Ministry of Environment
of Denmark
Environmental
Protection Agency

Supporting Technical Document: Sources of PFAS and their exchange between sediment and surface water in the lakes *Furesø, Bagsværd sø, Lyngby sø* and the river *Mølleåen*

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Contents

1. Summary

This technical report contains background information supporting the study 'Sources of PFAS and their exchange between sediment and surface water - in the lakes Furesø, Bagsværd sø, Lyngby sø and the river Mølleåen', DK EPA (2024).

2. Analyses of PFAS

This section describes the targeted analyses from previous studies, targeted LC-MS analyses of water and sediment (by Eurofins), and suspect screening LC-QTOF MS analyses of sediment (by UCPH).

2.1.1 Materials and chemicals and Sample pre-treatment

Details are provided on Chemicals and materials/Sample pre-treatment in Appendix 2 and in Analytical standards in Appendix 5. Care was taken to wash all plastic equipment in ethanol before analyses (and dry it before use) to minimise blank carry-over.

In summary the principle of the sample treatment was a slightly modified method by Langberg et al. (2021), where subsamples were weighed out, porewater centrifuged, internal standards (IS) added, twice acetonitrile was added followed by ultrasonification/shaking/centrifuging, decanting of acetonitrile, evaporation to 5 mL, filtration, evaporation to 100 μL, recombination to 700 μL of 1:1 water:methanol.

2.1.2 LC-ESI$^-$- QTOF MS analyses - quantification

Details are provided on Analyses by suspect/non-targeted analyses by UCPH in Appendix 3, and Method performance (calibration curves etc.) is provided in Appendix 4.

The Waters software called 'TargetLynx' v4.1 was used to extract ions and quantify PFSAs and PFCAs and their precursors.

Appendix 4 contains examples of chromatograms showing retention times, extracted ions, and calibration curves showing with standard and IS were used for the quantification. A weighing of 1/X (giving more significance to the lower concentrations) and with quadratic curves were used. As can be seen from the calibration curves, the use of IS spiked to the sediments from the beginning resulted in rather good/repeatable calibration curves. Blanks were also included and were automatically used to correct for blanks in the quantification. Detection limits were not very low, but comparable to those from Eurofins. The variation was in most cases good, so the issue is more blanks for some of the compounds.

A challenge for the quantification is that IS have small impurities of the PFAS (eg. PFOS-IS may have a bit of PFOS in it), and also the FOSE/FOSA/FOSAA standards. While these in principle may be corrected for this is rather complicated and time-consuming so typically, they will add to the 'blank' value. Isotopically labelled standards may also have degradation products that are identical to the product ions of the PFAS, which means that quantification ions have to be selected *very* carefully – and sometimes the most intense product ion cannot be used, which hampers the LOD.

Variation in the areas of the internal standards affecting the quantification

For some compounds the area of the internal standard – spiked at a constant concentration - varied a lot, between standards and samples, but also between the sub-samples. This was the case for instance for PFTeDA-IS (C14 PFCA-IS). Several situations could cause this:

a. Matrix effects of co-eluting compounds affecting the ionisation efficiency
b. Decrease in intensity over the run, due to the instrument getting more dirty
c. Drift in m/z accuracy meaning that the m/z peak would slightly fall outside the mass (m/z) extraction window of 0.05 Da.
d. Matrix effects due to variations in the subsample constitution, combined with a less than 100% extraction efficiency

Ad a) Matrix effects lowering the ionisation efficiency could explain some but not all the variance, namely that a higher area is seen for the 'clean' standards vs. the samples: Figure S1a hence shows a 2 ng/mL standard (230102_052) and below the chromatograms for the subsamples 3A (230102_031), 3B (230102_084) and 3C (230102_132). The S/N is at least 4 for the 669.961 peak (in the extracted ion chromatogram) for the standard. However, the variation in the S/N between the subsamples is up to a factor 12. This cannot be explained by matrix effects alone, if we assume that the same constituents are present in the 3 subsamples – since the same 'interferences' hence would be eluting at the same time. But matrix effects does matter somewhat, between the standard in solvent to the sample.

Ad b) Decrease in signal intensity over the sequence/run is not observed. Figure S1b hence shows that the lowest signal is for 3A (-031), with the signal being 10 times higher at the end of the run for the last subsample 3C (_0132), shown in Figure S1a.

Ad c) Drift in m/z over the run was not observed either. Figure S1b hence shows extremely consistent m/z of PFTeDA-IS target ion (zoomed in at 669.961 Da) for the 3 subsamples plus for another sample 5A (230102_093).

Ad d) Matrix effects due to subsamples material composition could explain the variance, due to PFTeDAs strong partitioning to some parts of the solid sample (e.g. with high TOC) – if the extraction is not 100%. This effect could be expected more for the long chain than for the short chain PFAS. For comparison the areas of PFTeDA-IS (long chain) vs. PFHxA-IS (short chain) are shown in Figure S2.

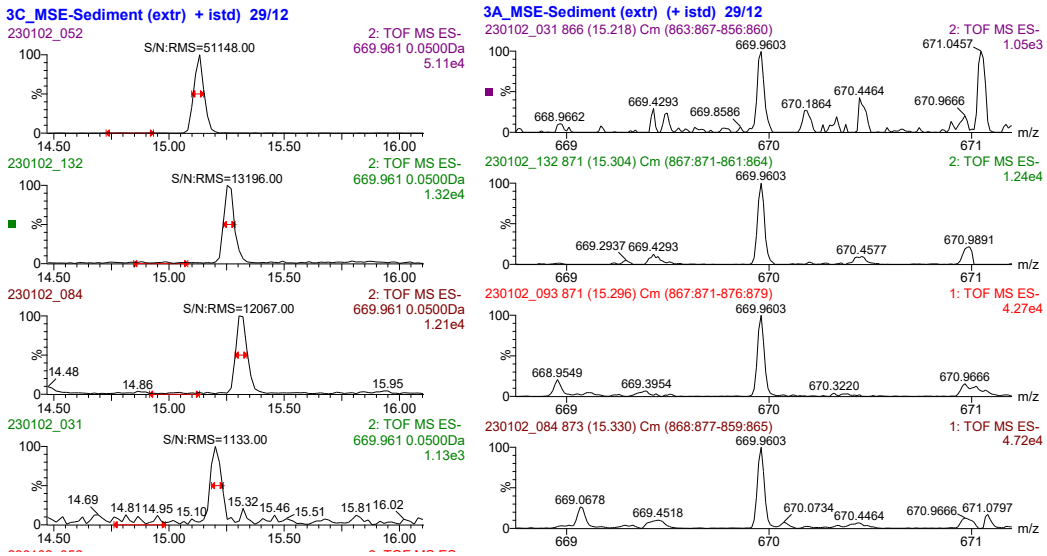


FIGURE S1a (left): Variations in the areas and S/N of the internal standards of PFTeDA.
FIGURE S1b (right): The very consistent accurate m/z for PFTeDA over the run of sequences.

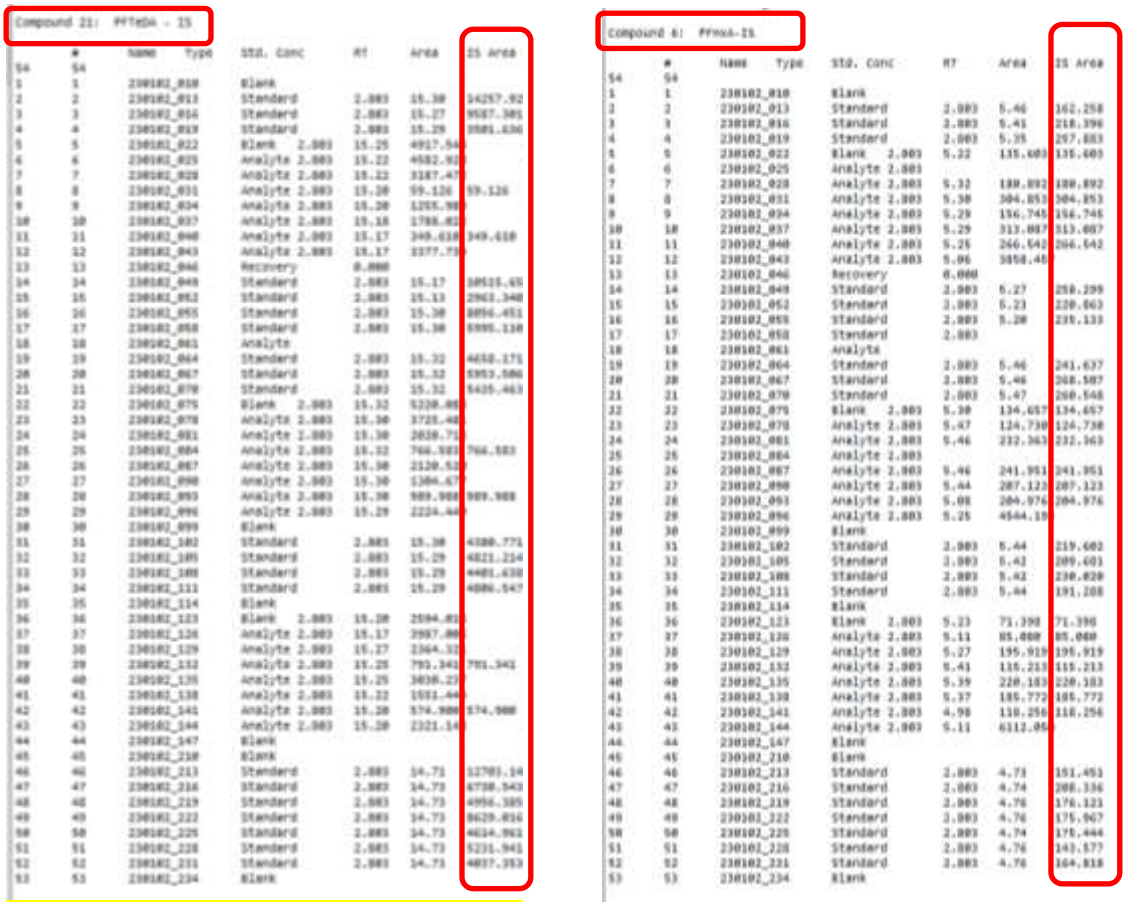


FIGURE S2: Comparison the areas of PFTeDA-IS (long chain) vs. PFHxA-IS (short chain), showing that the variance is lower in the area of the short-chain PFAS with less strong affinity to solid matter, than for the long-chain PFTeDA-IS.

2.1.3 LC-ESI⁻- QTOF MS analyses - identification:

For some PFAS the identification of the peaks and their respective retention times were not certain enough so these were not included in the reported PFAS. This was in part because many of the PFAS have the same product ions, which complicated the annotation of peaks. Another issue is that the collision energy ramp used (10-60 eV, cf. the experimental file provided in Appendix 2.10) may not have been sufficient to effectively fragment the larger PFAS, e.g. prePFOS such as SAmPAPs, and therefore product ions lacked for the larger PFAS. Shifts in retention times also made it difficult to assign peaks with high certainty, which may have been caused by a very high LC backpressure, due to the use of a long 150 mm column and methanol as the organic solvent. Here the internal standards (IS) helped annotation, since the distances to the IS's were rather constant.

Another challenge in the identification is, that ISs have small impurities of the PFAS (eg. PFOS-IS may have a bit of PFOS in it), and in some cases they form the same fragments (product ions) that may interfere with the quantifier ions and hence the quantification. Also standards such as the FOSEs had impurities of PFOS, which could be seen because the standards were not mixed (PFOS was in the 'PFAC-24PAR' and FOSEs were combined into a separate mix.

The lack of certainty on retention times, and the lack of product ions for confirmation meant that rather large retention time spans had to be used in the search for e.g. SAm-PAPS. This combined with the presence of high levels of other contaminants (obviously organo-chlorine/bromine compounds signified by their characteristic isotopic patterns – e.g. at Mortonsvej (5) and Kulsviervej (6)) meant that the extraction of the accurate masses of the stable precursor ions was not sufficient to determine the potential identify of other prePFOS such as SAm-PAPS.

Presence of other organohalogens in the samples affecting the identification

Many peaks showed up in the suspect screening analyses, but were eventually discarded because it could not be ruled out that they were caused by the presence of other compounds with similar m/z values. The Figure S3 below shows some examples, of peaks which mass spectra show obvious isotopic patterns for chlorinated and/or brominated pollutants as illustrated for some with green circles. Unfortunately, these organohalogens also have negative mass defects which happen to have ions similar to precursor ions of PFCAs, such as 712.9523 Da (PFTeDA) and 812.9497 Da (PFHxDA), and for diPAPs x+y=12 (788.9902 Da) and also for a number of SAmPAPs (1102.95, 1202.95 Da), as shown in Figure S3.

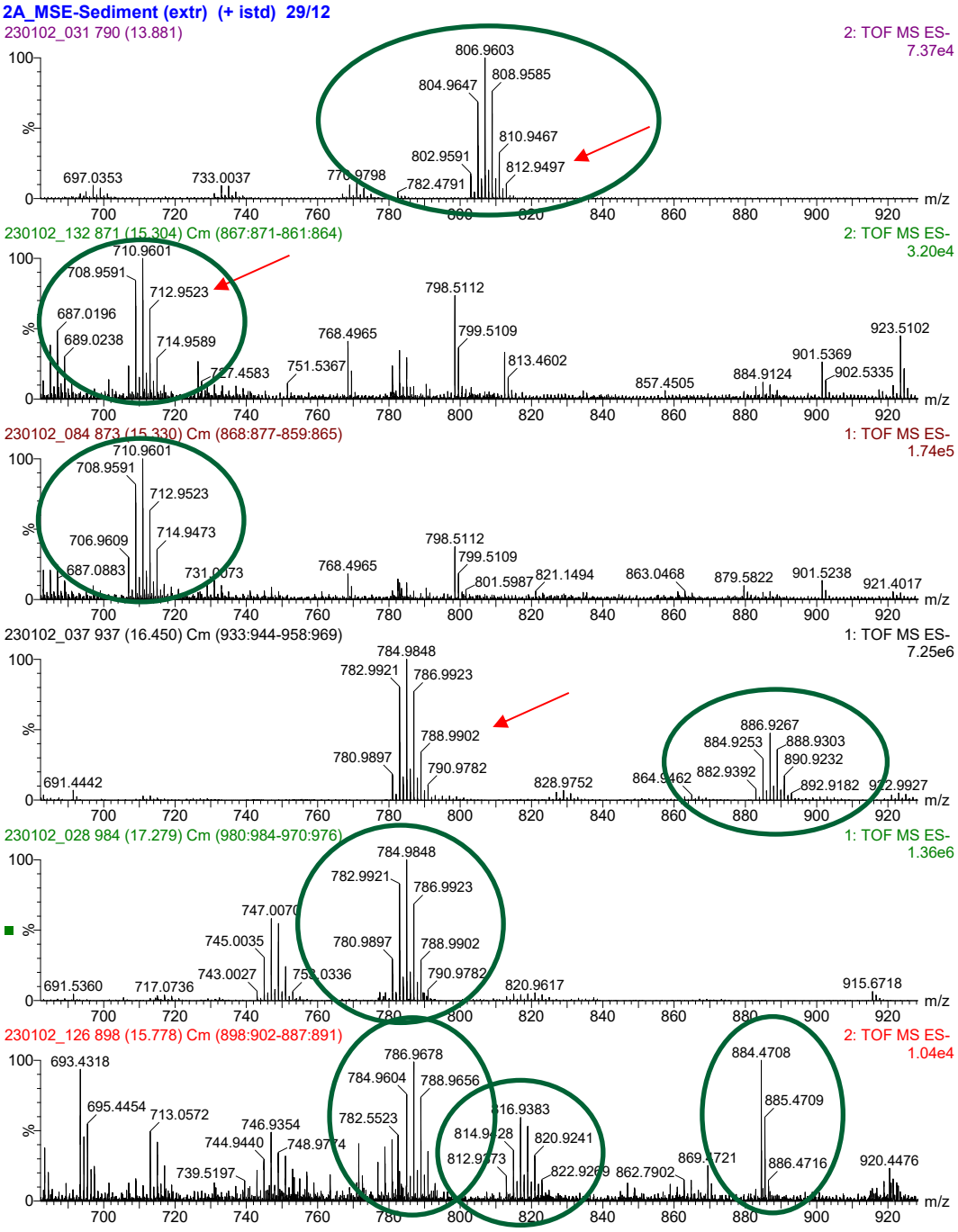


Figure S3: Examples of typical organo-chlorine/bromine isotopic patterns that points towards the 'matching' m/z values not being caused by a PFAS.

Therefore, these substances were for some samples not reported as detected, namely because
1) the mass spectras isotopic patterns were suspiciously looking like organochlorine compounds, not PFAS
2) for many of the suspect screened compounds we lacked standards to confirm their retention times and fragmentation spectra and
2) in the case of PFTeDA the retention time did match but the target/product ions area ratio of ca. 57 (area of 712.9473/668.9574) s did not match with the ratio of ca. 0.8 for the PFTeDA-IS (area of 714.9540/668.9608) as shown in Figure S4.

