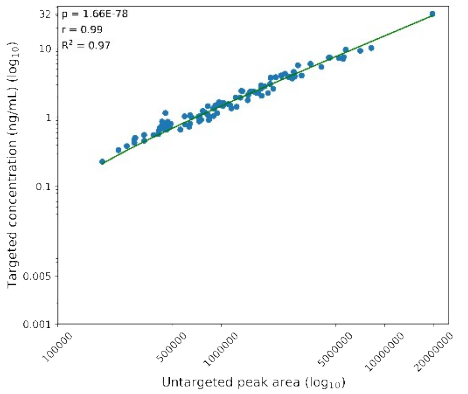


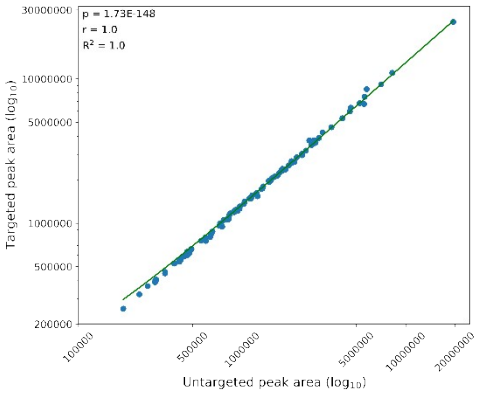
PFTrDA



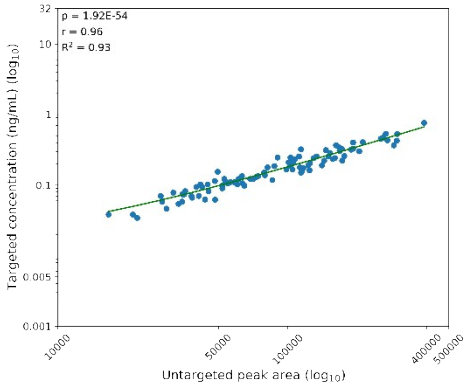



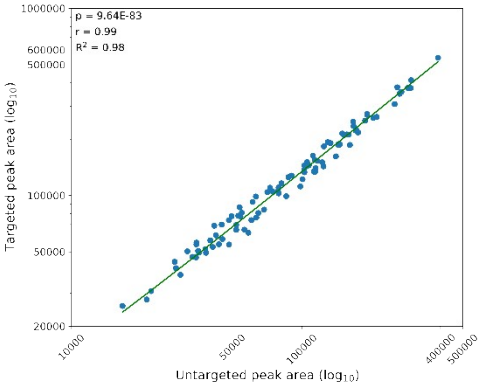

PFHxS



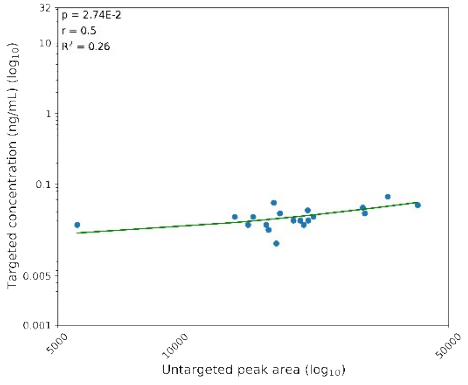



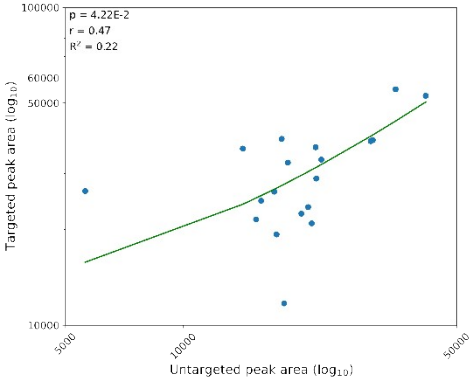

PFUdA




PFDoA



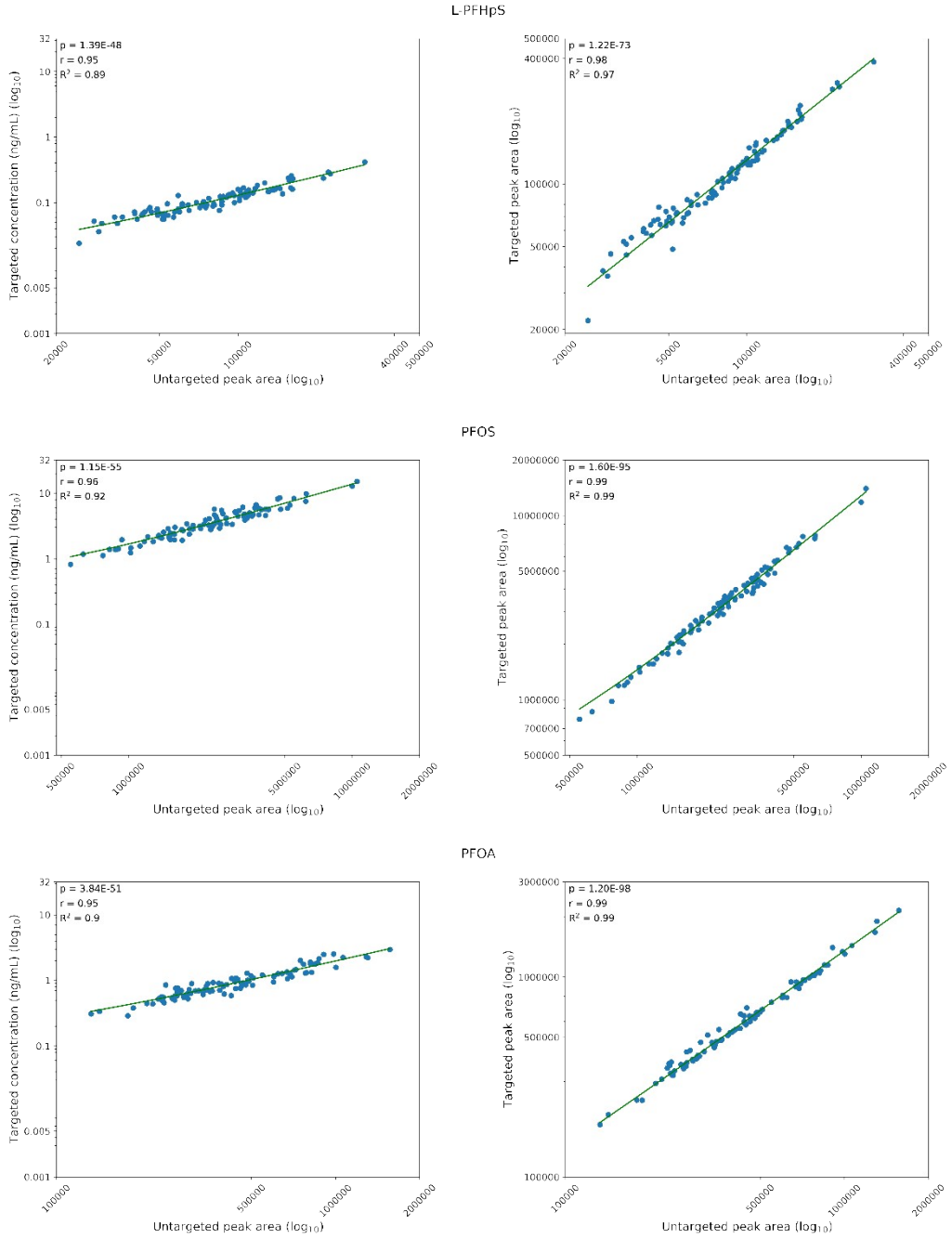
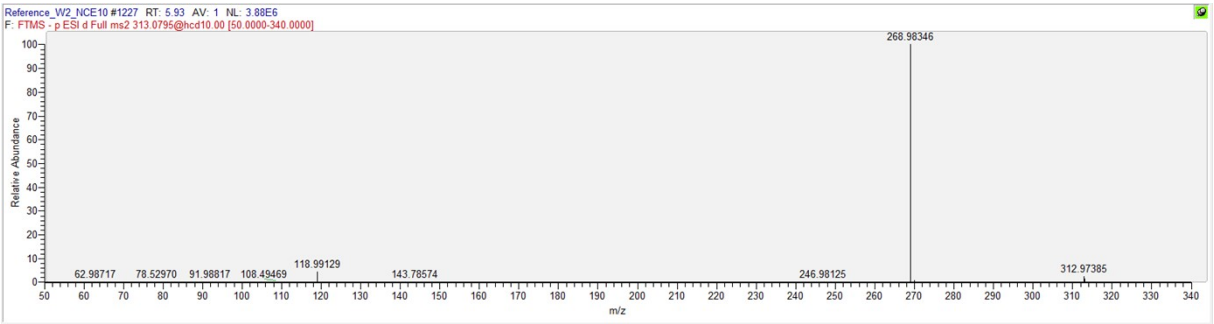



**Figure S5.** Correlation plots for the compounds detected using both the targeted and untargeted methods. The correlation plots to the left