ASMS 2023, WP 446

Comprehensive 4D workflow for targeted and untargeted screening of PFAS in organisms from different trophic levels utilizing LC-VIP HESI(-)-TIMS-QToF MS

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Introduction

Per- and polyfluoroalkyl substances (PFAS)

easily transportable

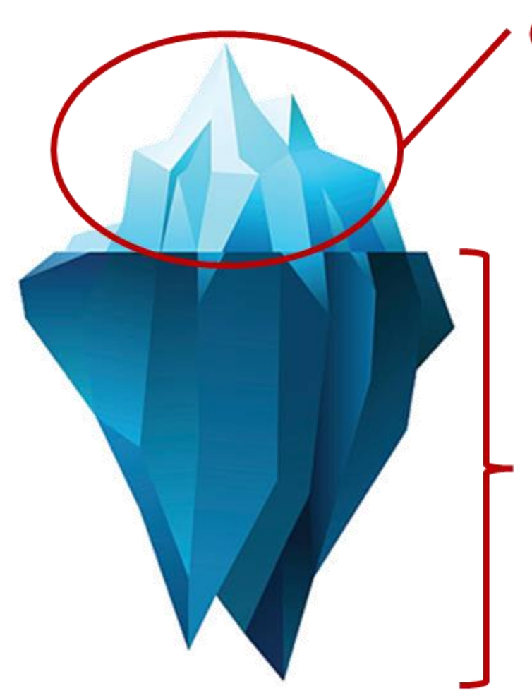
persistent

non-biodegradable

bioaccumulated & biomagnified

adverse effect on human health

> 4,700 registered PFAS plus degradation products



commonly monitored PFAS

PFCAs (n=4-13), PFSAs (n=4-13), ADONA, GenX

*restricted within EU (water DIRECTIVE (EU) 2020/2184)

emerging PFAS

Methods

generic sample preparation protocol

Androulakakis et al 2022

Simultaneous extraction of PFAS from different sub-classes

(different physicochemical properties)

RPLC - VIP HESI(-) – TIMS - QTOF MS

TargetScreener 4D

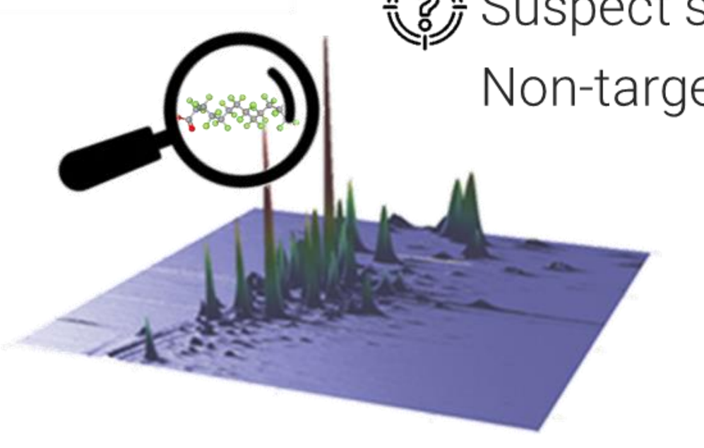
acquisition modes:

- ✓ *bbCID*
- ✓ *PASEF*

Target analysis

Suspect screening

Non-target screening



Results

Target Analysis

Database

m/z	formula	name	rt	Qual1	Qual2	Qual3	Qual4	ccs	oneOverK0
298.9430	C4HF9O3S	Perfluorobutanesulfonic acid (L-PFBS)	5.68	98.9558	79.9574			133.83	0.6390
448.9334	C7HF15O3S	Perfluoroheptanesulfonic acid (L-PFHpS)	8.64	168.9894	79.9574	98.9558		160.00	0.7752
498.9302	C8HF17O3S	Perfluorooctanesulfonic acid (L-PFOS)	9.47	79.9574	98.9558			168.84	0.8204
368.9766	C7F15^1	Perfluorooctanoic acid (L-PFOA) Fragm 369	8.63	168.9894	368.9766	218.9862	118.9926	157.25	0.7570
326.9743	C6H4F9O	4:2 Fluorotelomer sulfonic acid (4:2 FTS)	6.52	306.9681	80.9652			150.81	0.7228
426.9679	C8H4F	6:2 Fluorotelomer sulfonic acid (6:2 FTS)	8.59	406.9617	80.9652	386.9554		168.81	0.8166
526.9615	C10H4		10.23	506.9553	80.9652			186.00	0.9051
630.0237									
616.0081									
250.9760									