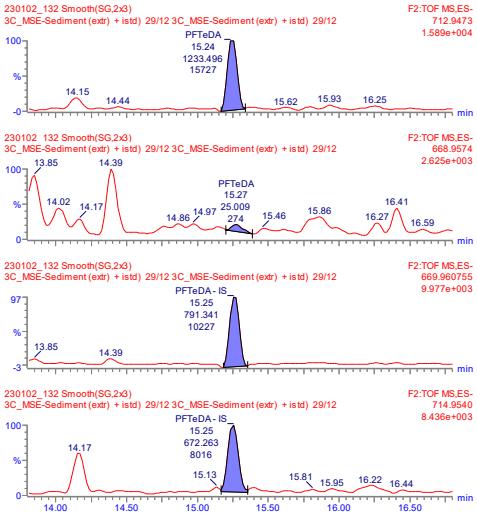
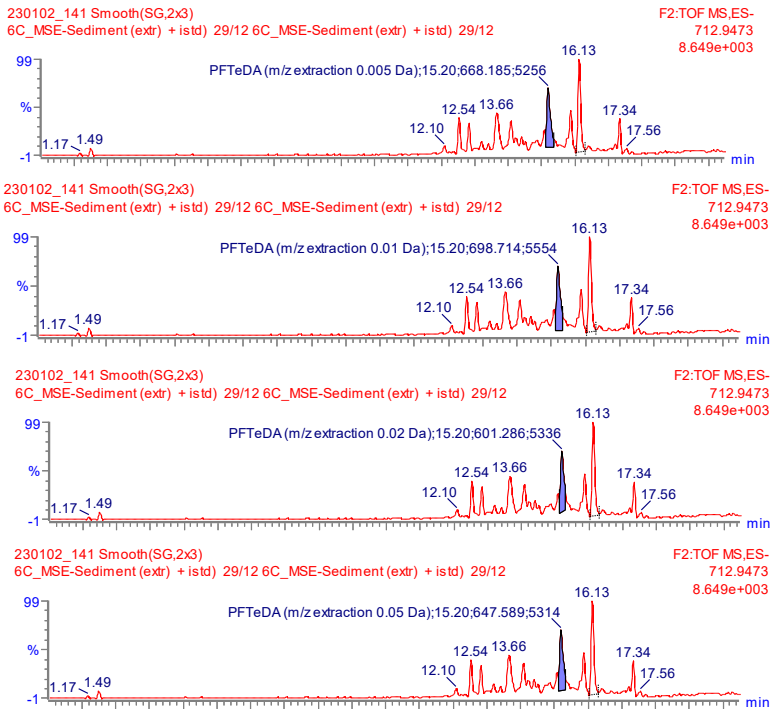


Figure S4: Differences in the ratio of quantification vs. target ion areas between PFTeDA-IS and the potential PFTeDA, supports the dismissal of PFTeDA being present in the sample.

Check if the mass extraction window could be lowered to get rid of interferences

We tried for one compound (PFTeDA) to extract the *m/z* with different settings in the TargetLynx method, by adding the same compound but with different mass windows ranging from 0.01-0.1 Da. The Extracted ion chromatograms are shown below in Figure S5, and shows that no peaks are 'removed' as we lower the mass extraction window.



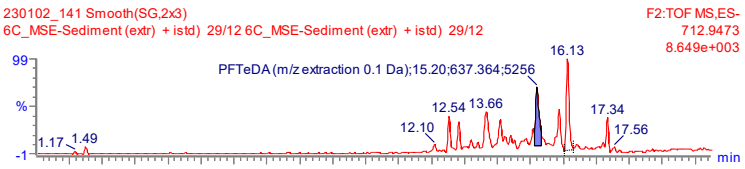


Figure S5: Mass extraction windows from 0.01 Da to 0.1 Da do not affect the fingerprint of the peaks for PFTeDA (712.9473 Da). Lowering the mass extraction window can therefore not be used to 'clean up' the spectrum – but on the good side sensitivity has not been sacrificed by use of a window of 0.05 Da.

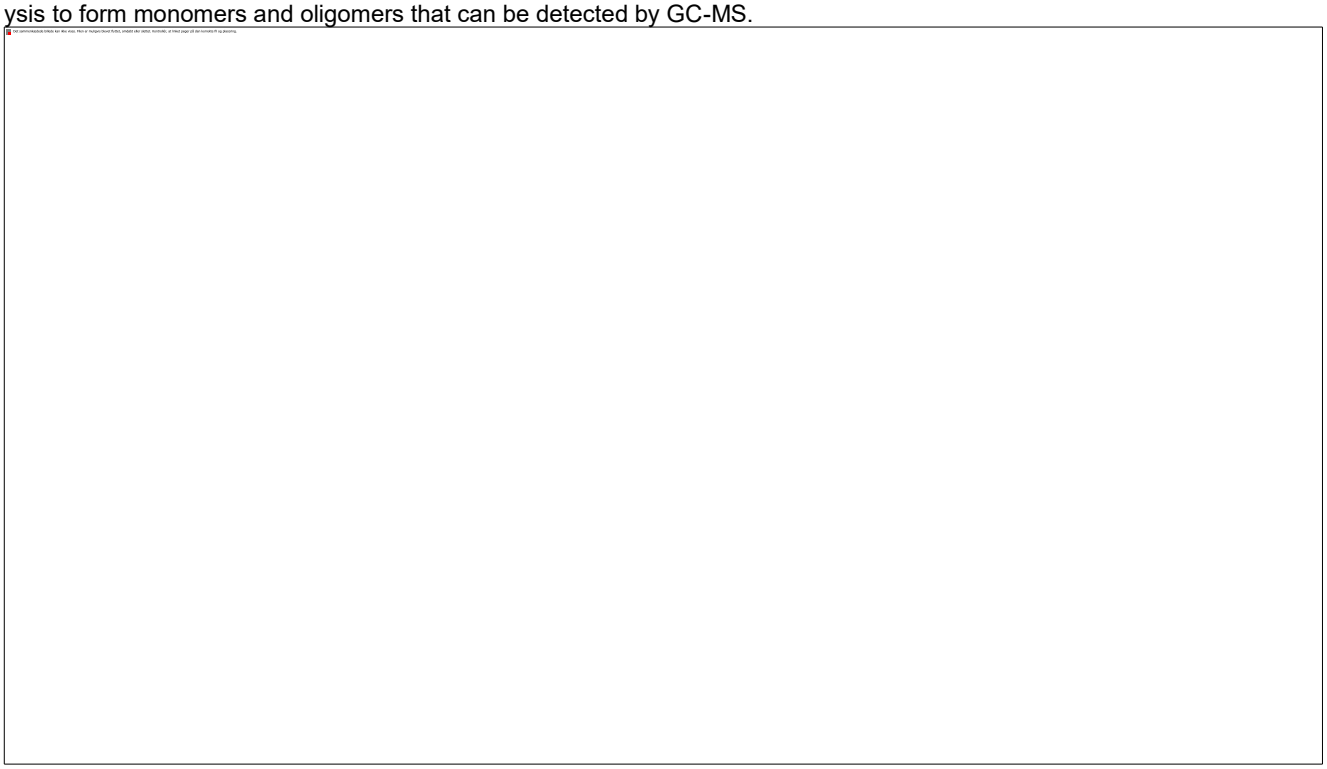
2.1.4 Future options for improvement of analyses

Sample pre-treatment:

- Try out other extraction methods, e.g. QuEChERS.
- Optimise extraction efficiency of long-chain PFAS, e.g. by alkaline digestion, and test of second/third extraction batch
- Measure the total organic carbon (TOC)
- Use SPE (e.g. on-line SPE – see comment under quantifications)

Quantification:

Getting standards for more PFAS, including prePFOS. The PFAS 'Handbook' (Danish Regions, 2022), provides suggestions for which PFAS to include. In addition, it would be key to include the PFAS which according to the Danish Product registry have been used the most in Denmark (DK, EPA 2016). The neutral compounds including the polyfluoropolyethers (PFPEs, CAS no. 65545-80-4, 26655-00-5) and 143372-54-7) and N-MeFOSE (CAS no. 24449-09-7) would be easiest detected by GC-MS methods. The polymers CAS no. 9011-17-01, 69991-67-9 and potentially 26655-00-5) may need to be analysed by pyrolysis to form monomers and oligomers that can be detected by GC-MS.



- Generate mass spectra at different collision energies and establish their retention times.
- Test higher collision energies, e.g. up to 100 eV, to enable generation of higher intensity product ions for the larger PFAS
- Careful selection of precursor and product ions, including potential adduct ions, to minimise the risk of interference from other PFAS including IS.

- Further prediction of retention times and mass spectral fragmentation patterns
- Use acetonitrile for the LC-method, as the mobile phase's organic solvent with the aim to lower the UHPLC back-pressure and thereby minimise the risk of shifts in retention times and blocking of the system
- Set up an on-line SPE and add a pre-filter. This would minimise the risk of transferring dirt into the system (resulting in build-up of back pressure over time). It could also increase the signal which may result in lower LODs and in more certain identifications. For efficiency (regenerating the SPE column while running the analytical column) a 10- and a 6-port valve system would have to be used.
- Run all the samples twice: with and without IS (to avoid blanks added from the standards) – this is however time-consuming and costly.

Identification of suspect/unknown PFAS by accurate mass spectrometry:

- Run the samples in Data Dependent Acquisition (DDA) mode to ensure generation of clean product ion spectra.
- Prediction of retention times by information of known pKa values and test runs with the mobile phase and LC conditions. Some pKa values are available from 4 Physical and Chemical Properties – PFAS — Per- and Polyfluoroalkyl Substances (itrcweb.org). See also *PFAS-håndbogen 2022_Final (miljoeogressourcer.dk).
- Perform data treatment with FluoroMatch to search for PFAS with typical Kendricks mass defects. This requires data ideally run in the DDA mode – or alternatively to extract the suspect peaks by MSDial from MSE spectra.
- Explore the possibility to use presence of impurities and homologous series from known synthesis routes, as fingerprints for PFAS – and use these as additional identification points.
- Knowledge of typical adduct ions can help to identify PFAS for which there are no pure reference standards. Adducts can also increase sensitivity for the neutral compounds such as FTOHs and FOSEs.
- The series of $C_3F_7^-$ (*m/z* of 168.9894 Da) can be used to search for PFCAs and precursors, and SO_3^- (*m/z* of 79.9568 Da) to search for PFSAs and precursors. High collision energies may be needed, but advanced acquisition setups with daughter ion scans may be used (so when one of these fragments are identified, it triggers an MS scan). In this way it may not be needed to degrade the PFAS in the sample preparation, as done in e.g. Total Oxidizable Precursor Assays (TOPA).
- Set up workflows on different platforms that allows for automatic search in data bases with, e.g. the NORMAN SLE (*m/z* values for precursors, and in some cases product ions and retention times); and massbanks e.g. the Massbank Of North America (MONA).

3. References

Danish Regions (2022). Håndbog om undersøgelse og afværge af forurening med PFAS-forbindelser, Teknik og Administration, Nr. 1 2022, Regionernes Videncenter for Miljø og Ressourcer. PFAS-håndbogen 2022_Final (miljoeogressourcer.dk)

DK EPA (2016) Kortlægning af brancher der anvender PFAS, Miljøprojekt nr. 1905, Miljøministeriet, Miljøstyrelsen.

DK EPA (2024) Sources of PFAS and their exchange between sediment and surface water, The Danish Environmental Protection Agency.

EUROFINS – Sorbisense and Sorbisense feltvejledning: What is Sorbisense? - Eurofins Danmark. Accessed March 30th 2024.

IRTC 2022. US Interstate Technology and Regulatory Councils website on PFAS, including physical-chemical properties. 4 Physical and Chemical Properties – PFAS — Per- and Polyfluoroalkyl Substances (itrcweb.org) Accessed April 5th 2023.

Appendix 1. Sampling

Figure A1: Scouting sampling locations

Appendix 1.1 Shortlist of potential sampling sites discussed with authorities

Appendix 1.2 Sampling of water by Sorbicells

The temperature was about 10-11 C when cages were set out in November, and about 5-6 C when collected in December 2022.

Table A1: Sampling information about locations, type of tube, depth, amount and comments

Water sampled by sorbicells								
No	Site	Sampled	GIS location	Tube type	Sampling depth	Tube A (g water collected)	Tube B (g water collected)	Comment
1	Nørreskoven	8/11-5/12-22	55.80367/ 12.40846	102	4.02 m	271 g	301 g	
2	Stavnsholt WWTP	8/11-5/12-22	55.80957/ 12.40856	102	2.90 – 3.10 m	231 g	244 g	Cage moved about 100 m East by wind
3	Bagsværd Rostadion	8/11-5/12-22	55.77272/ 12.44247	101	1.50 m	101 g	102 g	Very dirty at the top
4	Nybrovej	9/11-5/12-22	55.77136/ 12.46791	101	1.20 m	82 g	90 g	Dirty at the top

5	Mortonsvej	9/11-5/12-22	55.46230/12.29284	101	1.40 m	95 g	81 g + 20 cm	Black at the “filter”
6	Dybendal WWTP	9/11-6/12-22	55.80272/12.53664		0.5 m	342 g	461 g	
7	Kulsviervej	9/11-6/12-22	55.80197/12.95873	101	0.56 m	257 g	135 g	

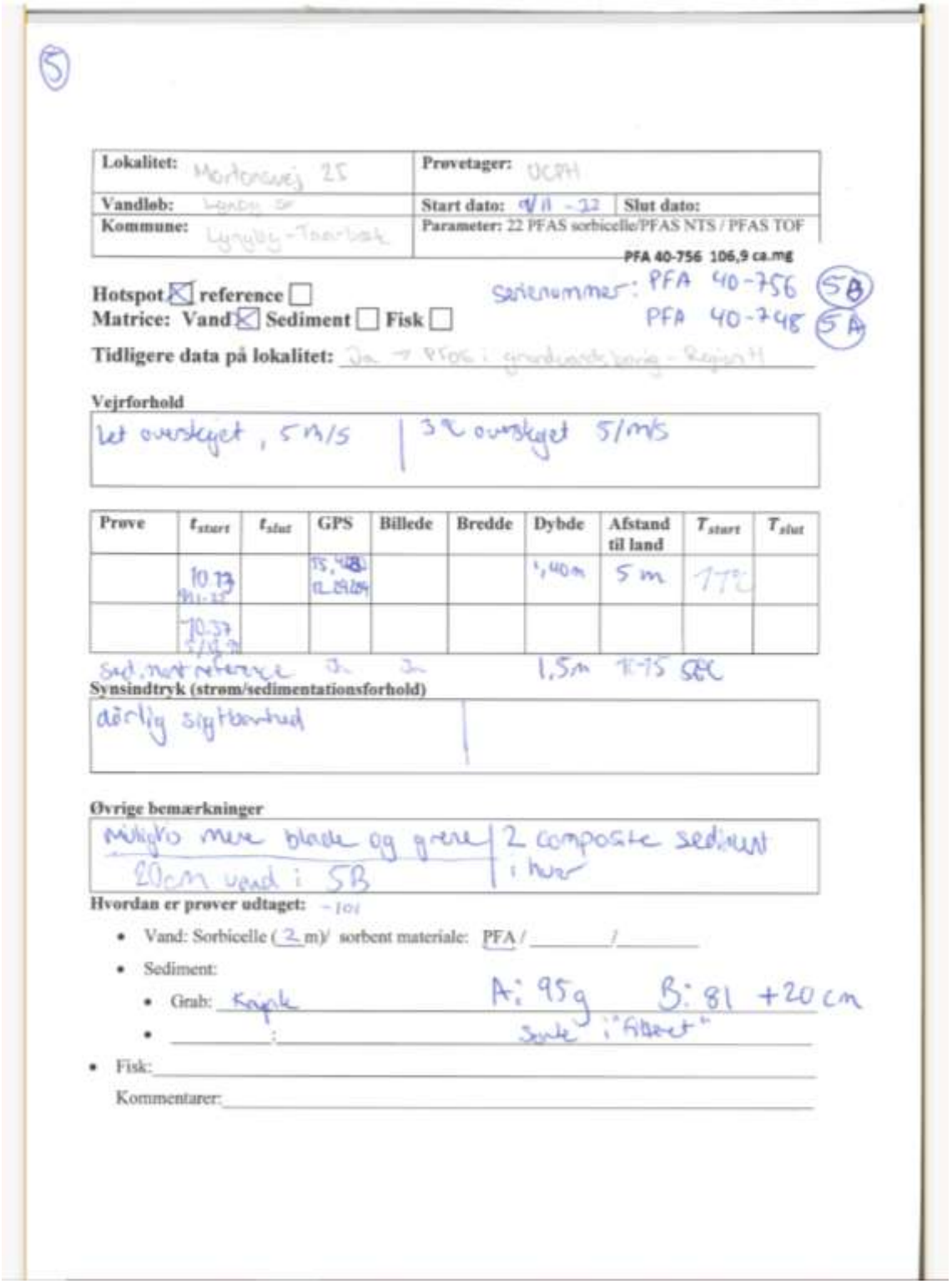
Appendix 1.3 Sampling of sediment

Table A2: Information about sediment samples

Sediments									
No	Site	Sampled	Mass-A (g)	Mass-B (g)	Total mass (g)	Eurofins mass (g)	Eurofins %dw	UCPH mass (g)	UCPH %dw
1	Nørresko-ven	5/12-2022	188	150	338		68		77
2	Stavnsholt WWTP	5/12-2022	80	98	178		49		66
3	Bagsværd Rostadion	5/12-2022	109	98	207		6.9		17
4	Nybrovej	5/12-2022	93	86	179		63		77
5	Mortonsvej	5/12-2022	107	104	211		23		62
6	Dybendal WWTP	6/12-2022	103	100	203		9.6		68
7	Kulsviervej	6/12-2022	107	112	219		26		49

Appendix 1.4 Example of a field journal

Example of Field journal for sampling of water by sorbicells. Weight of water for sub-sample A is 95g and subsample B is 81 g, plus 20 cm in the tubing.