show the correlations between the concentrations estimated using the targeted approach ($log_{10}$ transformed) and the peak areas from the untargeted approach ($log_{10}$ transformed). The correlation plots to the right show the correlations between the peak areas from the targeted approach ($log_{10}$ transformed) and the peak areas from the untargeted approach ($log_{10}$ transformed).

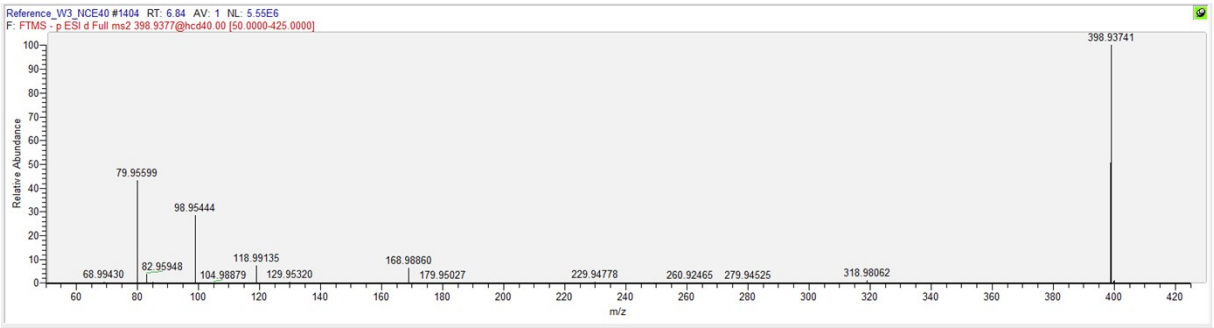
# 6. Example MS2 spectra: Perfluoro-n-hexanoic acid vs Perfluorohexanesulfonic acid