↲ 12 PFAS sub-classes

✓ 60 PFAS

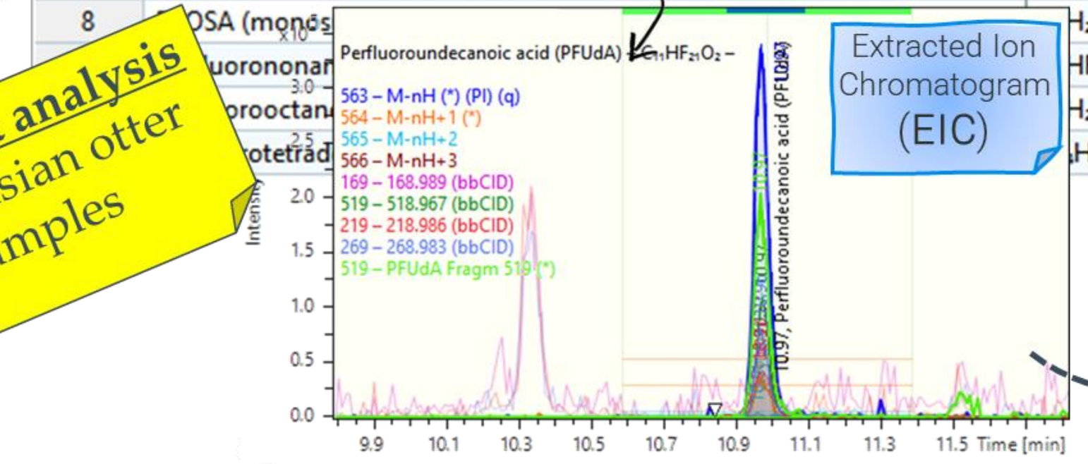
✓ 21 PFAS I.S.

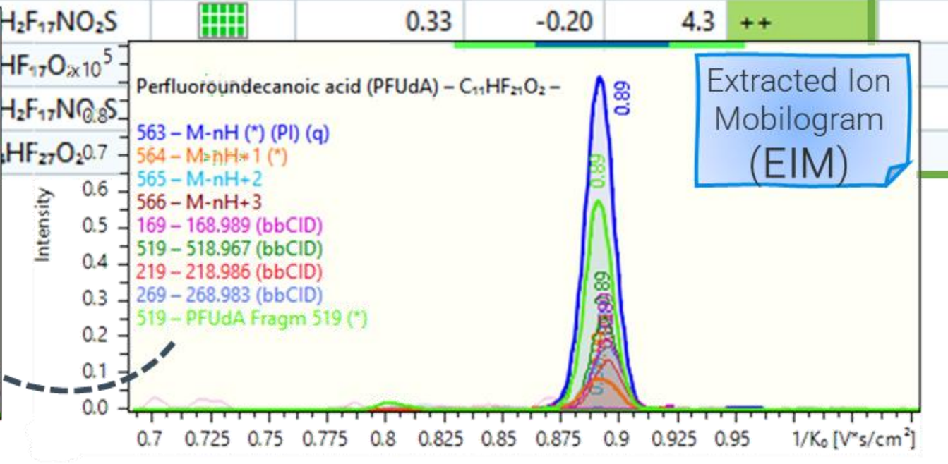
↰ representative isotopically labeled I.S.