5

Lokalitet: Mortensvej 25	Prøvetager: UCPH	
Vandløb: Lerdrup Sø	Start dato: 9/11 - 22	Slut dato:
Kommune: Lyngby-Taarbæk	Parameter: 22 PFAS sorbicelle/PFAS NTS / PFAS TOF	

PFA 40-756 106,9 ca.mg

Hotspot ☒ reference ☐
Matrice: Vand ☒ Sediment ☐ Fisk ☐

Serienummer: PFA 40-756 (5B)
PFA 40-748 (5A)

Tidligere data på lokalitet: Ja - PFOS i grundvandsboring - Region H

Vejrforhold

let overskyet, 5 m/s	3/8 overskyet 5/m/s

Prøve	t_{start}	t_{slut}	GPS	Billede	Bredde	Dybde	Afstand til land	T_{start}	T_{slut}
	10.17		55,48[illegible] 12,[illegible]			1,40 m	5 m	7°C	
	10.37								

Sed. ref. reference 2. 2. 1,5m 11-15 SEC

Synsindtryk (strøm/sedimentationsforhold)

dårlig sigtbarhed

Øvrige bemærkninger

Muligvis mere blade og grene 20cm vand i SB	2 composite sediment i hver

Hvordan er prøver udtaget: -10i

- Vand: Sorbicelle (2 m)/ sorbent materiale: PFA / ____ / ____
- Sediment:
  - Grab: Kajak A: 95g B: 81 +20 cm
  - ____: Sonde; "Albert"
- Fisk: ____

Kommentarer: ____

Lokalitet:	**Prøvetager:**	
Vandløb:	**Start dato:**	**Slut dato:**
Kommune:	**Parameter:** 22 PFAS sorbicelle/PFAS NTS / PFAS TOF	

Hotspot reference

Matrice: Vand Sediment Fisk

Tidligere data på lokalitet:

_____

Vejrforhold

Prøve	t_{start}	t_{slut}	GPS	Billede	Bredde	Dybde	Afstand til land	T_{start}	T_{slut}

Synsindtryk (strøm/sedimentationsforhold)

Øvrige bemærkninger

Hvordan er prøver udtaget:

- Vand: Sorbicelle (___m)/ sorbent materiale: PFA / _____/_____
- Sediment:
  - Grab:

    _____
  - _____:_____
- Fisk:_____

  Kommentarer:_____

5.4.10 Bilag 2 - Sedimentoplysninger

Institution: _____

Stationsnr.: **Dato** (for prøvetagning):

BESKRIVELSE AF SEDIMENTOVERFLADEN

Overflade

Obsansvarlig: **Institution:** _____

Obs. tidspunkt: _____ GMT

farve	struktur	tekstur
☐ sort	☐ jævn	☐ grus
☐ hvid	☐ ujævn	☐ sand
☐ grå	☐ sprækket	☐ silt & ler
☐ lysebrun	☐ flaget	
☐ mørkebrun	☐ tottet	

største mineral partikel (mm): _____

Sedimentbelægning

belægning	dækningsgrad
☐ diatoméer	_____ /8
☐ blågrøn alger	_____ /8
☐ Beggiatoa	_____ /8

Sedimentmakrofauna

makrofauna	type	dækningsgrad
☐ levende	_____	_____ /8
☐ døde	_____	_____ /8
☐ fækalier	_____	_____ /8
☐ faunarør	_____	_____ /8
☐ skaller	_____	_____ /8

Appendix 1.5 Sorbicells /Sorbisense description of principle and use

Sorbisense-Surfacewater-Monitoring UK.pdf

Close

Get started

First check the local conditions at the monitoring site. The water depth should preferably be >0.5 m and you will need a fixing point for the sampling unit. Then choose the correct Sorbicell sorbent type that corresponds to the solutes to be monitored. Finally choose the correct hydraulic resistance and mounting unit depending on the sampling depth (we recommend GWS-40/70 for sampling depth >10 m). Now you can choose your correct ordering number (see table below). Please note that SorbiCells are shipped in aluminium sealed bags with 6 pcs. ready for use.

Solute type	SorbiCell Type	Depth under water table	Sorbicell order no.	Sorbisystem
Per- and polyfluoralkyl substances:	SorbiCell PFAS	0,5-10 m >10 m	092-101 (6 pcs) 092-102 (6 pcs)	WW-50 GWS-40/70
Nutrients, SO4:	SorbiCell NiP	0,5-10 m >10 m	012-101 (6 pcs) 012-102 (6 pcs)	WW-50 GWS-40/70
Organics:	SorbiCell VOC	0,5-10 m >10 m	042-101 (6 pcs) 042-102 (6 pcs)	WW-50 GWS-40/70
Metals, NH4-N:	SorbiCell CAN	0,5-10 m >10 m	072-101 (6 pcs) 072-102 (6 pcs)	WW-50 GWS-40/70

Table 1. *Products suitable for surface water with minimum depth of 0,5m.*

Laboratory analyses

Finally, list your list of solutes and send your project information with the above information as a quotation request to sorbisense@eurofins.dk.

Along with the products we send standard field operating procedures. Further, we offer free on-line services for advice on installations and the optimal choice of analysis packages.

Eurofins A/S
Smedeskovvej 38
8464 Galten
Denmark
www.eurofins.dk

About us
Eurofins Scientific is a global market leader in food, environment and pharmaceutical products testing as well as in the fields of agro-science, genomics, and pharmacology. With over 30,000 staff in 400 laboratories across 42 countries, Eurofins offers a portfolio of over 150,000 analytical methods.
Sorbisense was founded as a Danish spin-off company from Aarhus University in 2004 by Dr. Hubert de Jonge and Prof. Dr. Gadi Rothenberg. Sorbisense was aquired by Eurofins in 2017, and has its headquarters in Denmark. We offer online and worldwide support for planning your projects and interpretation of the results, as well as advice on a growing number of laboratory tests for SorbiCell.

Feltvejledning Sorbisense WW-50 i vandløb.

Hubert de Jonge, Eurofins Miljø. 2022.

Udstyr og værktøj

- Sorbicells og feltprotokoller, transportrør (ved optagning)
- Værktøj: målestok, skævbider eller kniv, skruetrækker med lige kærv, strips, hammer
- Målebæger eller målecylinderglas til opsamling af feltvolumen.
- Reserveslang PE 6x8 mm og gummipropper (Eurofins Sorbisense)

Feltprotokol, opsætning prøvetagning

1. Tjek og mål vanddybde.
2. Montér luftslangen på beholdere ca. 1,5 m længde. Tjek at luftslangen sidder helt fast.
3. Montér SorbiCellen i beholderen og tryk fast med transportrør af Sorbicell. Sorbicell VOC/POL kan monteres direkte efter aftagning af bund- og top-propper. Ved anvendelse af Sorbicell PFA/NiP/CAN skal der ske en opfugtning af cellen før montering med vand fra målested eller evt. demineraliseret vand. Skriv type og serienr. af Sorbicells på feltprotokol. Bevar transportrør til optagning og transport. OBS til WW-50 montage bruges hydraulisk modstand der passer med måledybde – for overfladevand type "101", for regnvand med periodiske tørperioder anvendes type "090". Hydraulisk modstand kan ses på etiketten på poserne, ligesom udløbsdato og serienummer er angivet på emballagen:

SorbiCell™ produktinfo på emballage:

- På posen fremgår endvidere:
  Varenummer (OBS hydraulisk modstand og montage):
  - 998 montage i kugle ("Instream")
  - 090 regnvandsbrønd
  - 101 overfladevand 115-0072-101
  - 102 grundvand
- Produktions- og holdbarhedsdato best before 24-06-2022
- Unik serienummer der skal fremgår på rekvisitionen: CAN 31-605..
- Sorbicellerne kan sendes i transportrør, og i en plast-

Feltmontage Sorbisense WW50 i vandløb 2022, Eurofins Miljø. Kundeservice tlf. 7022 4231
www.eurofins.dk

4. På hver målested installeres antal 1,2 eller flere montage systemer efter behov. Ved flere montagesystemer, kan de beholdere evt. strippes "løst sammen" og sættes i en metal kurve, se billede herunder.

5. Øverste kurve sættes på som låg, og luftslanger samles og trækkes igennem låget. Kurver befæstes med strips.

6. Ved enden af luftslanger laves viklinger for at forhindre at fx regnvand kan trænge ind.

7. Kurven sættes på bund af vandløb med WW-50 beholdere nedstrøms.

8. Montager kan holdes på plads med 2 hegnspæle som bankes ned i vandbund. Luftlange befæstes på én af pælene med strips.

Feltmontage Sorbisense WW50 i vandløb 2022, Eurofins Miljø. Kundeservice tlf. 7022 4231
www.eurofins.dk

9. Lav gerne billede dokumentation ifm. eventuelle forespørgsler til Eurofins salgsteam.

Prøveskift

10. Mål vanddybde og vandsøjle over Sorbicell. Gerne billede dokumentation
11. Optag pæler og løft montage ud på åbrinken. Fjern strips der holder de 2 kurver sammen og løft låget. Fjern strips som holder de WW-50 sammen.
12. Tjek om luftslange er stadig korrekt monteret og gummi propper sidder ok. Evt. udskiftning af disse dele hvis der er tegn op beskadigelse.
13. Fjern evt. sediment der har lagt sig på SorbiCells og fjern Sorbicells med en lille skruetrekker med lige kærv, evt. spidstang. Tjek at der har været tab af sporsalt (se billede herunder), og tjek serie nummer af Sorbicell.

Billede af Sorbiceller efter prøvetagning. Udvaskning af sporsalt viser om der er tilstrækkeligt prøvevolumen. Venstre: meget lidt gennemstrømning og udvaskning af sporsalt, volumen < 0,05L. Middel og højre billede, tydelige udvaskning af sporsalt, god prøvevolumen. Prøvevolumen skal ligge mellem 100 og 500 ml for en god måling.

14. Fjern gummiprop og prøvevolumen måles ved at hælde vandet fra reservoiret over i et målebæger. Sorbicell nummer og notér volumen på feltprotokol.

15. Gentag punkt 3-9 som ovenfor.

16. Alle Sorbicells samles i en køleboks. I køleboksen skal medfølge en udfyldt rekvisition inden prøveafhentning bestilles.

Sidste prøveoptagning

17. Gentag punkt 10 - 14 og 16 som ovenfor.

NB. Når vanddybde er mindre en ca. 35 cm bruges ikke kurver men WW-50 beholderen kan evt. graves ned i vandbunden. Slangen fæstnes til pælen på sammen måde.

Prøvelogistik

Sorbiceller opbevares på køl inden transport til laboratorie. Sorbicell prøver puljes gerne sammen og sendes/afhentes i separat køletaske til laboratoriet efter aftale med kundeservice.

Appendix 2. Sample preparation for LC-MS suspect screening of PFAS

Figure A2: Precious final samples. Note that PFAS stick to surfaces, especially to glass and metal. To avoid losses, minimize the total surface area that PFAS are in contact with from sampling, sample pretreatment to analyses. To achieve the highest recovery, clean tubes with extra solvent and recombine with extract.

Appendix 2.1 Materials and instruments

A. Metal spoon to mix the samples with
B. Sarstedt tubes of PP – 50 mL, Cap of HDPE, product number 62.548.004 from Hounisen
C. Macherey-Nagel vials (700 μL with conical insert) and caps (septa PP inside, silicone on the outside), product number MN 702010 and MN 702402 from Mikrolab
D. Eppendorph tubes, 1.5 mL, material of polypropylene(PP), and with markings of 100, 500, 1000 μL
E. AcN – LCMS grade (preheated in a water bath to 40℃) with product number 34967 from Honewell
F. Macherey-Nagel 0.2 μm filters, d: 25 mm, Chromafil Xtra sprøjtefiltre, PES (polyethersulfon), MN729240, from Mikrolab

G. Mikrolab plastic syringes for the filters – 10 and 20 mL, material: PP, with product number ML 384610L and ML 384620L from Mikrolab
H. Plastic boxes for storage and for ethanol baths
I. IS: MPFAC-24PAR + 3 FOSE and 3 FTCA sediment samples and method blanks spiked with 2 ng, i.e. 10 μL 200 ng/mL used for spiking, from Wellington...
J. Pipettes with volumes ranges of 2-20 μL , 20-200 μL, 100-1000 μL and 0.5-5 mL. including tips, Thermofisher Finntips, model: F1
    a. Pipette calibration checked by weighing 3*smallest volumes
K. Aluminum foil
L. 5800 ultrasonication bath, from Branson
M. Thermometer
N. IKA KS 260 basic shaking-table for 50 mL tubes, from IKA-WERKE
O. 6-16K centrifuge, 11000 rpm, from Sigma
P. N_2 evaporation setup with small plastic pipettes, incl. block for heating from Glas-Col
Q. Oven for drying sediments, from BINDER
R. Weight for weighing sediment (ca. 5-15 g)
S. 96% ethanol (EtOH), with product number 83804.360 from VWR Chemicals
T. LC-MS grade water for eluents, with product number 455.2500 from ChemSolute
U. LC-MS grade methanol (MeOH)for eluents, with product number 34966 from Honeywell
V. LC-MS grade acetonitrile (AcN) for eluents, with product number 34967 from Honeywell

    a. Analytical chemical standards and internal standards (IS) from Wellington Laboratories, please see section 4 and refer to annexes for precise names and concentrations

Appendix 2.2 Cleaning of materials

All metal utensils, plastic tubes, caps, pipettes, beakers etc. in contact with the samples need to be cleaned twice in ethanol (96%). Use metal tweezers (pincet) to lift stuff out of the batch. Plastic pipettes should only be rinsed, not soaked. Do not wash the filters.[1]

1. Bath: 1-24 hrs;
2. Bath: just rinse
    2.1. Leave in plastic box to dry overnight in the fume hood, lightly covered by aluminum foil
    2.2. Use directly or store in clean plastic box. Vials can be put in LC-MS glass bottles.

Appendix 2.3 Sediment samples

Characterisation of sediments
Visual inspection noted.
Carbon content – determined by weighing in an oven in soil lab:
1. 24h at 105 ℃ - weigh to determine dry-weight (dw)
2. 16h at 450 degrees – weigh to determine total organic content (TOC) by subtraction of final weight from dw.
Removal of pore water
Weigh out 54-80 g of sample in one (or two if needed) 50 mL Sarstedt tube(s) depending of estimated dw. If sample is distributed in two tubes, take a bit from each for every sub-sample.

[1] if large background of some contaminant, they may be cleaned and blown dry with N2

Centrifuge for 45 min at 11,000 rpm
Decant pore water into 50 mL tube
Weighing of sediment
Mix the sample with a metal spoon, and take subsamples from different parts of the mixed sample
Weigh out 10 g of each of the sub-samples into 50 mL Sarstedt tubes
Add 10 μL of 200 ng/mL IS – this corresponds to a mass of 2 ng (and a final concentration in the injected sample of approximately 2 ng/700 μL)
Weigh out 2*5 g of the remaining sample to determine the dry-weight (see 3.1 above)

Appendix 2.4 Extraction

Following the method by Langberg et al. 2021 (PFAS in Tyrifjord sediments) with some modifications.
Applied to each sample and for blanks.

b) 1st Extraction: Add 15 mL of AcN into the Sarstedt tubes containing the sub-samples.
   ii) Place for 30 min in an ultrasonic bath at 40 C.
   iii) Place for 30 min at a shaking table.
   iv) Centrifuge for 3 min at 11000 rpm
   v) Decant into a new Sarstedt tube
c) *2nd Extraction*: Repeat the 1. Extraction and combine the extracts[2]
d) Concentrate the combined extract under N_2 to ca. 5 mL at ca. 40C. Fit single-use plastic pipette tips onto the N_2 metal tips. Ensure to blow in a way that avoids splattering. (if low recovery wash off the tips).
e) Suck up the remaining approx.. 5 mL of sample into a 10 mL syringe
f) Filter through 0.2 um filters ca. 1.35 mL into an Eppendorph tube; blow down to ca. 100 μL; Repeat twice until 100 μL left (i.e. to the 100 μL level pre-existing mark at the Eppendorphs).
g) Use ca. 500 uL to rinse pipette tips into the used Saarsted tube and filter it through into the Eppendorph tube. Blow down to 100 μL.
h) Concentrate under N2 to 100 μL
i) Add 600 uL of 1:1 water:AcN
j) Centrifuge Eppendorf vials at ca. 10C for 6 min to precipitate dissolve organic matter.
k) Transfer supernatant to 700 μL vials to ensure particles will stay in Eppendorf.

[2] In case of very poor recovery, add a 3. Extraction step: As 1. Extraction, but with 0.1% CH3COONH4 added to the AcN

Appendix 3. LC-QTOF MS suspect screening of PFAS

Appendix 3.1 LC and sequences

1. **LC Instrumentation**

a. Waters Acquity UHPLC – Synapt G2-Si, ESI^- QTOF MSMS high resolution mass spectrometry (HRMS)

b. Analytical column: Waters C18 CSH 150 mm*2.1 mm*1.7 μm

c. Mobile phases/Eluents

Mobile phases were prepared in advance and kept in a refrigerator until use

i. Mobile phase A: 5%/95% water/methanol with 20 mM Ammonium Formate buffer
ii. Mobile phase B: 100% methanol with 20 mM Ammonium Formate buffer

d. Preparation of stock buffer solutions: According to WATERS guidelines

Mobile phase A:
In a 1000 mL graduated cylinder 900 mL filtered MS-grade water was measured.
With a pipette 13.9 mL of 28% ammonium hydroxide solution were added to the cylinder and afterwards mixed. 1.62 mL formic acid was added with a pipette to the cylinder and mixed again.
MS-grade water was added to the 950 mark and then methanol was added to the 1000 mL mark.
The prepared buffer was hereby transferred to a 1-L blue cap bottle, mixed, and labeled according to guidelines.

Mobile phase B:
Procedure for mobile phase B is the same as mobile phase, but instead of water use methanol so the buffer composition will be 20 mM ammonium formate buffer in methanol.