The value of using a range of collision energies to collect high-quality MS2 spectra is illustrated by the examples of Perfluoro-n-hexanoic acid (PFHxA) and Perfluorohexanesulfonic acid (PFHxS). Linear perfluorocarboxylic acids, e.g. PFHxA, are almost fully fragmented even at low collision energies (here NCE 10; the lowest possible option on the used instrument) while perfluroalkylsulfonates, e.g. PFHxS require high collision energies (here NCE 40, the highest NCE used in the present study).

**PFHxA, NCE: 10**




**PFHxS, NCE: 40**



# 7. Nontargeted PFAS species detected from untargeted data

**Table S3. Nontargeted PFAS species detected from untargeted data**

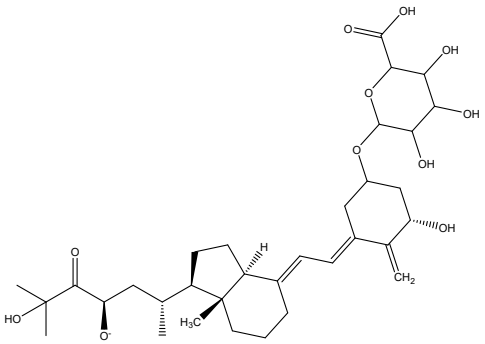
| Unknown ID | Observed m/z | rt min | Predicted logKow | Correlated with | Correlation coefficient (p-value)[a] | Identity (confidence level)[b] | Molecular formula | Mass error ppm | Listed logKow |
|---|---|---|---|---|---|---|---|---|---|
| 1772 | 368.977 | 7.36 | 4.89 | PFOA | 0.99 (p= 6E-89 | PFOA-$CO_2$ (in-source fragment, level 2) | C7H1F15 | 0.99 | 4.89 |
| 4424 | 418.9741 | 7.83 | 5.33 | PFNA, PFOS, PFDA, PFUdA | 0.98 (p= 1.4E-62) , 0.83 (p= 1.8E-24), 0.90 (p= 3.8E-34), 0.76 (p= 6.1E-18) | PFNA-$CO_2$ (in-source fragment, level 2) | C8H1F17 | 1.65 | 5.56 |
| 1673 | 498.931 | 7.69 | 5.23 | L-PFHpS | *0.94 (p= 2.0E-47)* | PFOS Branched (level 2) | C8H1F17O3S1 | 1.56 | 4.68 |

[a] p-values for correlation coefficients. All p-values are presented in the Supplementary information.

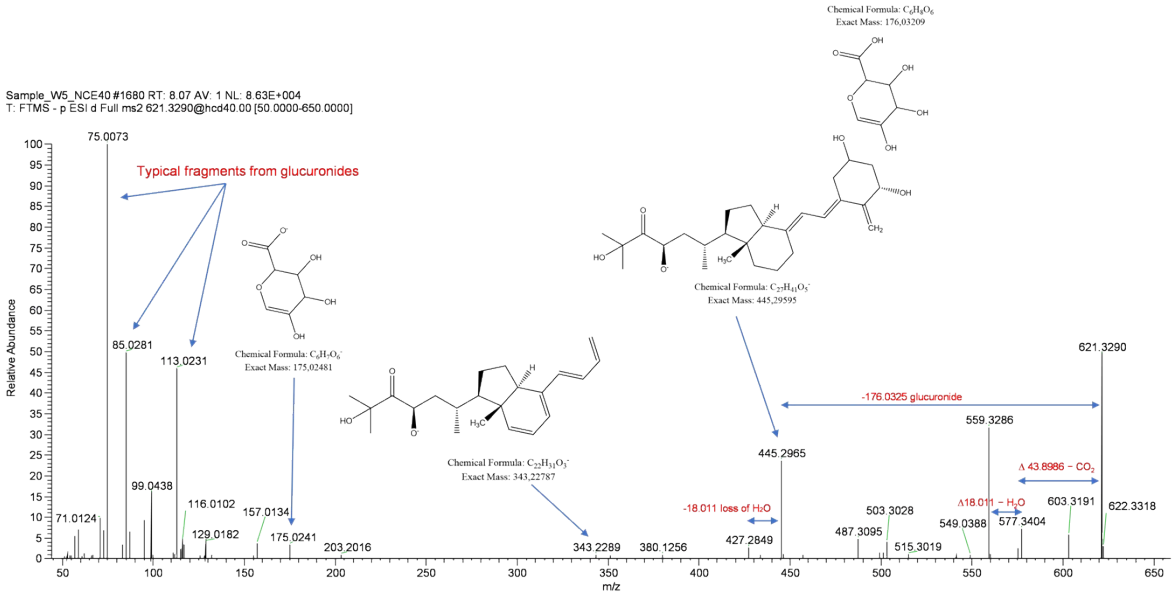
[b] Identification confidence levels according to Schymanski et al. 2014