Screening results

#	Name	Formula	MRSQC	Δm/z [mDa]	ΔRT [min]	mSigma	Ions Score	ΔCCS [%]
1	br-PFOS (monosubstituted isomers)	$C_8HF_{17}O_3S$		0.27	-0.02	25.6	++	0.36
2	Perfluorodecanoic acid (PFDA)	$C_{10}HF_{19}O_2$		0.83	-0.03	22.0	++	-0.01
3	Perfluorododecanoic acid (PFDoA)	$C_{12}HF_{23}O_2$		0.50	-0.02	3.8	++	-0.02
4	Perfluorohexanesulfonic acid (L-PFHxS)	$C_6HF_{13}O_3S$		-0.13	-0.01	17.9	++	0.31
5	Perfluorooctanesulfonic acid (L-PFOS)	$C_8HF_{17}O_3S$		-0.10	-0.03	14.7	++	0.08
6	Perfluorooctanoic acid (L-PFOA) Fragm 369	$C_7F_{15}^{1-}$		0.58	-0.14	1.1	++	0.12
7	Perfluoroundecanoic acid (PFUdA)	$C_{11}HF_{21}O_2$		0.45	-0.02	1.8	++	-0.01
8	…OSA (mono…	$…H_2F_{17}NO_2S$		0.33	-0.20	4.3	++	1.61
9	…uorononan…	$…HF_{17}O_2$						0.09
10	…rooctan…	$…H_2F_{17}NO_2S$						0.53
11	…otetrad…	$…HF_{27}O_2$						0.09

4D target analysis in Eurasian otter samples





ΔCCS typically ≤ 1%

Mass accuracy

Retention time

Sigma value

Qualifier ions

CCS value agreement

additional identification criterion

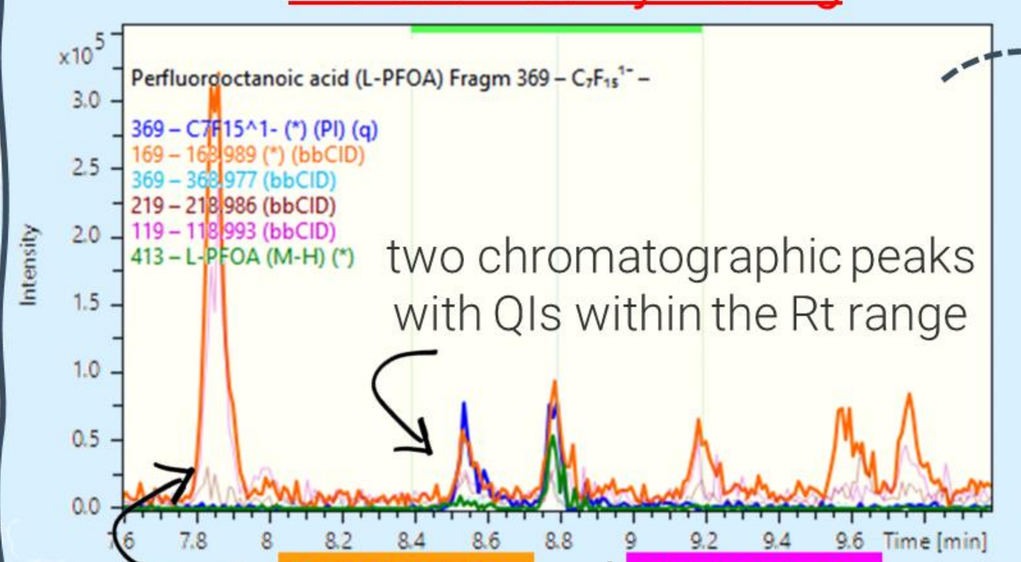
Effect of **mobility filtering** during data processing