2. **Sequences and inlet programs**

Before each injection of a sample, blank or analyse, two rinse runs were made:

a. Rinse 1 min (high organic (B) strength) – and one full cycle to wash needle and valves
b. Rinse 3 min (high water (A) to equilibrate at initial conditions – and clean out salts) – and one full cycle to wash needle and valves
c. Analysis 22 min – LC program is 22 min, but MS program 20.5 min (to avoid collection of data)

Samples were set up in this 'system' to avoid cross contamination and to have calibration curves around the samples.

d. Loading of PFAS high content (50 μg/L PFAC-24PAR), 2*(1 min rinse), 1*(3 min rinse)
e. Half a calibration curve (0-50-5-1 ng/mL) (e.g. PFAC-24PAR)
f. Run 8 samples – A sub-samples, starting and ending with extraction blanks
g. Half a calibration curve (20-2-0.5-0 ng/mL) (e.g. PFAC-24PAR)
h. Half a calibration curve (0-50-5-1 ng/mL) (e.g. FOSEs)
i. Run 8 samples – B sub-samples, starting and ending with extraction blanks
j. Half a calibration curve (20-2-0.5-0 ng/mL) (e.g. FOSEs)
k. Half a calibration curve (0-50-5-1 ng/mL) (e.g. MXFs)

l. Run 8 samples – C sub-samples, starting and ending with extraction blanks
m. Half a calibration curve (20-2-0.5-0 ng/mL) (e.g. MXFs)
n. A full calibration curve (0-50-5-1-20-2-0.5-0 ng/mL) (e.g. FTOHs)
o. A full calibration curve (0-50-5-1-20-2-0.5-0 ng/mL) (e.g. FTCAs)

Table A3: Rinse program – 1 min

Time(min)	Flow Rate	%A	%B	Curve
Initial	0.200	0	100	Initial
1.0	0.200	100	100	1

Table A4: Rinse program – 3 min

Time(min)	Flow Rate	%A	%B	Curve
Initial	0.200	0	100	Initial
0.5	0.200	95	5	2
3.0	0.200	95	5	1

Table A5: Analysis inlet program – 22 min

Time(min)	Flow Rate	%A	%B	Curve
0.5	0.280	95	5	Initial
1.5	0.280	55	45	1
2.5	0.280	50	50	6
4.5	0.280	45	55	6
14.5	0.280	15	85	6
15.5	0.300	7	93	6
16.5	0.300	2	98	6
17.0	0.300	1	99	1
17.5	0.400	0	100	1
20.5	0.400	0	100	1
21.0	0.300	95	5	1
22.0	0.300	95	5	1

Appendix 3.2 QTOF MS settings

1. MS Tune settings

ESI (Neg), optimized for small molecules. The source and cone were cleansed weekly. CapV = -3 kV, Cone 35 eV, OffSet V= 60 V, Source temp: 120 C, Desolvation gas temp: 500 C, Desolvation gas flow: 800 L/hr, Cone gas flow: 100 L/hr, Nebulizer gas pressure: 5.5 bar, Ion Energy 1: 1.0. Collision energy: 2 V (MS), or collision energy ramp (MSE), See Appendix 3.7 for Experimental file.

Calibration: *m/z* 50-1200 Da every day with NaF negative calibration in *Resolution* mode, to obtain < 1ppm mass accuracy (typically 0.2-0.4 ppm). Lockspray used with LeuEnk (neg mode 554.2615 Da). Detector voltage tuned beforehand (approx. 2800 V in Neg mode).

2. MS Method

See Appendix 3.7 for full information on parameters

Data were acquired in the MSE Resolution mode, with an *m/z* 70-1250 Da; scan time 0.5, continuum, t=20.5 min; Event: @20.5 min sample sent to waste to protect detector.

Function 1: MS analyses, at Col E = 2 V

Function 2: MSE analyses; Low Collision energy = 10 V (Transfer collision energy off), High Collision energy = 60 V (Transfer collision energy off)

Appendix 3.3 Analytical chemical standards

Standards were provided by Wellington Laboratories. Please refer to Appendix 5 for an overview of standards. Please note that some concentrations are provided for the salts (e.g. K or Na salts), and for the dissolved acids (which is the concentration used for the calibration curves)

l) PFAC-24PAR: Mix of a total of 24 stds of
   i) C6-C14 PFCAs (stock solution: 2000 ng/mL)
   ii) 4:2, 6:2, 8:2 FTS (stock solution: 2000 ng/mL)
   iii) FOSAs, N-MeFOSAA, N-EtFOSAA (stock solution: 2000 ng/mL)
   iv) Linear PFSAs (C4- C10), (stock solution: 2000 ng/mL for the salt – the dissolved acid concentrations vary, see table)
   v) Branched PFSAs (C6, C8) (stock solutions for the acids: C6 (1480 ng/mL), C8 (1460 ng/mL))

m) FOSEs: N-MeFBSE-M, N-MeFOSE-M, N-EtFOSE-M (stock solution: 50000 ng/mL),

n) FTCAs: 4:2, 6:2, 8:2 FTS (stock solution: 50000 ng/mL) – *data collected but not extracted for this study*

o) FTOHs: 6:2, 8:2, 10:2 FTOHs (stock solution: 50000 ng/mL) - *data collected but not extracted for this study*

p) MXF (Mix of replacements for PFCAs), (stock solution: 2000 ng/mL) - *data collected but not extracted for this study*

2) Internal Analytical standards:

Standards were provided by Wellington Laboratories. Please refer to Appendix 5.2 and 5.3 (PFAS structures) for an overview of standards. Please note that some concentrations are provided for the salts (e.g. K or Na salts), and for the dissolved acids (which is the concentration used for the calibration curves).

MPFAC-24ES (ISTDs: used for PFAC-24PAR, incl. FOSA used for FOSEs; also used for MXF) stock solution: 1000 ng/mL

MFTCA (6:2, 8:2, 10:2): stock solution: 50000 ng/mL

MFTOH (6:2, 8:2, 10:2): stock solution 50000 ng/mL

Appendix 3.4 Calibration curves for semi-quantification by LC-QTOF MS

a. External calibration curves with analytical stds (+ IS)
   Concentrations: 0.5-1-2-5-10-20-50 ng/mL and same IS concentration spiked to calibration stds as for samples.
b. Linearity please see Appendix 4.1 and Appendix 4.2

Appendix 3.5 Validation:

c. Uncertainty: Precision by analysis of triplicates, and double calibration curves
d. Blanks: 1:1 MeOH/water, and extraction blanks (+IS).
e. Validation of the uncertainty and recovery of semi-quantification by spiking PFAC-24PAR and FOSEs to a sediment (Mortonsvej), and extraction efficiency by spiking a sediment (Mortonsvej) with two concentration levels and comparison with external calibration curve were performed, by the IS levels looked odd, so these data were not used. In any case the IS will correct for extraction efficiency, matrix effects etc.
f. Comparison with Eurofins analyses
g. Dry-weight determined by duplicate weight analyses

Appendix 3.6 Data analyses

a) Quantification
WATERS TargetLynx V4.1 using ISTDs, automatic integration of peak area and manual check, and generation of calibration curves.

b) Idenfication
Alignment: Chemometric in-house MatLab software
NTS: FluoroMatch open source software: FluoroMatch Flow – Innovative Omics

Appendix 3.7 Example of Acquisition Experiment Report

File:c:\masslynx\xenia pfas 2022.pro\data\230102_016.raw

Note: This file contains a record of the instrument parameters used at the start of the acquisition.
Note: Where parameters are varied through the experimental method, refer to that method or the spectrum header for details. These include, but are not limited to Use of LockSpray, Use of EDC, Collision Energy ramping, and Pusher frequency.

Header
Acquired File Name: 230102_016
Acquired Date: 03-Jan-2023
Acquired Time: 18:39:24
Job Code: 2022_PFAS_230102
Task Code:
User Name:
Laboratory Name:
Instrument: SYNAPTG2-Si#NotSet
Conditions:
Submitter:
SampleID: MSE - Cal 5 - PFAC-24PAR (+ istd)
Bottle Number: 1:4
Description: MSE - Cal 5 - PFAC-24PAR (+ istd)

Instrument Calibration:
Calibration File:
Parameters
MS1 Static: None
MS1 Scanning:
Mass: 50 Da to 1200 Da.
Resolution: 0.0/0.0
Ion Energy: 0.0
Reference File: ESI_NaFormate_Neg
Acquisition File: Small Molecules (test XT)-2023-0o~9
MS1 Scan Speed Compensation: None
MS2 Static: None
MS2 Scanning: None
MS2 Scan Speed Compensation: None
Calibration Time: 17:30
Calibration Date: 01/03/23
Coefficients
MS1 Static: None
MS2 Static: None
Function 1: -0.000000000185*x^5 + 0.000000020609*x^4 + -0.000000841756*x^3 + 0.000015487366*x^2 + 1.000224762288*x +-0.006232105302, Root Mass
Function 2: -0.000000000185*x^5 + 0.000000020609*x^4 + -0.000000841756*x^3 + 0.000015487366*x^2 + 1.000224762288*x +-0.006232105302, Root Mass
Function 3: -0.000000000185*x^5 + 0.000000020609*x^4 + -0.000000841756*x^3 + 0.000015487366*x^2 + 1.000224762288*x +-0.006232105302, Root Mass

Parameters for C:\MassLynx\Xenia PFAS 2022.PRO\ACQUDB\PFAS_221220_MSe_Res_Col_(20-5 min).EXP
Created by 4.1 SCN 924

Lock Spray Configuration:

Reference Scan Frequency(sec)	30.000	
Reference Cone Voltage(V)	30.000	
Reference Trap Collision Energy	4.000	
Reference DRE Setting		9.580

Temperature Correction:

Temperature Correction	Disabled

Instrument Configuration:

Lteff	1800.0

Veff			7204.49
Resolution		20000	
Min Points in Peak	2		
Acquisition Device	WatersADC		
Acquisition Algorithm		ADC Mode	
ADC Trigger Threshold (V)	-1.00		
ADC Input Offset (V)		-1.56	
Average Single Ion Intensity	29		
ADC Amplitude Threshold		2	
ADC Centroid Threshold		-1	
ADC Ion Area Threshold		3	
ADC Ion Area Offset	10		
ADC Pushes Per IMS Increment	1		
EDC Delay Coefficient		1.4100	
EDC Delay Offset	0.4000		
Experimental Instrument Parameters			
Instrument Parameter Filename	C:\MassLynx\Xenia PFAS 2022.PRO\ACQUDB\Xenia PFAS opstart 2022_MS_4.IPR (MODIFIED)		
Polarity		ES-	
Capillary (kV)		3.0000	
Source Temperature (°C)		120	
Sampling Cone		25.0000	
Source Offset		60.0000	
Source Gas Flow (mL/min)	0.00		
Desolvation Temperature (°C)	500		
Cone Gas Flow (L/Hr)		100.0	
Desolvation Gas Flow (L/Hr)	800.0		
Nebuliser Gas Flow (Bar)	5.5		
LM Resolution		4.7	
HM Resolution		15.0	
Aperture 1		0.0	
Pre-filter		2.0	
Ion Energy		1.0	
Manual Trap Collision Energy	FALSE		
Trap Collision Energy		4.0	
Manual Transfer Collision Energy	FALSE		
Transfer Collision Energy	2.0		
Manual Gas Control	FALSE		
Trap Gas Flow (mL/min)		2.00	
HeliumCellGasFlow	180.00		
IMS Gas Flow (mL/min)		90.00	
Detector		2800	
DetectorCache		0	
Sample Infusion Flow Rate (µL/min)	20		
Sample Flow State	LC		
Sample Fill Volume (µL)		250	
Sample Reservoir	C		
LockSpray Infusion Flow Rate (µL/min)	20		
LockSpray Flow State		Infusion	
LockSpray Reservoir		B	
LockSpray Capillary (kV)	2.5		
Use Manual LockSpray Collision Energy	FALSE		
Collision Energy	4.0		
Acceleration1		70.0	
Acceleration2		200.0	
Aperture2		70.0	
Transport1		70.0	
Transport2		70.0	
Steering		0.07	
Tube Lens		62	
Pusher			1900.0
Pusher Offset		0.33	
Puller			1370.0
Pusher Cycle Time (µs)		Automatic	
Pusher Width (µs)	Automatic		
Collector		60	
Collector Pulse		10.0	
Stopper			10
Stopper Pulse		20.0	
Entrance		60	
Static Offset		180	
Puller Offset		0.00	

Reflectron Grid (kV)		1.480		
Flight Tube (kV)		10.00		
Reflectron (kV)			3.780	
Use Manual Trap DC			FALSE	
Trap DC Entrance		0.0		
Trap DC Bias			2.0	
Trap DC				-2.0
Trap DC Exit			2.0	
Use Manual IMS DC		FALSE		
IMS DC Entrance			-20.0	
Helium Cell DC			1.0	
Helium Exit			-20.0	
IMSBias				2.0
IMS DC Exit			20.0	
USe Manual Transfer DC			FALSE	
Transfer DC Entrance			5.0	
Transfer DC Exit		15.0		
Trap Manual Control			OFF	
Trap Wave Velocity (m/s)		300		
Trap Wave Height (V)			0.5	
IMS Manual Control		OFF		
IMS Wave Velocity (m/s)			300	
IMS Wave Height (V)			0.0	
Transfer Manual Control			OFF	
Transfer Wave Velocity (m/s)		247		
Transfer Wave Height (V)		0.2		
Step Wave 1 In Manual Control		OFF		
Enable Reverse Operation		OFF		
Step Wave 1 In Velocity (m/s)		300.0		
Step Wave 1 In Height			15.0	
Step Wave 1 Out Manual Control		OFF		
Step Wave 1 Out Velocity (m/s)		300.0		
Step Wave 1 Out Height			15.0	
Step Wave 2 Manual Control		OFF		
Step Wave 2 Velocity (m/s)		300.0		
Step Wave 2 Height		1.0		
Use Manual Step Wave DC			OFF	
Step Wave TransferOffset		25.0		
Step Wave DiffAperture1			3.0	
Step Wave DiffAperture2			0.0	
Use Automatic RF Settings		TRUE		
StepWave1RFOffset		300.0		
StepWave2RFOffset		350.0		
Target Enhancement Enabled		FALSE		
Target Enhancement Mode			EDC	
Target Enhancement Mass			556.0	
Target Enhancement Trap Height (V)	4.0			
Target Enhancement Extract Height (V)	15.0			
Mobility Trapping Manual Release Enabled	FALSE			
Mobility Trapping Release Time (µs)	500			
Mobility Trap Height (V)		15.0		
Mobility Extract Height (V)		0.0		
Trag Gate LUT table enabled		FALSE		
TriWave Trap Gate LookUp Table				
Using Drift Time Trimming		FALSE		
Drift Time Bins			0	
Using Mobility Delay after Trap Release	TRUE			
IMS Wave Delay (µs)			1000	
Variable Wave Height Enabled		FALSE		
Wave Height Ramp Type			Linear	
Wave Height Start (V)			10.0	
Wave Height End (V)			40.0	
Wave Height Using Full IMS		TRUE		
Wave Height Ramp (%)			100.0	
Wave Height Look Up Table				
Variable Wave Velocity Enabled		FALSE		
Wave Velocity Ramp Type			Linear	
Wave Velocity Start (m/s)		1000.0		
Wave Velocity End (m/s)			300.0	
Wave Velocity Using Full IMS		TRUE		
Wave Velocity Ramp (%)			100.0	
Wave Velocity Look Up Table				
Backing				2.82e0

Source			6.02e-3
Sample Plate		1.00e-6	
Trap			8.51e-3
Helium Cell		7.58e-4	
IMS			7.72e-4
Transfer		8.72e-3	
TOF			6.26e-7
IMSRFOffset		300	
IMSMobilityRFOffset		250	
TrapRFOffset		300	
Use Automatic RF Settings	TRUE		
AutoStepWave1RFOffset		300	
AutoStepWave2RFOffset		350	
TransferRFOffset	350		
MS Profile Type		Auto P	
MSProfileMass1		100	
MSProfileDwellTime1		20	
MSProfileRampTime1		20	
MSProfileMass2		300	
MSProfileDwellTime2		20	
MSProfileRampTime2		40	
MSProfileMass3		500	
PusherInterval		69.000000	
PusherOffset		0.250000	
LockMassValidSigma		5	
Acquisition mass range			
Start mass		70.000	
End mass		1250.000	
Calibration mass range			
Start mass		113.046	
End mass		1132.445	
Experiment Reference Compound Name: Leucine Enkephalin Single Point MS			
Function Parameters - Function 1 - TOF PARENT FUNCTION			
[ACQUISITION]			
Survey Start Time	0.0		
Survey End Time		20.5	
Survey Ion Mode		ES Mode	
Survey Polarity		Negative	
[PARENT MS SURVEY]			
Survey Start Mass	70.0		
Survey End Mass		1250.0	
Parent Survey Low CE (V)	10.0		
TIC Threshold		5.0	
Survey Scan Time	0.5		
Survey Interscan Time		0.0	
Survey Data Format	Continuum		
Analyser		Resolution Mode	
ADC Sample Frequency (GHz)	3.0		
TargetEnhancementMass2		69.0	
TargetEnhancementMass3		1.75	
Survey Use Tune Page CV		YES	
[PRODUCT IONS]			
Use High CE Product Ions Mass List File NO			
High CE Product Ions Mass List Filename			
Product Ions Match Logic	NO		
Product Ions Switch Threshold (Intensity/s)	10.0		
Product Ions Switch Detection Window +/- (mDa)	100.0		
Product Ions Retention Time Window +/- (sec)	10.0		
[NEUTRAL LOSS]			
Use Neutral Loss Mass List File	NO		
Neutral Loss Mass List Filename			
Neutral Loss Match Logic	OR		
Neutral Loss Switch Threshold (Intensity/s)	10.0		
Neutral Loss Switch Detection Window +/- (mDa)	100.0		
[MS/MS]			
MSMS Start Mass		70.0	
MSMS End Mass		1250.0	
Number of components		0	
Use MSMS to MS Switch After Time	NO		
MSMS Switch After Time (sec)	10.0		