# 8. Annotated MS2 spectra of non-targeted compounds

- **<u>24-oxo-1,23,25-trihydroxyvitamin $D_3$-glucuronide</u>**

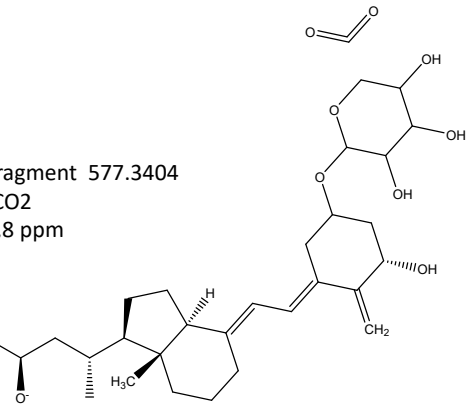


**Structure for 24-oxo-1,23,25-trihydroxyvitamin $D_3$-glucuronide**



## Proposed structures for observed fragments

Chemical Formula: $CO_2$
Exact Mass: 43,98983



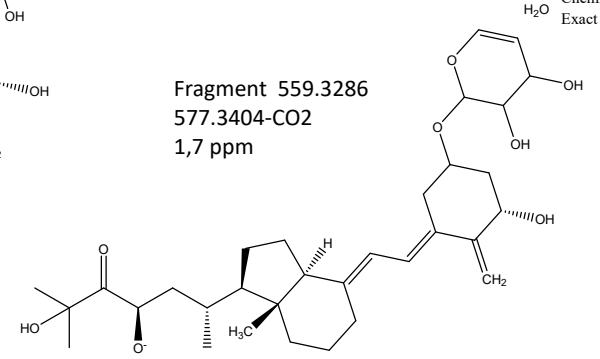
Fragment 577.3404
-CO2
3,8 ppm

Chemical Formula: $C_{32}H_{49}O_9^-$
Exact Mass: 577,33821

Chemical Formula: $CO_2$
Exact Mass: 43,98983

Chemical Formula: $H_2O$
Exact Mass: 18,01056

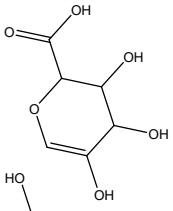
$H_2O$



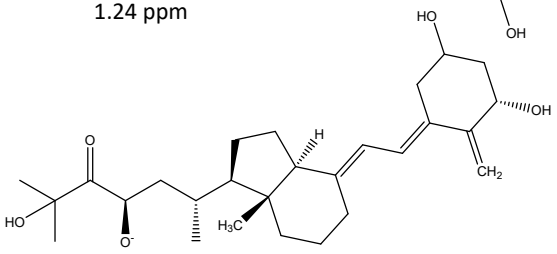
Fragment 559.3286
577.3404-CO2
1,7 ppm

Chemical Formula: $C_{32}H_{47}O_8^-$
Exact Mass: 559,32764

Chemical Formula: $C_6H_8O_6$
Exact Mass: 176,03209



Fragment 445.2965
621.3290 - glucuronide
1.24 ppm

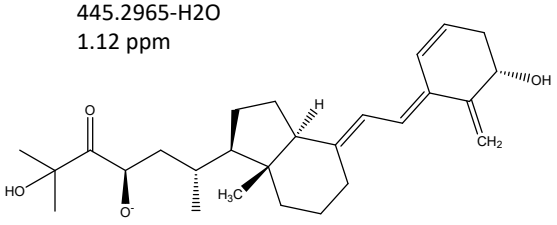


Chemical Formula: $C_{27}H_{41}O_5^-$
Exact Mass: 445,29595

Chemical Formula: $H_2O$
Exact Mass: 18,01056

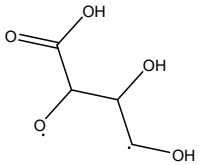
$H_2O$

Fragment 427.2849
445.2965-H2O
1.12 ppm

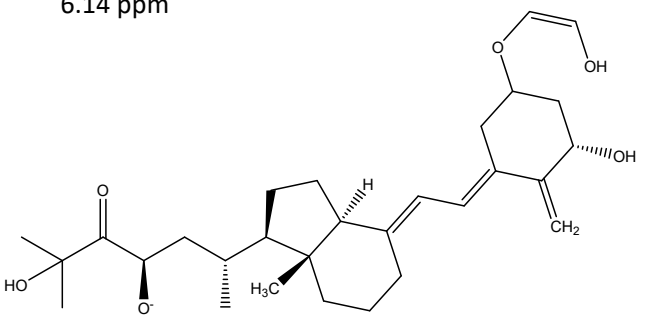


Chemical Formula: $C_{27}H_{39}O_4^-$
Exact Mass: 427,28538

Fragment 487.3095
6.14 ppm



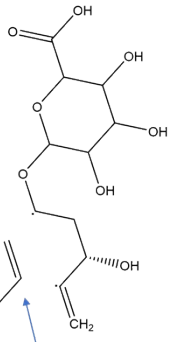
Chemical Formula: $C_4H_6O_5^{2\bullet}$
Exact Mass: 134,02152