Increased reliability of results

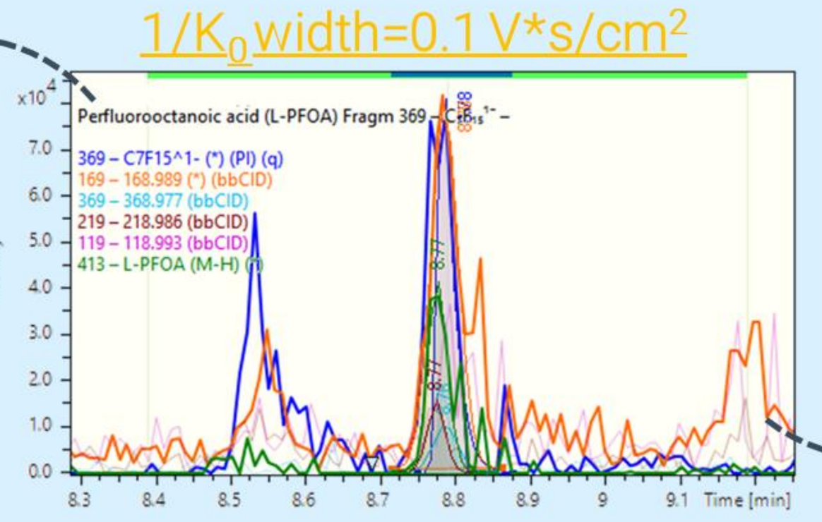
Reduced time for data review

l-PFOA ($C_{instr.}$=10 ng/mL)