Absence of Neutral Loss			NO
Absence of Product Ion			NO
MSMS Scan Time (sec)			1.0
MSMS Interscan Time (sec)		0.0	
MSMS Data Format		Continuum	
Use Tune Page Cone Voltage		YES	
Use MS/MS ipr File		NO	
Instrument Parameter Filename			
[PEAK DETECTION]			
Peak Detection Window			1.0
Use Intensity based Peak Detection	YES		
Charge State Tolerance Window		0.2	
Charge State Extraction Window		4.0	
Deisotope Tolerance Window		0.2	
Deisotope Extraction Window		4.0	
Discard survey data		NO	
[COLLISION ENERGY]			
Trap MS Collision Energy (eV)		10.0	
Using Auto Transfer MS Collision Energy (eV)	2.000000		
[INCLUDE]			
Precursor Selection		Everything	
[EXCLUDE]			
Use Exclude Masses List			NO
Exclude Mass Range			
Use Exclude File Masses			NO
Exclude Mass Filename			
Exclude Window +/- (mDa)		100.0	
Exclude Retention Time Window		10.0	
Reference Centroid Average			
Reference Frequency			0.0
Reference Cone Voltage			0.0
Calibration			Dynamic 2
Function Parameters - Function 2 - TOF PARENT FUNCTION			
[ACQUISITION]			
Survey Start Time		0.0	
Survey End Time			20.5
Survey Ion Mode			ES Mode
Survey Polarity			Negative
[PARENT MS SURVEY]			
Survey Start Mass		70.0	
Survey End Mass			1250.0
Ramp High Energy from			10.0 to 60.0
TIC Threshold			5.0
Survey Scan Time		0.5	
Survey Interscan Time			0.0
Survey Data Format		Continuum	
Analyser			Resolution Mode
ADC Sample Frequency (GHz)		3.0	
TargetEnhancementMass2			69.0
TargetEnhancementMass3			1.75
Survey Use Tune Page CV			YES
[PRODUCT IONS]			
Use High CE Product Ions Mass List File	NO		
High CE Product Ions Mass List Filename			
Product Ions Match Logic		NO	
Product Ions Switch Threshold (Intensity/s)	10.0		
Product Ions Switch Detection Window +/- (mDa)	100.0		
Product Ions Retention Time Window +/- (sec)	10.0		
[NEUTRAL LOSS]			
Use Neutral Loss Mass List File	NO		
Neutral Loss Mass List Filename			
Neutral Loss Match Logic		OR	
Neutral Loss Switch Threshold (Intensity/s)	10.0		
Neutral Loss Switch Detection Window +/- (mDa)	100.0		
[MS/MS]			
MSMS Start Mass			70.0
MSMS End Mass			1250.0
Number of components			0
Use MSMS to MS Switch After Time	NO		
MSMS Switch After Time (sec)		10.0	
Absence of Neutral Loss			NO
Absence of Product Ion			NO

MSMS Scan Time (sec)			1.0
MSMS Interscan Time (sec)		0.0	
MSMS Data Format		Continuum	
Use Tune Page Cone Voltage		YES	
Use MS/MS ipr File		NO	
Instrument Parameter Filename			
[PEAK DETECTION]			
Peak Detection Window			1.0
Use Intensity based Peak Detection	YES		
Charge State Tolerance Window		0.2	
Charge State Extraction Window		4.0	
Deisotope Tolerance Window		0.2	
Deisotope Extraction Window		4.0	
Discard survey data		NO	
[COLLISION ENERGY]			
Trap MS Collision Energy Low (eV)	10.0		
Trap MS Collision Energy High (eV)	60.0		
Using Auto Transfer MS Collision Energy (eV)	2.000000		
[INCLUDE]			
Precursor Selection		Everything	
[EXCLUDE]			
Use Exclude Masses List			NO
Exclude Mass Range			
Use Exclude File Masses			NO
Exclude Mass Filename			
Exclude Window +/- (mDa)		100.0	
Exclude Retention Time Window		10.0	
Reference Centroid Average			
Reference Frequency			0.0
Reference Cone Voltage			0.0
Calibration			Dynamic 2
Function Parameters - Function 3 - TOF PARENT FUNCTION			
[ACQUISITION]			
Survey Start Time		0.0	
Survey End Time			20.5
Survey Ion Mode			ES Mode
Survey Polarity			Negative
[PARENT MS SURVEY]			
Survey Start Mass		70.0	
Survey End Mass			1250.0
Parent Survey High CE (V)		30.0	
TIC Threshold			5.0
Survey Scan Time		0.5	
Survey Interscan Time			0.1
Survey Data Format		Continuum	
Analyser			Resolution Mode
ADC Sample Frequency (GHz)		3.0	
TargetEnhancementMass2			69.0
TargetEnhancementMass3			1.75
Survey Use Tune Page CV			YES
[PRODUCT IONS]			
Use High CE Product Ions Mass List File	NO		
High CE Product Ions Mass List Filename			
Product Ions Match Logic		NO	
Product Ions Switch Threshold (Intensity/s)	10.0		
Product Ions Switch Detection Window +/- (mDa)	100.0		
Product Ions Retention Time Window +/- (sec)	10.0		
[NEUTRAL LOSS]			
Use Neutral Loss Mass List File	NO		
Neutral Loss Mass List Filename			
Neutral Loss Match Logic		OR	
Neutral Loss Switch Threshold (Intensity/s)	10.0		
Neutral Loss Switch Detection Window +/- (mDa)	100.0		
[MS/MS]			
MSMS Start Mass			100.0
MSMS End Mass			1500.0
Number of components			1
Use MSMS to MS Switch After Time	NO		
MSMS Switch After Time (sec)		10.0	
Absence of Neutral Loss			NO
Absence of Product Ion			NO
MSMS Scan Time (sec)			1.0

MSMS Interscan Time (sec)		0.1	
MSMS Data Format		Continuum	
Use Tune Page Cone Voltage		YES	
Use MS/MS ipr File		NO	
Instrument Parameter Filename			
[PEAK DETECTION]			
Peak Detection Window			1.0
Use Intensity based Peak Detection	YES		
Charge State Tolerance Window		0.2	
Charge State Extraction Window		4.0	
Deisotope Tolerance Window		0.2	
Deisotope Extraction Window		4.0	
Discard survey data		NO	
[COLLISION ENERGY]			
Using Auto Trap MS Collision Energy (eV)	4.000000		
Using Auto Transfer MS Collision Energy (eV)	2.000000		
[INCLUDE]			
Precursor Selection		Everything	
[EXCLUDE]			
Use Exclude Masses List			NO
Exclude Mass Range			
Use Exclude File Masses			NO
Exclude Mass Filename			
Exclude Window +/- (mDa)		100.0	
Exclude Retention Time Window		10.0	
Reference Centroid Average			
Reference Frequency			0.0
Reference Cone Voltage			0.0
Calibration			Dynamic 2

ACE Experimental Record

Inlet Method File: c:\masslynx\xenia pfas 2022.pro\acqudb\pfas_test_221220_22 min

---------- Run method parameters ----------

Waters Acquity SDS
Run Time: 22.00 min
Comment:
Solvent Selection A: A1
Solvent Selection B: B1
Low Pressure Limit: 0.000 bar
High Pressure Limit: 1034.200 bar
Solvent Name A: Water_20mM Am FA
Solvent Name B: MeOH_20mM Am FA
Switch 1: No Change
Switch 2: No Change
Switch 3: No Change
Seal Wash: 5.0 min
Chart Out 1: System Pressure
Chart Out 2: %B
System Pressure Data Channel: Yes
Flow Rate Data Channel: Yes
%A Data Channel: Yes
%B Data Channel: Yes
Primary A Pressure Data Channel: No
Accumulator A Pressure Data Channel: No
Primary B Pressure Data Channel: No
Accumulator B Pressure Data Channel: No
Degasser Pressure Data Channel: No
[Gradient Table]
Time(min) Flow Rate %A %B Curve
1. Initial 0.300 95.0 5.0 Initial
2. 0.50 0.280 95.0 5.0 1
3. 1.50 0.280 55.0 45.0 6
4. 2.50 0.280 50.0 50.0 6
5. 4.50 0.280 45.0 55.0 6

6. 14.50 0.280 15.0 85.0 6
7. 15.50 0.300 7.0 93.0 6
8. 16.50 0.300 2.0 98.0 6
9. 17.00 0.300 1.0 99.0 1
10. 17.50 0.400 0.0 100.0 1
11. 20.50 0.400 0.0 100.0 1
12. 21.00 0.300 95.0 5.0 1
13. 22.00 0.300 95.0 5.0 1
Run Events: Yes
Gradient Start (Relative to Injection): 0 uL
2D Repeat: No

Waters Acquity CM

Target Column Temperature: 40.0 C
Temperature Alarm Band: Off
Shutdown all columns: No
Column Valve Position: Column 1
Equilibration Time: 0.1 min
Active Preheater: Disabled
External Valve 1: No Change
External Valve 2: No Change
External Valve 3: No Change
Comment:
Column Temperature Data Channel: No
Preheater Temperature Data Channel: No

Waters ACQUITY FTN AutoSampler
Run Time: 22.00 min
Comment:
Load Ahead: Disabled
Loop Offline: Automatic min
Wash Solvent Name: MeOH_20mM Am FA
Pre-Inject Wash Time: 0.0 sec
Post-Inject Wash Time: 6.0 sec
Purge Solvent Name: MeOH_20mM Am FA
Dilution: Disabled
Dilution Volume: 0 uL
Delay Time: 0 min
Dilution Needle Placement: 1.0 mm
Target Column Temperature: Off C
Target Sample Temperature: 20.0 C
Sample Temperature Alarm Band: Disabled
Syringe Draw Rate: Automatic
Needle Placement: Automatic
Pre-Aspirate Air Gap: Automatic
Post-Aspirate Air Gap: Automatic
Column Temperature Data Channel: No
Room Temperature Data Channel: Yes
Sample Temperature Data Channel: Yes
Sample Organizer Temperature Data Channel: No
Sample Pressure Data Channel: No
Preheater Temperature Data Channel: No
Seal Force Data Channel: No
No Injection Mode Enabled: No
Autoaddition Mix Stroke Cycles: Automatic
Autoaddition Mix Stroke Volume: Automatic uL
Active Preheater: Disabled
Run Events: No

Sample Run Injection Parameter

Injection Volume (ul) - 5.00

---------- oOo ----------

End of experimental record.

---------- Waters Acquity SDS Postrun Report ----------

IcsVersion: 1.70.2864

FirmwareVersion: 1.65.273 (Feb 27 2015)

Checksum: 0x3462a3f3

SerialNumber: C14BUR988M

Minimum System Pressure: 427.3

Maximum System Pressure: 905.2

Average System Pressure: 729.6

Minimum Degasser Pressure: 0.0

Maximum Degasser Pressure: 0.0

Average Degasser Pressure: 0.0

---------- oOo ----------

---------- Waters Acquity CM Postrun Report ----------

Software Version: 1.69.2942

Firmware Version: 1.69.154 (Feb 17 2017)

Checksum: 0x17ac4a76

Serial Number: A14CMP051G

Valve Position: 1

ColumnType: ACQUITY UPLCr CSHT C18 1.7µm

Column Serial Number: 01813226515133

Column Part Number: 186005298

Total Injections on Column: 336

Minimum Column Temperature: 40.0 C

Maximum Column Temperature: 40.0 C

Average Column Temperature: 40.0 C

---------- oOo ----------

---------- Waters ACQUITY FTN Postrun Report ----------

Software Version: 1.69.2261

Firmware Version: 1.65.375 (Mar 26 2015)

Checksum: 0x34728d7d

Serial Number: L13USM352G

Sample Syringe Size: 250.0

Extension Loop Size: 0.0

Needle Size: 15.0

Minimum Sample Temperature: 19.8 C

Maximum Sample Temperature: 23.0 C

Average Sample Temperature: 21.1 C

Minimum Column Temperature: -0.2 C

Maximum Column Temperature: 0.0 C

Average Column Temperature: -0.2 C

---------- oOo ----------

Function 1
Scans in function: 1167
Cycle time (secs): 0.514
Scan duration (secs): 0.500
Inter Scan Delay (secs): 0.014
Start and End Time(mins): 0.000 to 20.500
Ionization mode: ES-
Data type: Enhanced Mass
Function type: TOF MS
Mass range: 70 to 1250

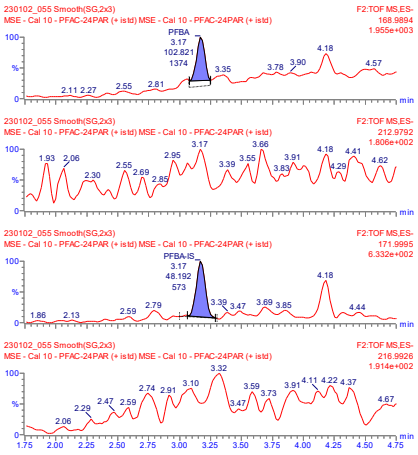
Function 2
Scans in function: 1167
Cycle time (secs): 0.514
Scan duration (secs): 0.500
Inter Scan Delay (secs): 0.014
Start and End Time(mins): 0.000 to 20.500
Ionization mode: ES-
Data type: Enhanced Mass
Function type: TOF MS
Mass range: 70 to 1250

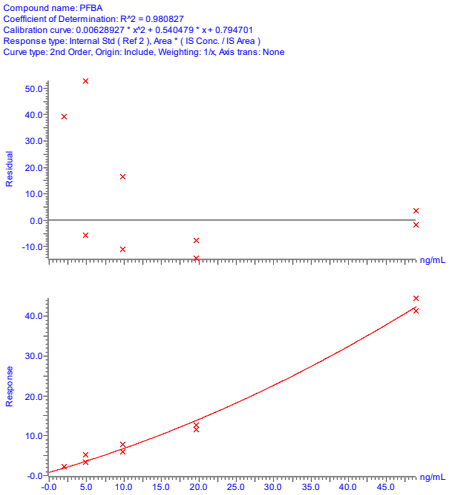
Function 3
Scans in function: 41
Cycle time (secs): 0.600
Scan duration (secs): 0.500
Inter Scan Delay (secs): 0.100
Start and End Time(mins): 0.000 to 20.500
Ionization mode: ES-
Data type: Enhanced Accurate Mass
Function type: TOF MS
Mass range: 70 to 1250

Appendix 4. Method performance

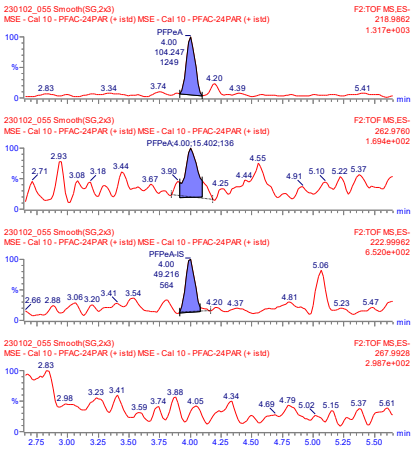
Appendix 4.1 Calibration curves – PFCAs (230102)