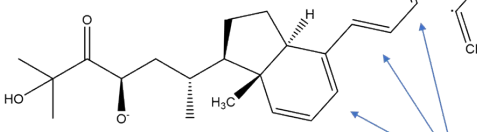


Chemical Formula: $C_{29}H_{43}O_6^-$
Exact Mass: 487,30651

Chemical Formula: $C_{11}H_{16}O_8^{2\bullet}$
Exact Mass: 276,08452

Fragment 343.2289
3.00 ppm

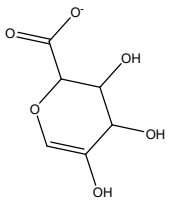




Chemical Formula: $C_{22}H_{31}O_3^-$
Exact Mass: 343,22787

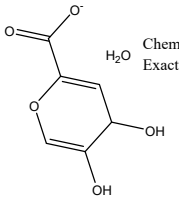
Rearrangement of double bonds

Fragment 175.0241
4.06 ppm



Chemical Formula: $C_6H_7O_6^-$
Exact Mass: 175,02481

Fragment 157.0134
5.41 ppm

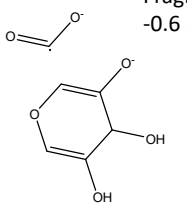


Chemical Formula: $H_2O$
Exact Mass: 18,01056

Chemical Formula: $C_6H_5O_5^-$
Exact Mass: 157,01425

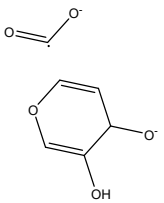
Chemical Formula: $CO_2^{\bullet-}$
Exact Mass: 43,99038

Fragment 129.0182
-0.6 ppm



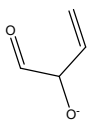
Chemical Formula: $C_5H_5O_4^-$
Exact Mass: 129,01933

Fragment 113.0231
2.21 ppm



Chemical Formula: $C_5H_5O_3^-$
Exact Mass: 113,02442

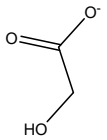
Fragment 85.0281
4.7 ppm



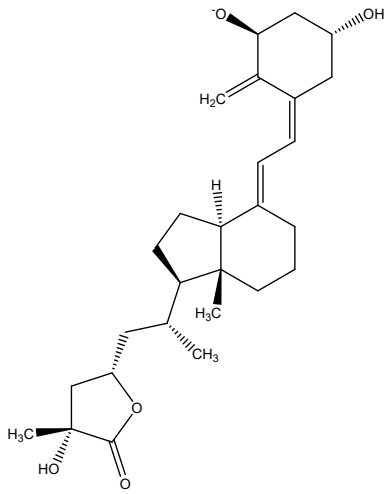
Chemical Formula: $C_4H_5O_2^-$
Exact Mass: 85,02950

Fragment 75.0073
-4.62 ppm

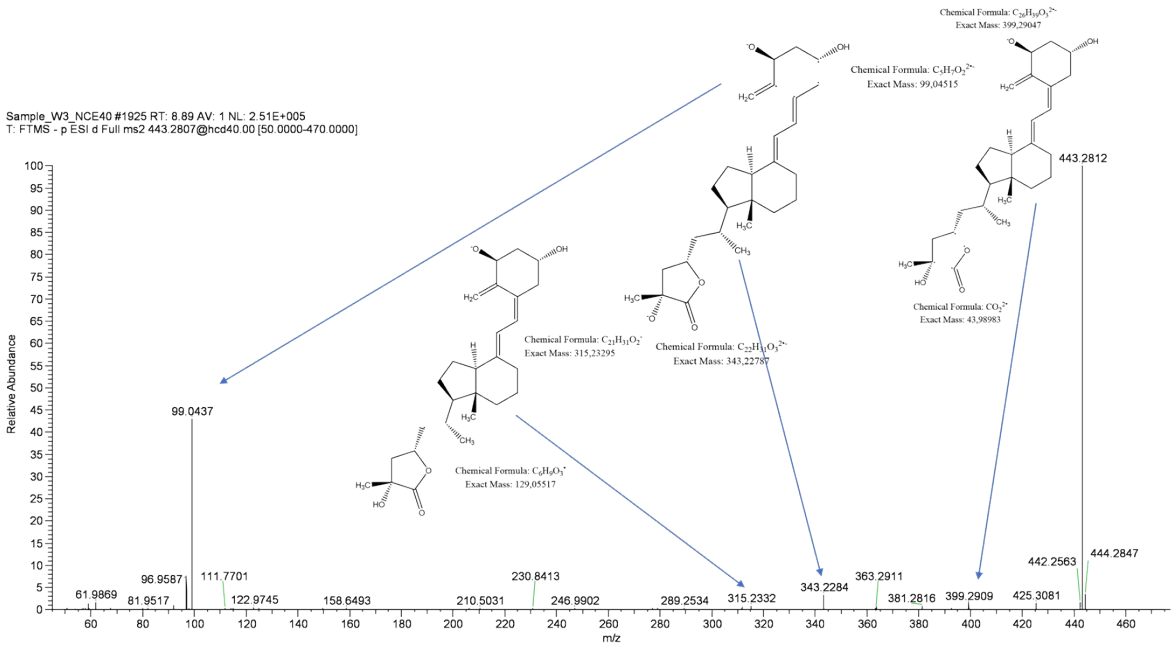
Chemical Formula: $C_2H_3O_3^-$
Exact Mass: 75,00877