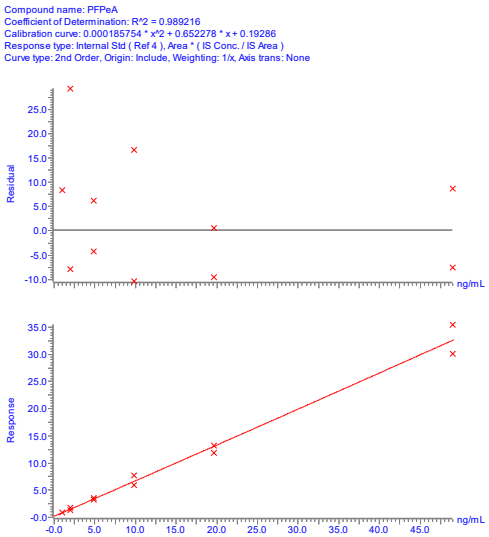
1. PFBA



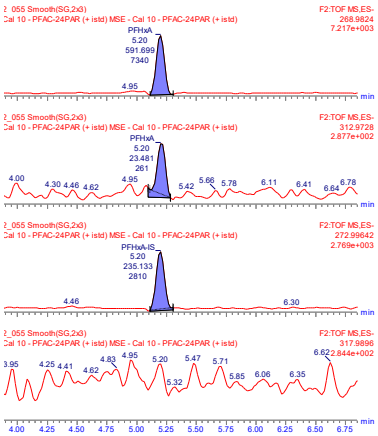


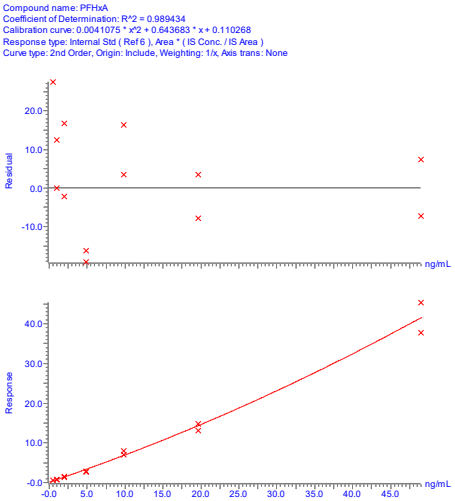
2. PFPeA



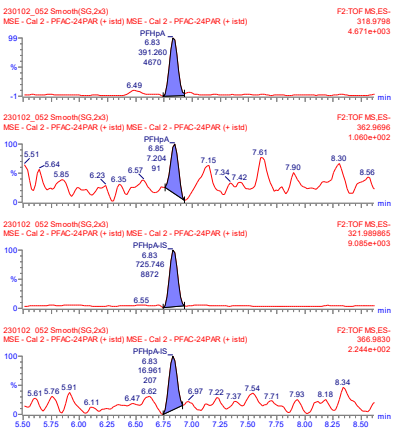


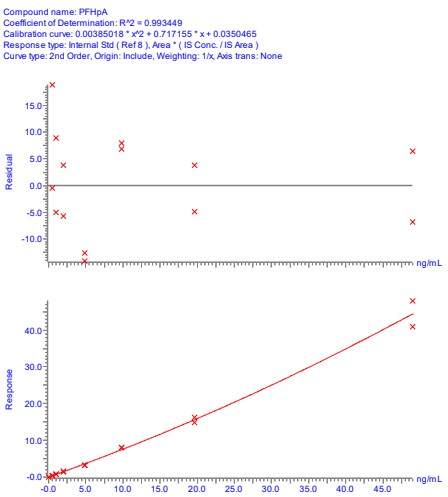
3. PFHxA



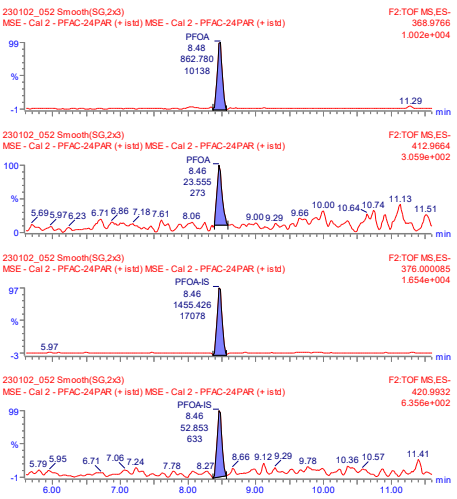


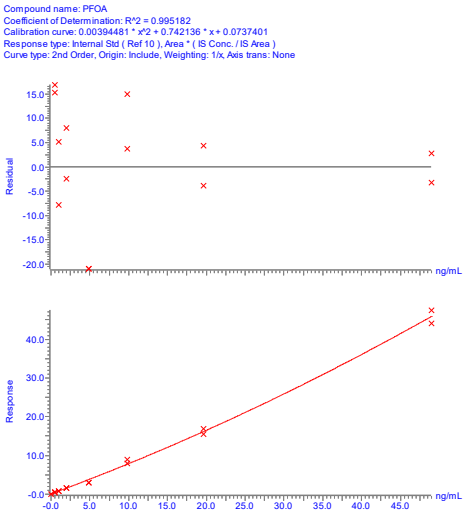
4. PFHpA



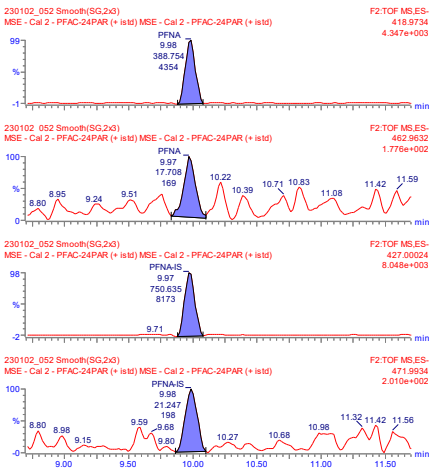


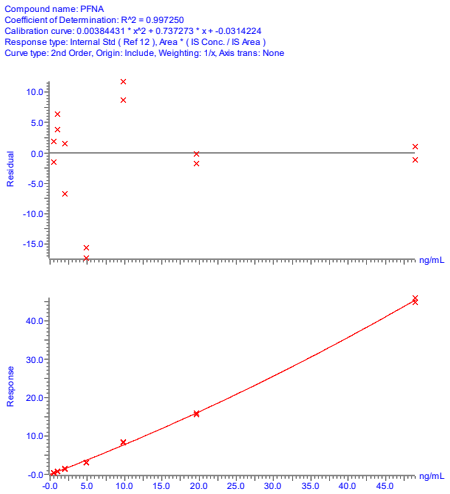
5. PFOA



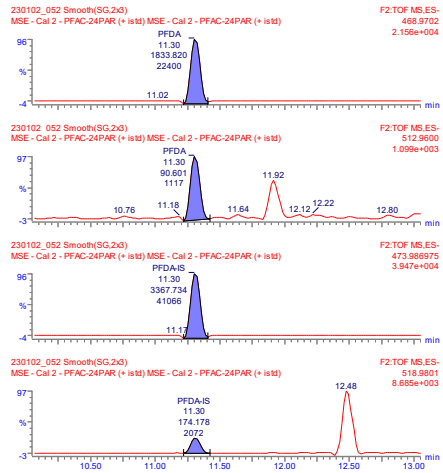


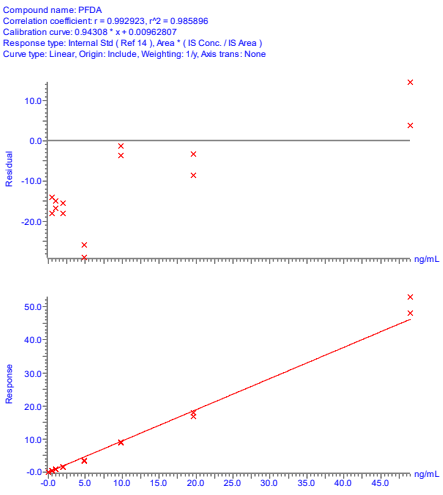
6. PFNA



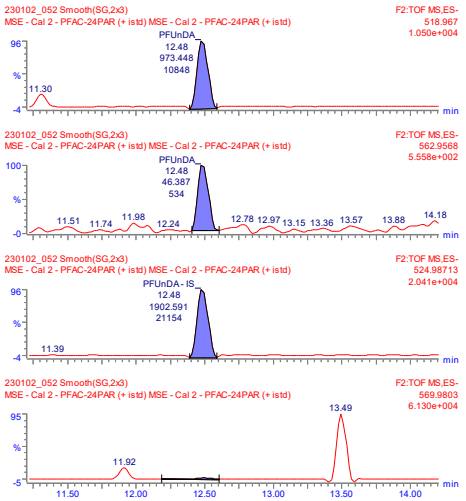


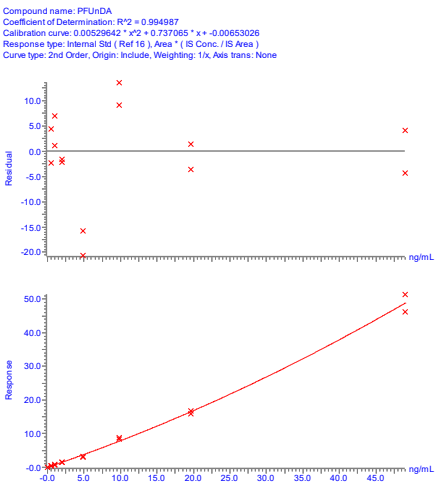
7. PFDA



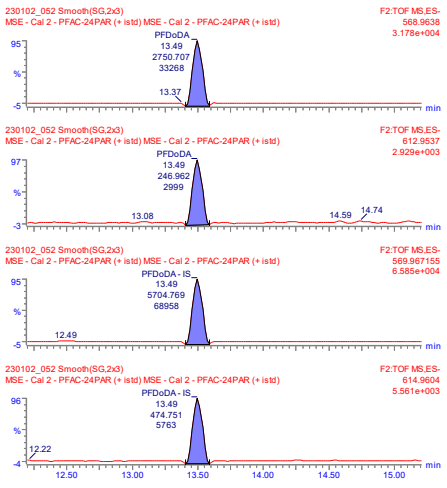


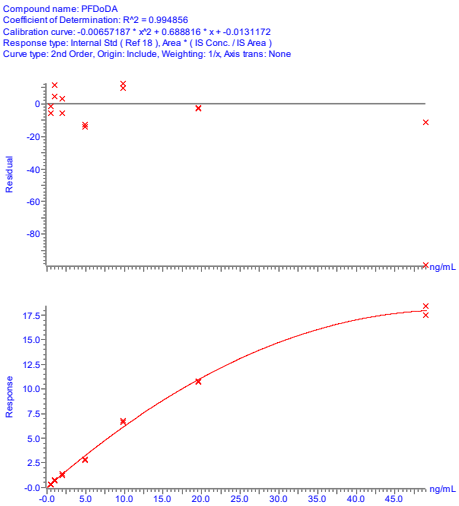
8. PFUnDA



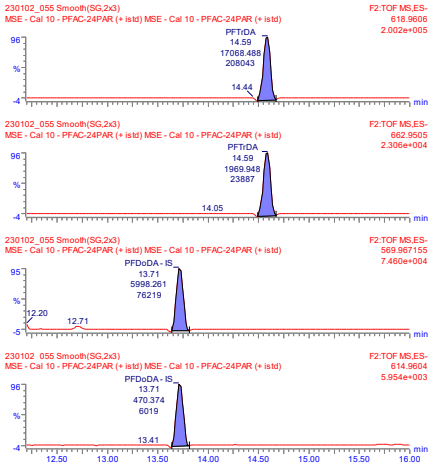


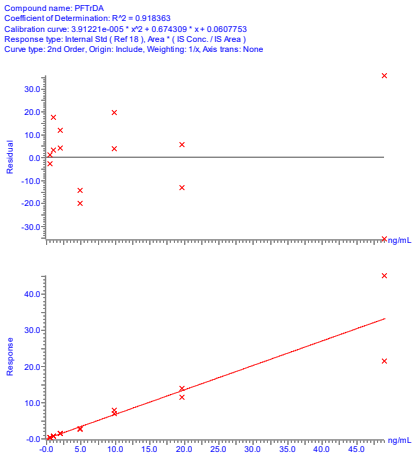
9. PFDoDA



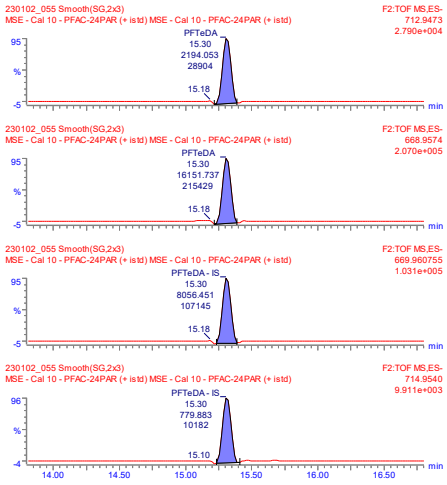


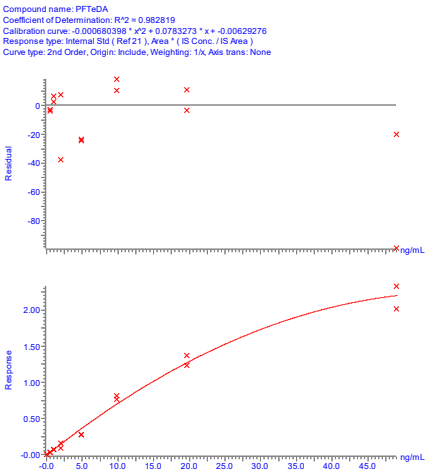
10. PFTrDA



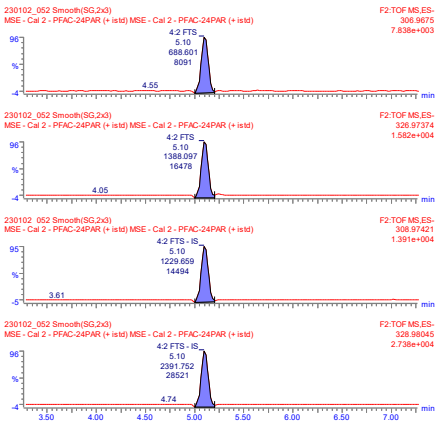


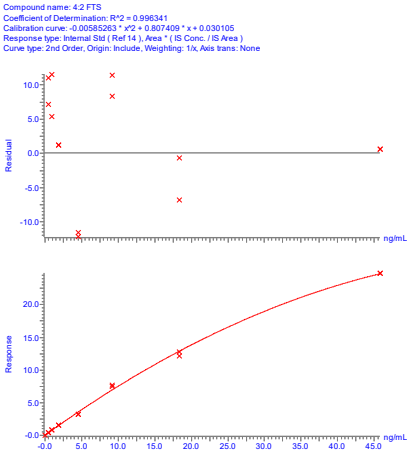
11. PFTeDA



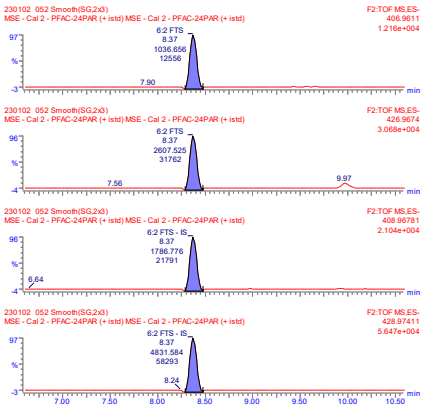


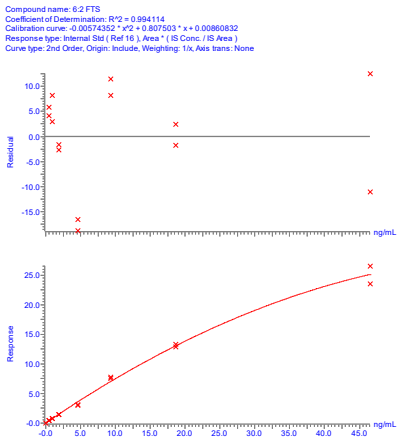
12. 4:2 FTS



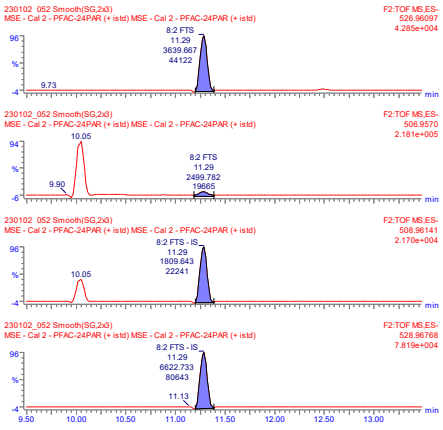


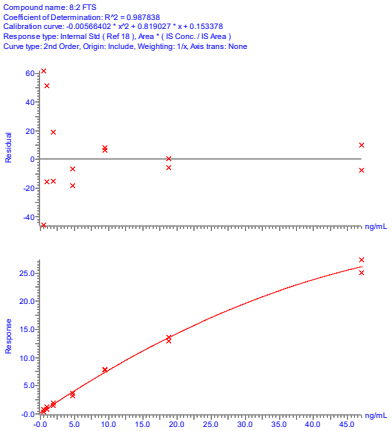
13. 6:2 FTS





14. 8:2 FTS

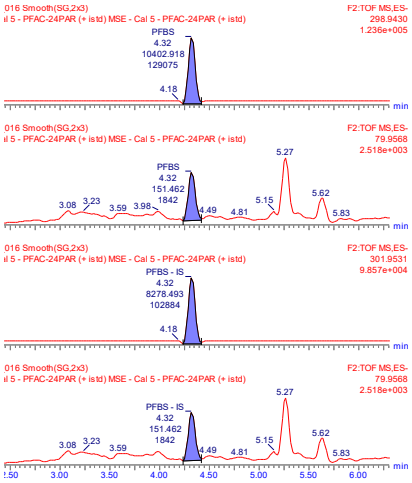


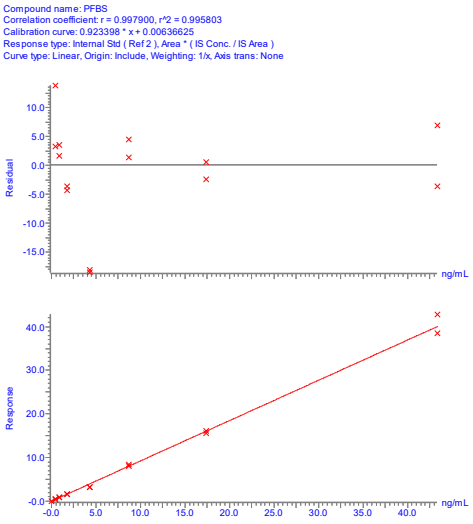


10:2 and 12:2 FTS were quantified against 8:2 FTS calibration curve and 8:2 FTS-IS.

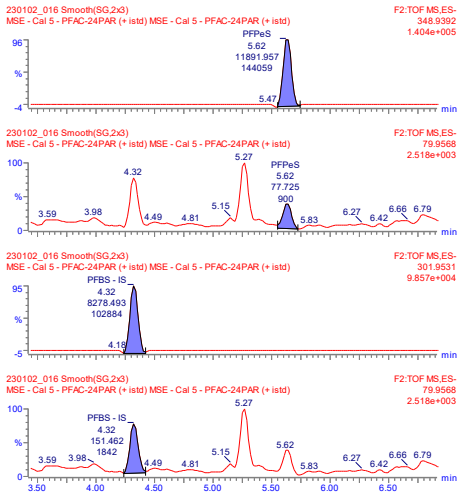
Appendix 4.2 Calibration curves – PFSAs (230102)

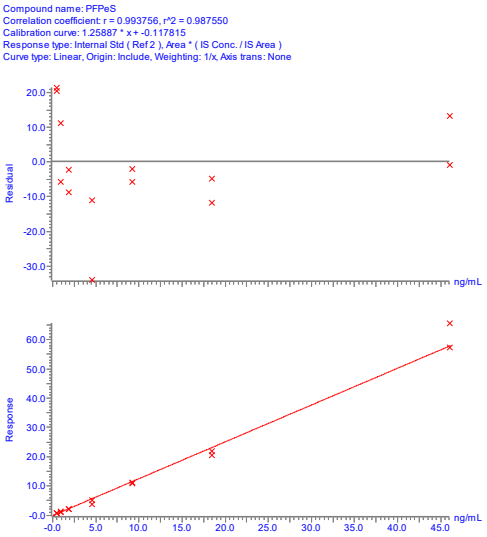
15. PFBS



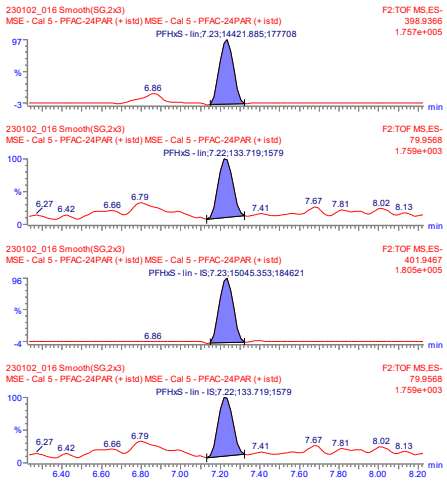


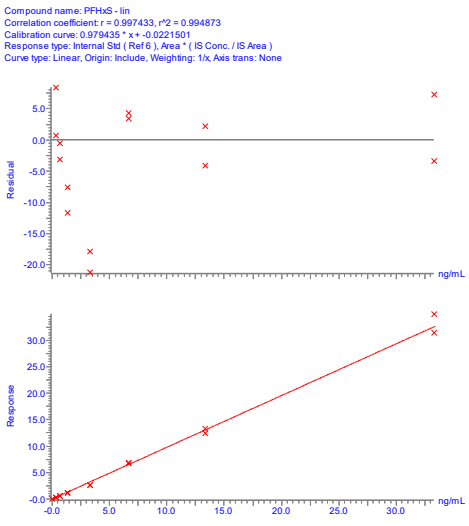
16. PFPeS



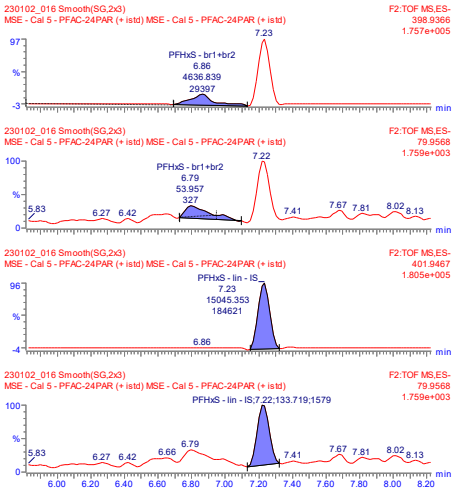


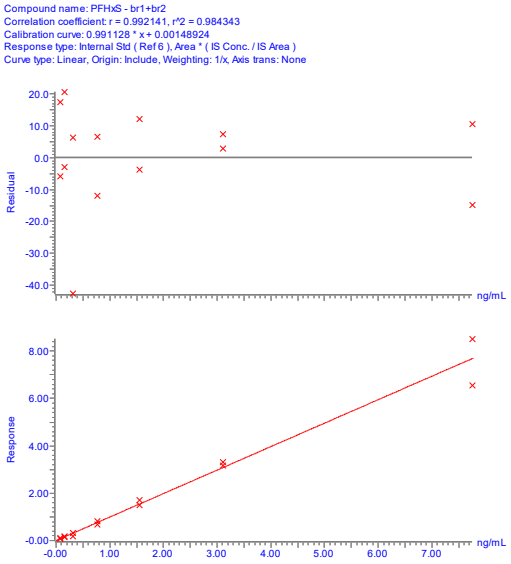
17. PFHxS – linear



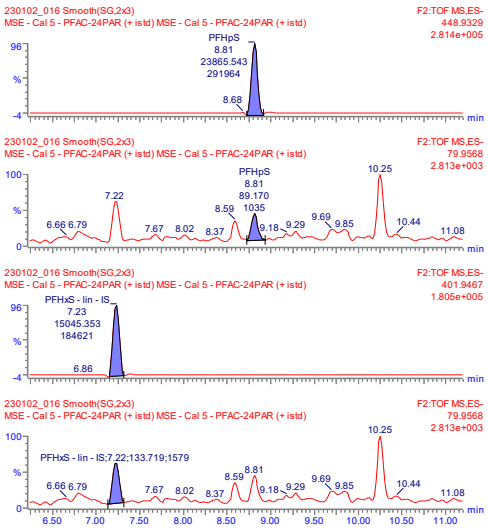


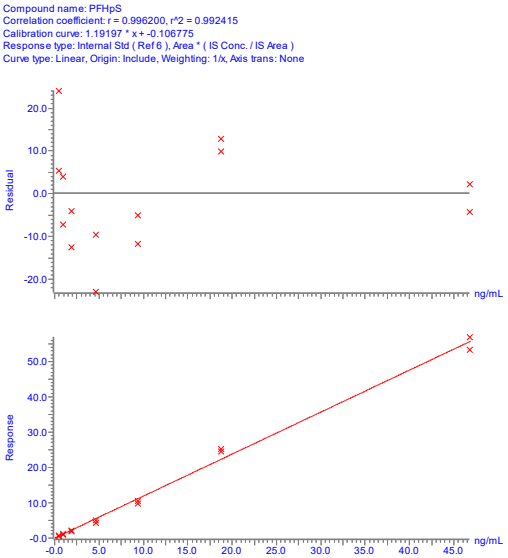
18. PFHxS – branched



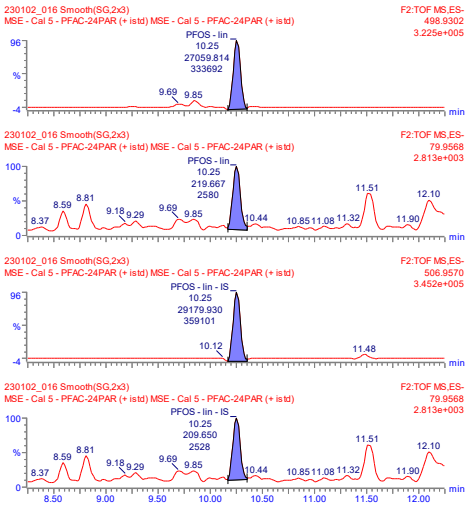


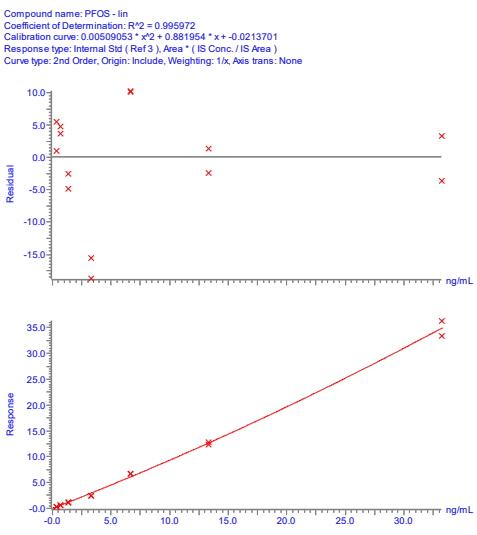
19. PFHpS



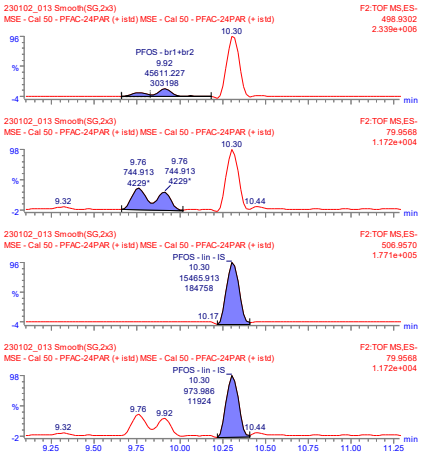


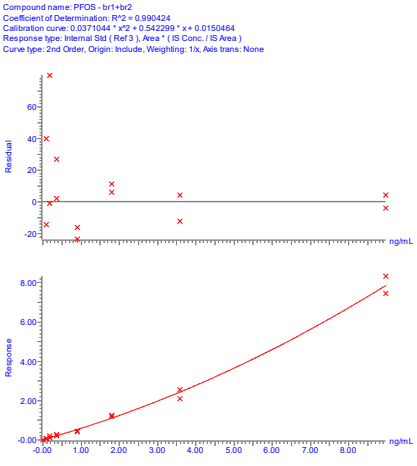
20. PFOS – linear



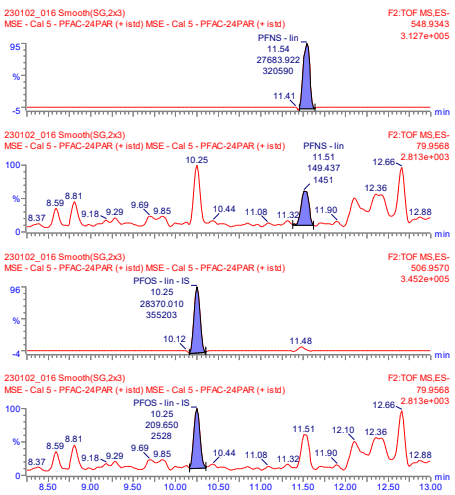


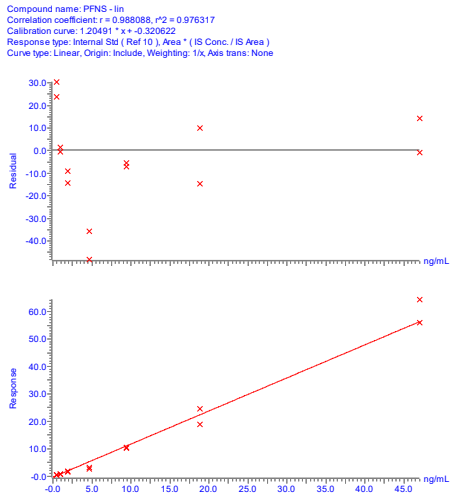
21. PFOS – branched



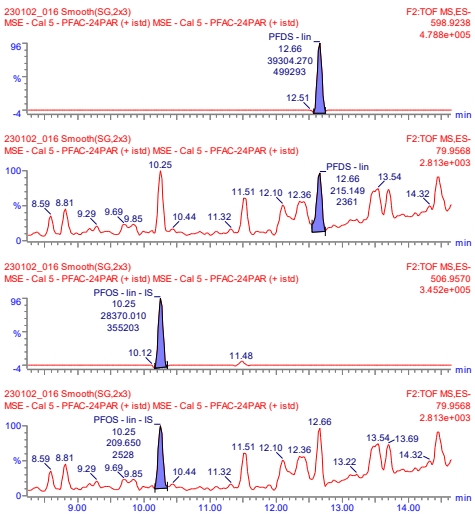


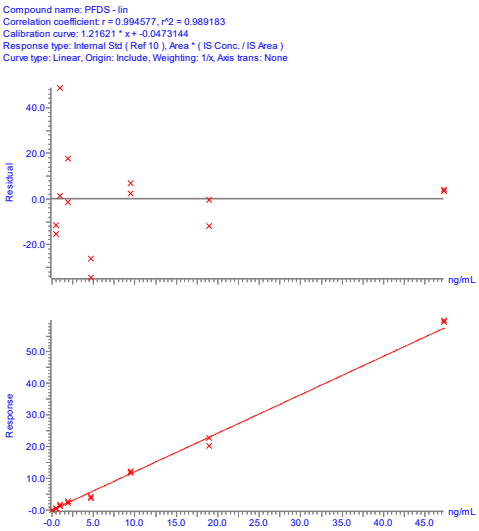
22. PFNS



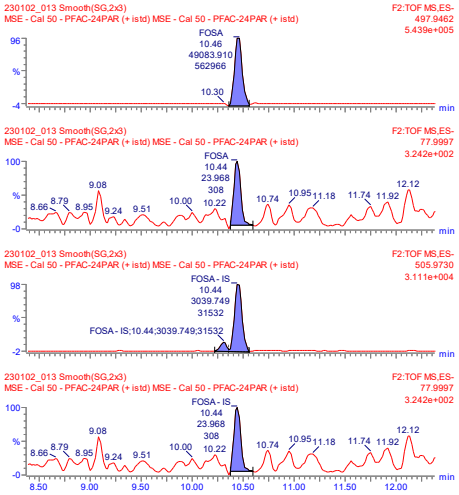


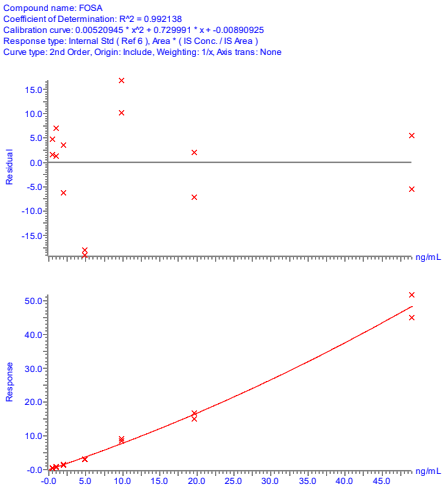
23. PFDS



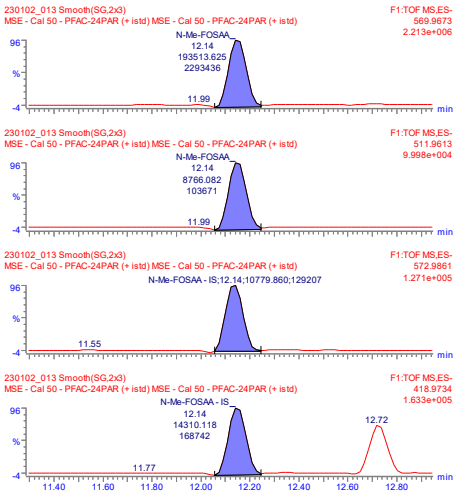


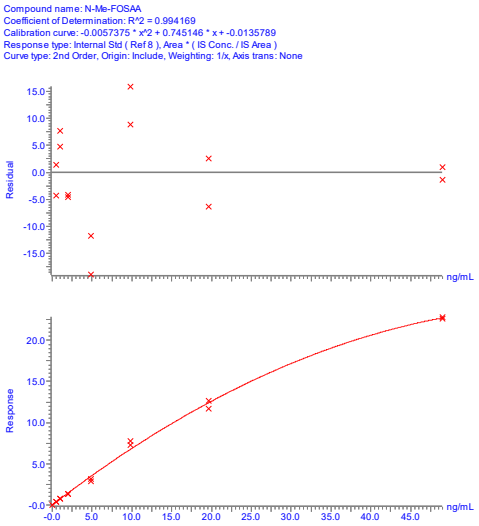
24. FOSA



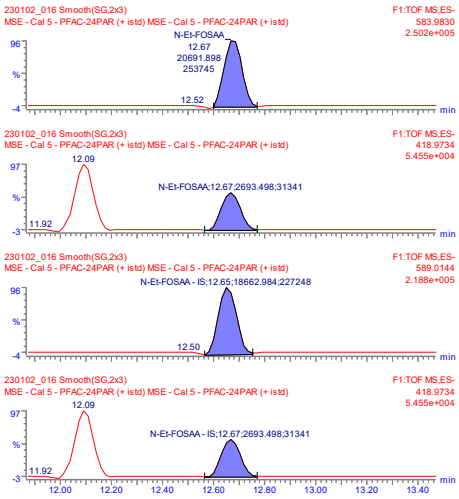


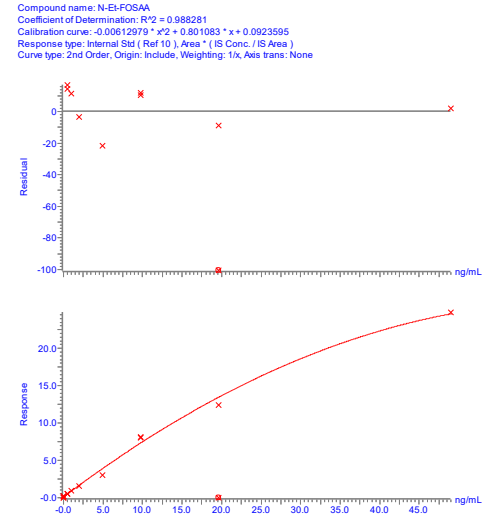
25. N-Me-FOSAA



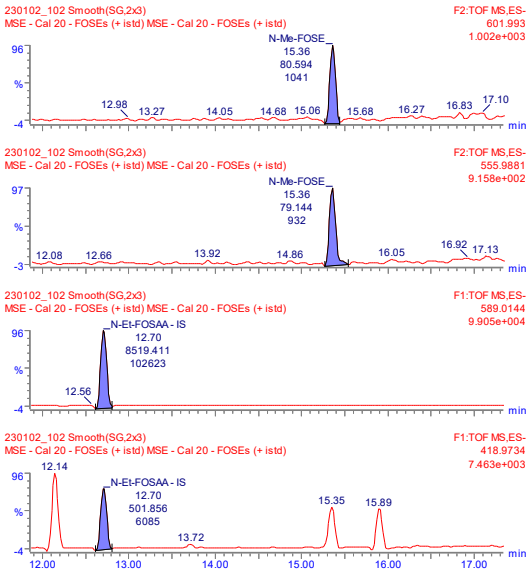


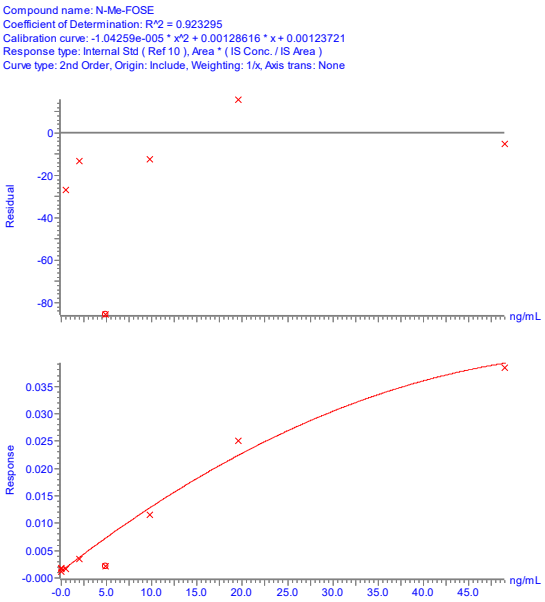
26. N-Et-FOSAA



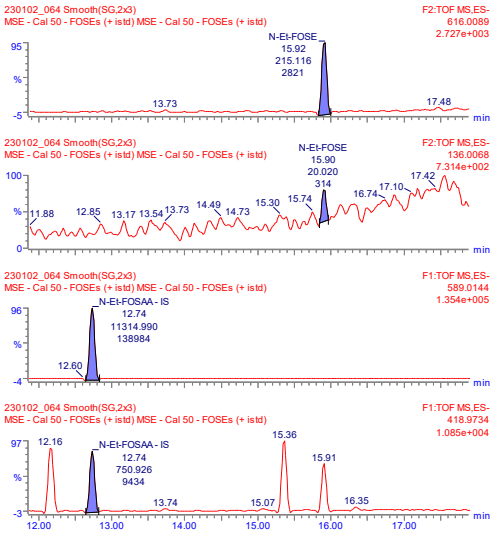


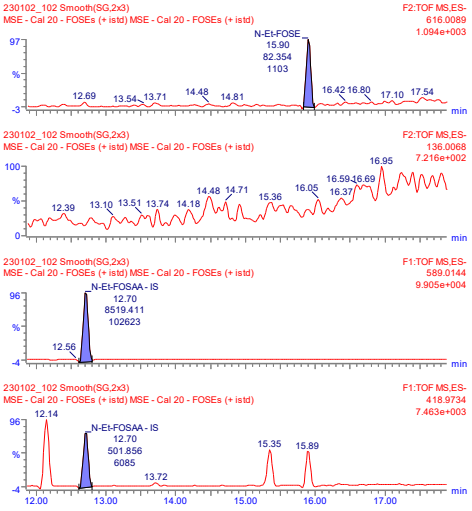
27. N-Me-FOSE

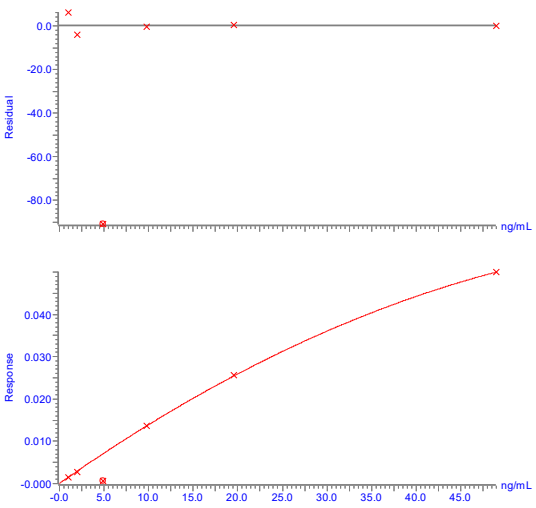




28. N-Et-FOSE







Appendix 5. Analytical standards

Appendix 5.1 Native analytical standards

PFAC-24PAR

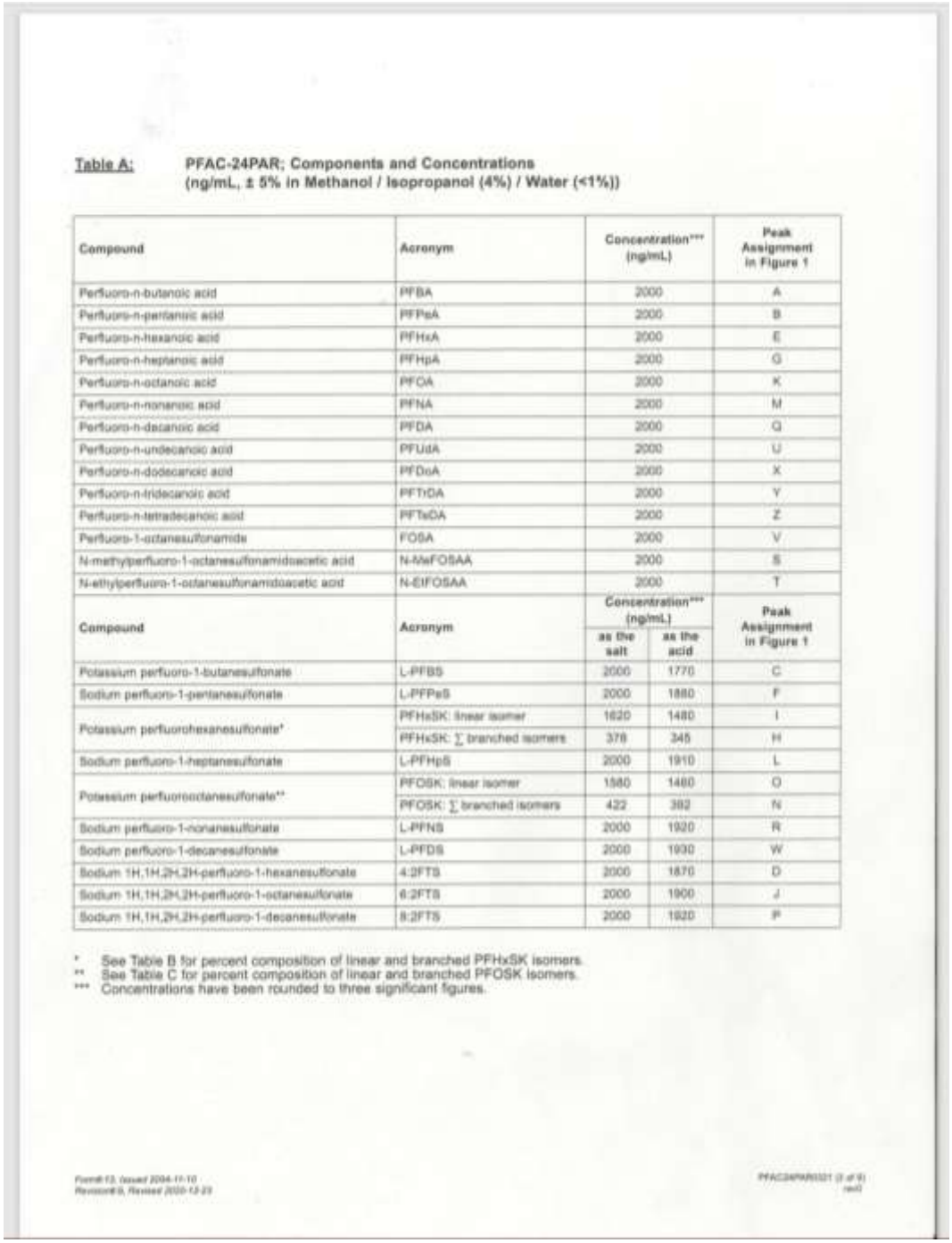


Table A: **PFAC-24PAR; Components and Concentrations (ng/mL, ± 5% in Methanol / Isopropanol (4%) / Water (<1%))**

Compound	Acronym	Concentration*** (ng/mL)	Peak Assignment in Figure 1
Perfluoro-n-butanoic acid	PFBA	2000	A
Perfluoro-n-pentanoic acid	PFPeA	2000	B
Perfluoro-n-hexanoic acid	PFHxA	2000	E
Perfluoro-n-heptanoic acid	PFHpA	2000	G
Perfluoro-n-octanoic acid	PFOA	2000	K
Perfluoro-n-nonanoic acid	PFNA	2000	M
Perfluoro-n-decanoic acid	PFDA	2000	Q
Perfluoro-n-undecanoic acid	PFUdA	2000	U
Perfluoro-n-dodecanoic acid	PFDoA	2000	X
Perfluoro-n-tridecanoic acid	PFTrDA	2000	Y
Perfluoro-n-tetradecanoic acid	PFTeDA	2000	Z
Perfluoro-1-octanesulfonamide	FOSA	2000	V
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	2000	S
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	2000	T

Compound	Acronym	Concentration*** (ng/mL)		Peak Assignment in Figure 1
		as the salt	as the acid	
Potassium perfluoro-1-butanesulfonate	L-PFBS	2000	1770	C
Sodium perfluoro-1-pentanesulfonate	L-PFPeS	2000	1880	F
Potassium perfluorohexanesulfonate*	PFHxSK: linear isomer	1620	1480	I
	PFHxSK: ∑ branched isomers	378	345	H
Sodium perfluoro-1-heptanesulfonate	L-PFHpS	2000	1910	L
Potassium perfluorooctanesulfonate**	PFOSK: linear isomer	1580	1460	O
	PFOSK: ∑ branched isomers	422	382	N
Sodium perfluoro-1-nonanesulfonate	L-PFNS	2000	1920	R
Sodium perfluoro-1-decanesulfonate	L-PFDS	2000	1930	W
Sodium 1H,1H,2H,2H-perfluoro-1-hexanesulfonate	4:2FTS	2000	1870	D
Sodium 1H,1H,2H,2H-perfluoro-1-octanesulfonate	6:2FTS	2000	1900	J
Sodium 1H,1H,2H,2H-perfluoro-1-decanesulfonate	8:2FTS	2000	1920	P

* See Table B for percent composition of linear and branched PFHxSK isomers.
** See Table C for percent composition of linear and branched PFOSK isomers.
*** Concentrations have been rounded to three significant figures.

N-MeFBSE-M

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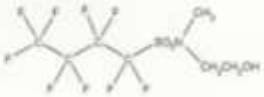
DOCUMENTATION

PRODUCT CODE:	N-MeFBSE-M	**LOT NUMBER:**	NMeFBSE0621M
COMPOUND:	2-(N-methylperfluoro-1-butanesulfonamido)-ethanol		
STRUCTURE:		**CAS #:**	34454-97-2



MOLECULAR FORMULA:	$C_7H_8F_9NO_3S$	**MOLECULAR WEIGHT:**	357.19
CONCENTRATION:	50.0 ± 2.5 µg/mL	**SOLVENT(S):**	Methanol
CHEMICAL PURITY:	>98%		
LAST TESTED:	06/09/2021 (HRGC/LRMS) 06/14/2021 (LC/MS)		
EXPIRY DATE:	Stability studies ongoing		
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place		

DOCUMENTATION/ DATA ATTACHED:

Figure 1: HRGC/LRMS Data (Full Scan and Mass Spectrum)
Figure 2: LC/MS Data (Full Scan and Mass Spectrum)
Figure 3: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- In order to see the molecular ion (adduct free), the LC mobile phase should be free of ammonium acetate buffer.

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Certified By: _____ **Date:** 07/27/2021
B.G. Chittim, General Manager

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N-MeFOSE-M

WELLINGTON LABORATORIES

CERTIFICATE OF ANALYSIS
DOCUMENTATION

PRODUCT CODE:	N-MeFOSE-M	**LOT NUMBER:**	NMeFOSE0522M
COMPOUND:	2-(N-methylperfluoro-1-octanesulfonamido)ethanol		
STRUCTURE:		**CAS #:**	24448-09-7

MOLECULAR FORMULA:	$C_{11}H_8F_{17}NO_3S$	**MOLECULAR WEIGHT:**	557.22
CONCENTRATION:	50.0 ± 2.5 µg/mL	**SOLVENT(S):**	Methanol
CHEMICAL PURITY:	>98%		
LAST TESTED:	05/13/2022 (HRGC/LRMS)		
	05/13/2022 (LC/MS)		
EXPIRY DATE:	05/13/2027		
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place		

DOCUMENTATION/ DATA ATTACHED:

Figure 1: HRGC/LRMS Data (Full Scan and Mass Spectrum)
Figure 2: LC/MS Data (Full Scan and Mass Spectrum)
Figure 3: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- In order to see the molecular ion (adduct free), the LC mobile phase should be free of ammonium acetate buffer.

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Certified By: B.G. Chittim, General Manager **Date:** 06/14/2022

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N-EtFOSE-M

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PRODUCT CODE:	N-EtFOSE-M	**LOT NUMBER:**	NEtFOSE0622M
COMPOUND:	2-(N-ethylperfluoro-1-octanesulfonamido)ethanol		
STRUCTURE:		**CAS #:**	1691-99-2

MOLECULAR FORMULA:	$C_{12}H_6F_{17}NO_3S$	**MOLECULAR WEIGHT:**	571.25