- **<u>1,25-dihydroxyvitamin D3-23,26 lactone</u>**

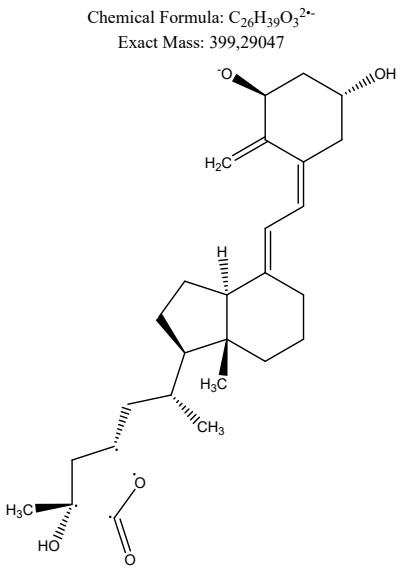


**Structure for 1,25-dihydroxyvitamin D3-23,26 lactone**

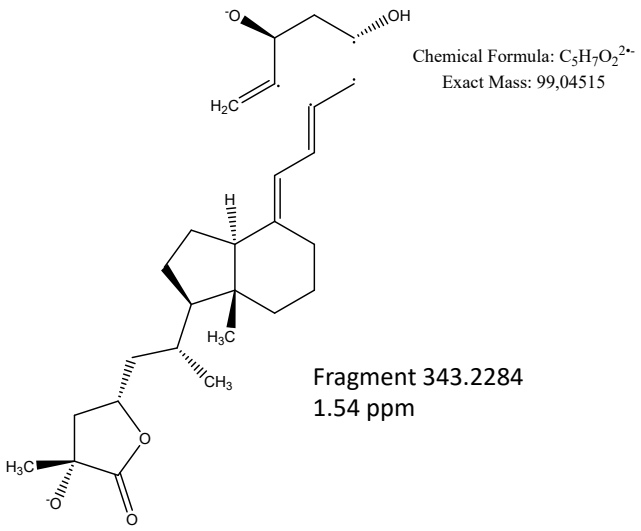


## Proposed structures for observed fragments

Fragment 399.2909
1.08 ppm

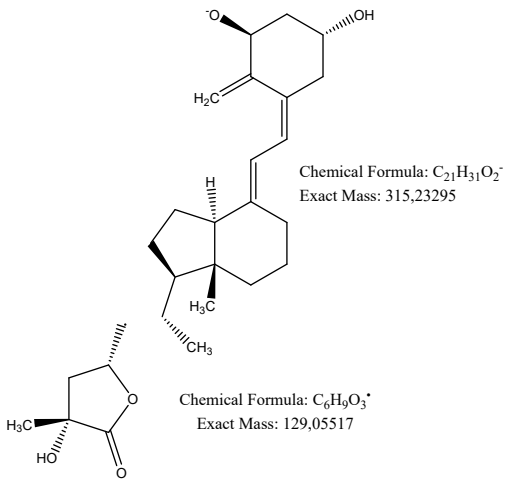



Fragment 99.0437

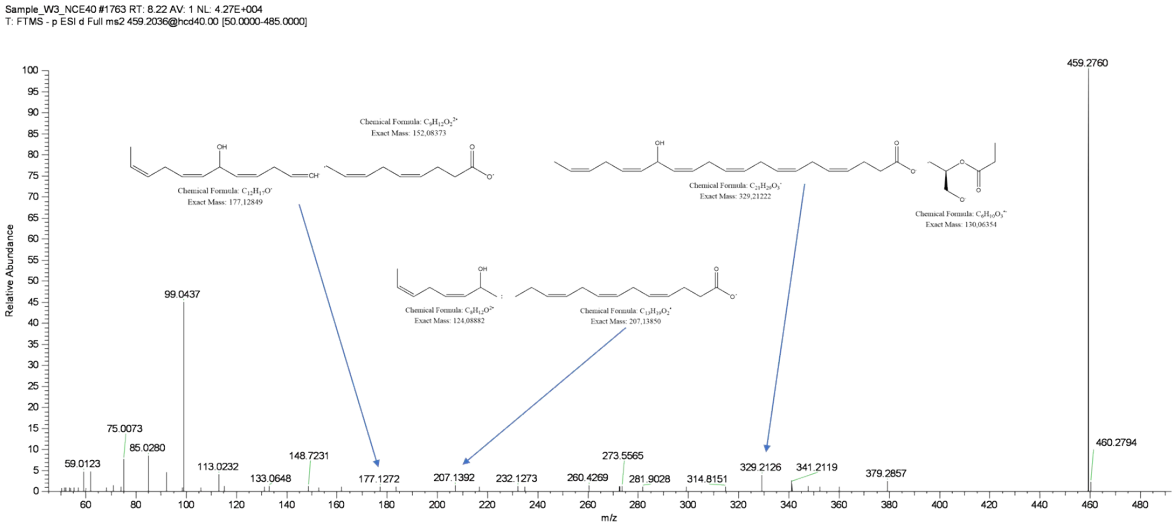