CONCENTRATION:	50.0 ± 2.5 µg/mL	**SOLVENT(S):**	Methanol
CHEMICAL PURITY:	>98%		
LAST TESTED:	05/13/2022 (HRGC/LRMS)		
	05/13/2022 (LC/MS)		
EXPIRY DATE:	05/13/2027		
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place		

DOCUMENTATION/ DATA ATTACHED:

Figure 1: HRGC/LRMS Data (Full Scan and Mass Spectrum)
Figure 2: LC/MS Data (Full Scan and Mass Spectrum)
Figure 3: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- In order to see the molecular ion (adduct free), the LC mobile phase should be free of ammonium acetate buffer.

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Certified By: _____ **Date:** 07/13/2022
B.G. Chittim, General Manager

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PFAC-MXF

Table A: **PFAC-MXF; Components and Concentrations (ng/mL; ± 5% in Methanol/Water (<1%))**

Compound	Acronym	Concentration* (ng/ml)	Peak Assignment in Figure 1
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA	2000	A

Compound	Acronym	Concentration* (ng/mL)		Peak Assignment in Figure 1
		as the salt	as the acid	
Sodium dodecafluoro-3H-4,8-dioxanonanoate	NaDONA	2000	1890	B
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS	2000	1870	C
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS	2000	1890	D

* Concentrations have been rounded to three significant figures.

Certified By: _____ Date: 01/12/2022

B.G. Chittim, General Manager

FHET (6:2 FTOH)

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DOCUMENTATION

PRODUCT CODE:	FHET (6:2 FTOH)	**LOT NUMBER:**	FHET0322
COMPOUND:	2-Perfluorohexyl ethanol		
STRUCTURE:		**CAS #:**	647-42-7

MOLECULAR FORMULA:	$C_8H_5F_{13}O$	**MOLECULAR WEIGHT:**	364.10
CONCENTRATION:	50.0 ± 2.5 µg/mL	**SOLVENT(S):**	Methanol
CHEMICAL PURITY:	>98%		
LAST TESTED:	03/18/2022 (HRGC/LRMS) 03/17/2022 (LC/MS)		
EXPIRY DATE:	03/18/2027		
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place		

DOCUMENTATION/ DATA ATTACHED:

Figure 1: HRGC/LRMS Data (Full Scan and Mass Spectrum)
Figure 2: LC/MS Data (Full Scan and Mass Spectrum)
Figure 3: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains ~0.6% of an unknown impurity.

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Certified By: _____ **Date:** 03/18/2022
B.G. Chittim, General Manager

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FOET (8:2 FTOH)

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DOCUMENTATION

PRODUCT CODE:	FOET (8:2 FTOH)	LOT NUMBER:	FOET0221
COMPOUND:	2-Perfluorooctyl ethanol		
STRUCTURE:		CAS #:	678-39-7

MOLECULAR FORMULA:	$C_{10}H_5F_{17}O$	MOLECULAR WEIGHT:	464.12
CONCENTRATION:	50.0 ± 2.5 µg/mL	SOLVENT(S):	Methanol
CHEMICAL PURITY:	>98%		
LAST TESTED:	02/11/2021 (HRGC/LRMS) 05/17/2021 (LC/MS)		
EXPIRY DATE:	05/17/2026		
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place		

DOCUMENTATION/ DATA ATTACHED:

Figure 1: HRGC/LRMS Data (Full Scan and Mass Spectrum)
Figure 2: LC/MS Data (Full Scan and Mass Spectrum)
Figure 3: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.

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FDET (10:2 FTOH)

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CERTIFICATE OF ANALYSIS
DOCUMENTATION

PRODUCT CODE:	FDET (10:2 FTOH)	**LOT NUMBER:**	FDET1021
COMPOUND:	2-Perfluorodecyl ethanol		
STRUCTURE:		**CAS #:**	865-86-1

MOLECULAR FORMULA:	$C_{12}H_5F_{17}O$	**MOLECULAR WEIGHT:**	564.13
CONCENTRATION:	50.0 ± 2.5 µg/mL	**SOLVENT(S):**	Methanol
CHEMICAL PURITY:	>98%		
LAST TESTED:	10/14/2021 (HRGC/LRMS) 11/17/2021 (LC/MS)		
EXPIRY DATE:	11/17/2026		
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place		

DOCUMENTATION/ DATA ATTACHED:

Figure 1: HRGC/LRMS Data (Full Scan and Mass Spectrum)
Figure 2: LC/MS Data (SIR)
Figure 3: LC/MS Data (Mass Spectrum)
Figure 4: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains ~1.4% total of two unknown impurities.

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Appendix 5.2 Internal standards

MPFAC-24ES

Table A: MPFAC-24ES; Components and Concentrations
(ng/mL, ± 5% in methanol / isopropanol (2%) / water (<1%))

Compound	Acronym	Concentration* (ng/mL)	Peak Assignment in Figure 1
Perfluoro-n-[$^{13}C_4$]butanoic acid	MPFBA	1000	A
Perfluoro-n-[$^{13}C_5$]pentanoic acid	M5PFPeA	1000	B
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	M5PFHxA	1000	E
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	M4PFHpA	1000	F
Perfluoro-n-[$^{13}C_8$]octanoic acid	M8PFOA	1000	I
Perfluoro-n-[$^{13}C_9$]nonanoic acid	M9PFNA	1000	J
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	M6PFDA	1000	M
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	M7PFUdA	1000	P
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	M2PFDoA	1000	R
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	M2PFTeDA	1000	S
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	M8FOSA	1000	Q
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	d3-N-MeFOSAA	1000	N
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	d5-N-EtFOSAA	1000	O

Compound	Acronym	Concentration* (ng/mL)		Peak Assignment in Figure 1
		as the salt	as the acid	
Sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	M3PFBS	1000	932	C
Sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	M3PFHxS	1000	948	G
Sodium perfluoro-1-[$^{13}C_8$]octanesulfonate	M8PFOS	1000	959	K
Sodium 1H,1H,2H,2H-perfluoro-1-(1,2-$^{13}C_2$]hexanesulfonate	M2-4:2FTS	1000	938	D
Sodium 1H,1H,2H,2H-perfluoro-1-(1,2-$^{13}C_2$]octanesulfonate	M2-6:2FTS	1000	951	H
Sodium 1H,1H,2H,2H-perfluoro-1-(1,2-$^{13}C_2$]decanesulfonate	M2-8:2FTS	1000	960	L

* Concentrations have been rounded to three significant figures.

Certified By: _____ Date: 03/31/2022
B.G. Chittim, General Manager

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