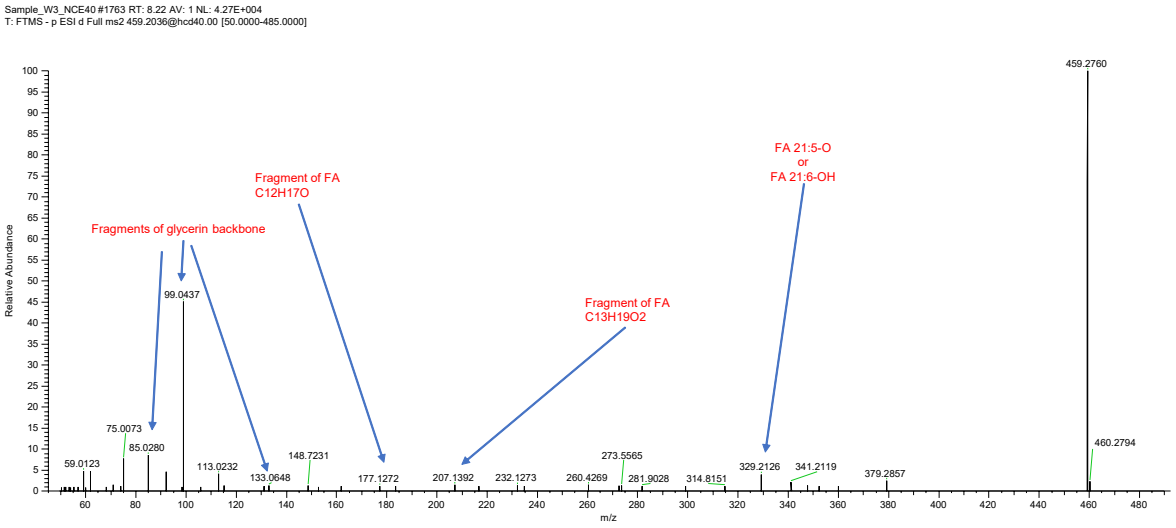

Fragment 343.2284
1.54 ppm

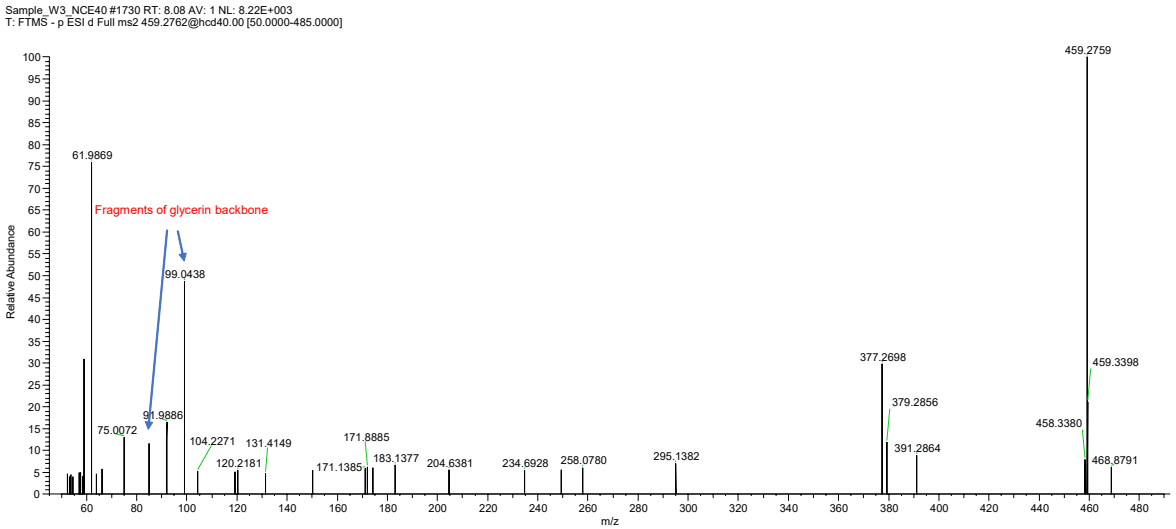
Fragment 315.2332
0.79 ppm



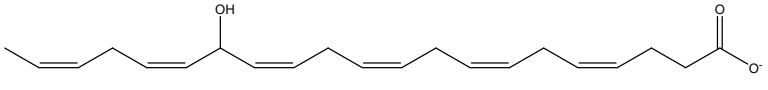
- **Diglyceride lipid: DG 24:6;O**



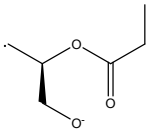



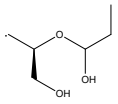
## Proposed structures for observed fragments



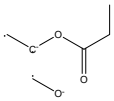

Chemical Formula: $C_{21}H_{29}O_3^-$
Exact Mass: 329,21222



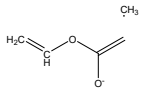

Chemical Formula: $C_6H_{10}O_3^{\bullet-}$
Exact Mass: 130,06354




Chemical Formula: $C_6H_{13}O_3^{\bullet}$
Exact Mass: 133,08647



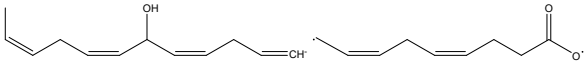

Chemical Formula: $C_5H_7O_2^{2-\bullet}$
Exact Mass: 99,04515



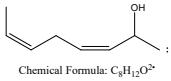

Chemical Formula: $C_4H_5O_2^-$
Exact Mass: 85,02950

Chemical Formula: $CH_3^{\bullet}$
Exact Mass: 15,02348

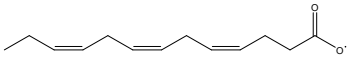
Chemical Formula: $C_9H_{12}O_2^{2-\bullet}$
Exact Mass: 152,08373




Chemical Formula: $C_{12}H_{17}O^-$
Exact Mass: 177,12849




Chemical Formula: $C_8H_{12}O^{2-\bullet}$
Exact Mass: 124,08882




Chemical Formula: $C_{13}H_{19}O_2^{\bullet}$
Exact Mass: 207,13850

# 9. Investigation of possible associations between PFAS and major vitamin D metabolites

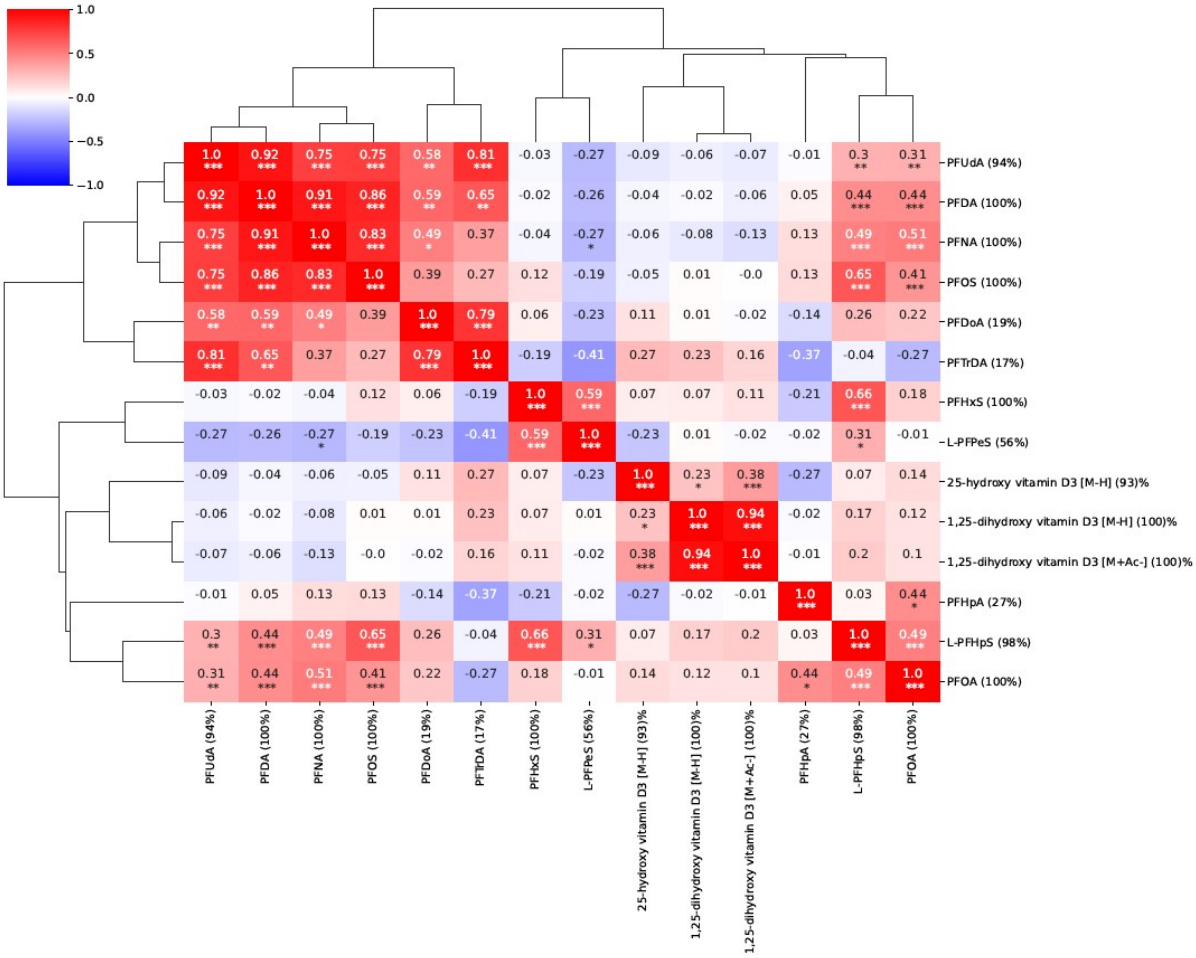



**Figure S6.** Possible associations between PFAS and major vitamin D metabolites 25-hydroxyvitamin D3 and 1,25-hydroxyvitamin D3 were investigated. No statistically significant associations were found.