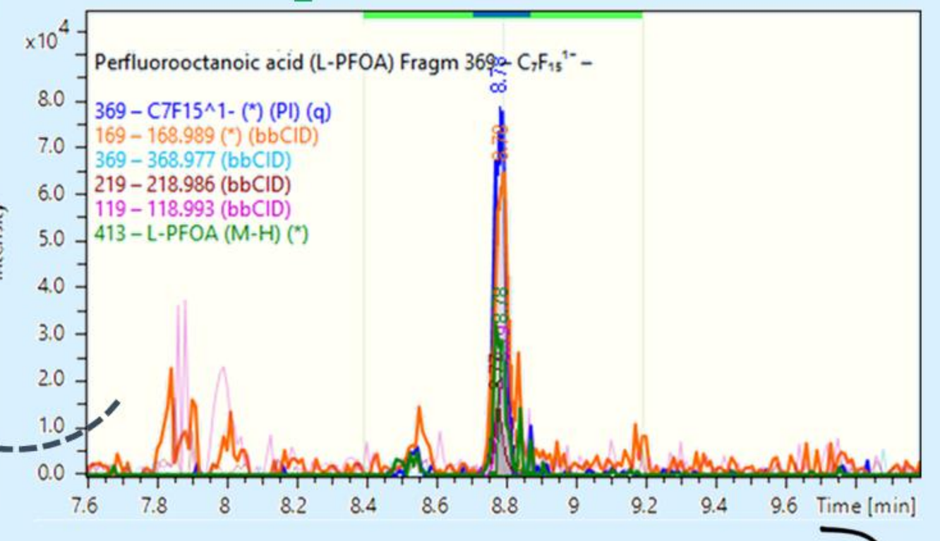
without mobility filtering



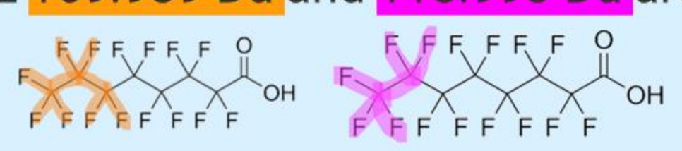
$1/K_0$ width=0.1 V*s/cm²



$1/K_0$ width=0.02 V*s/cm²



m/z 169.989 Da and 118.993 Da are common fragments of PFAS



background noise ✗

false positives

background noise

Method **Detection Limits**

low detection limits ⇔ good sensitivity

method* LODs

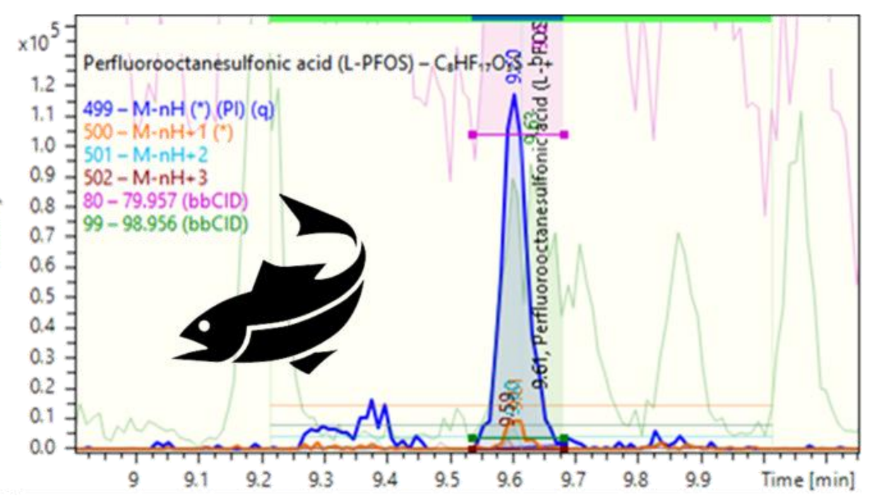
l-PFOS → 0.173 ng/g w.w.

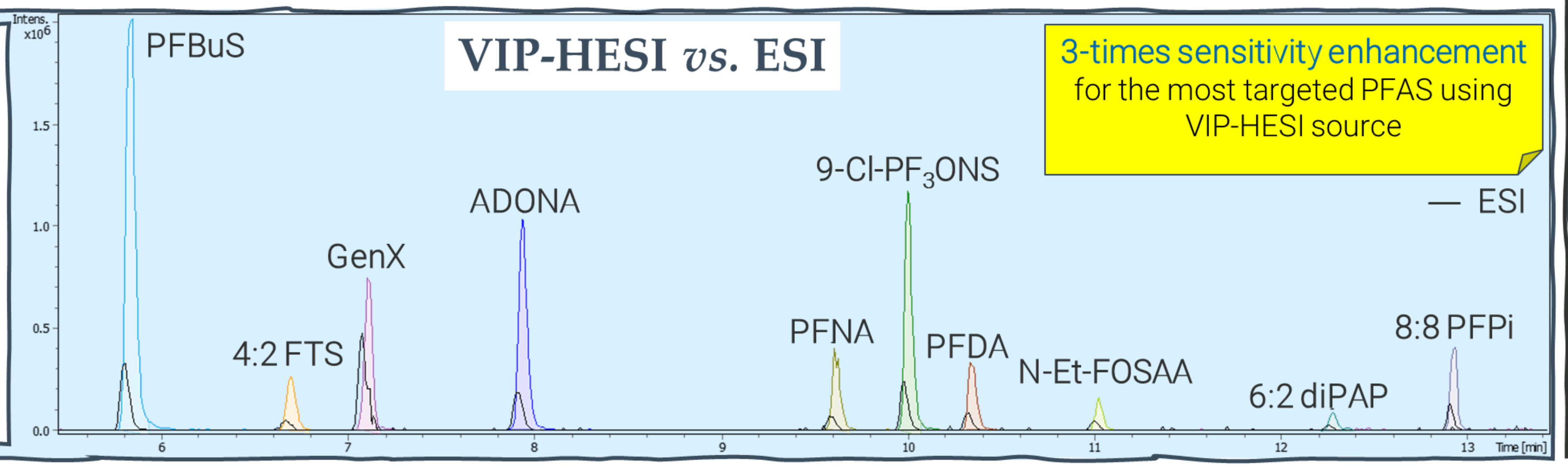
(EQS** = 9.1 ng/g w.w.)

10-times higher compared to the method LOD

*4-times pre-concentration

**Environmental Quality Standard

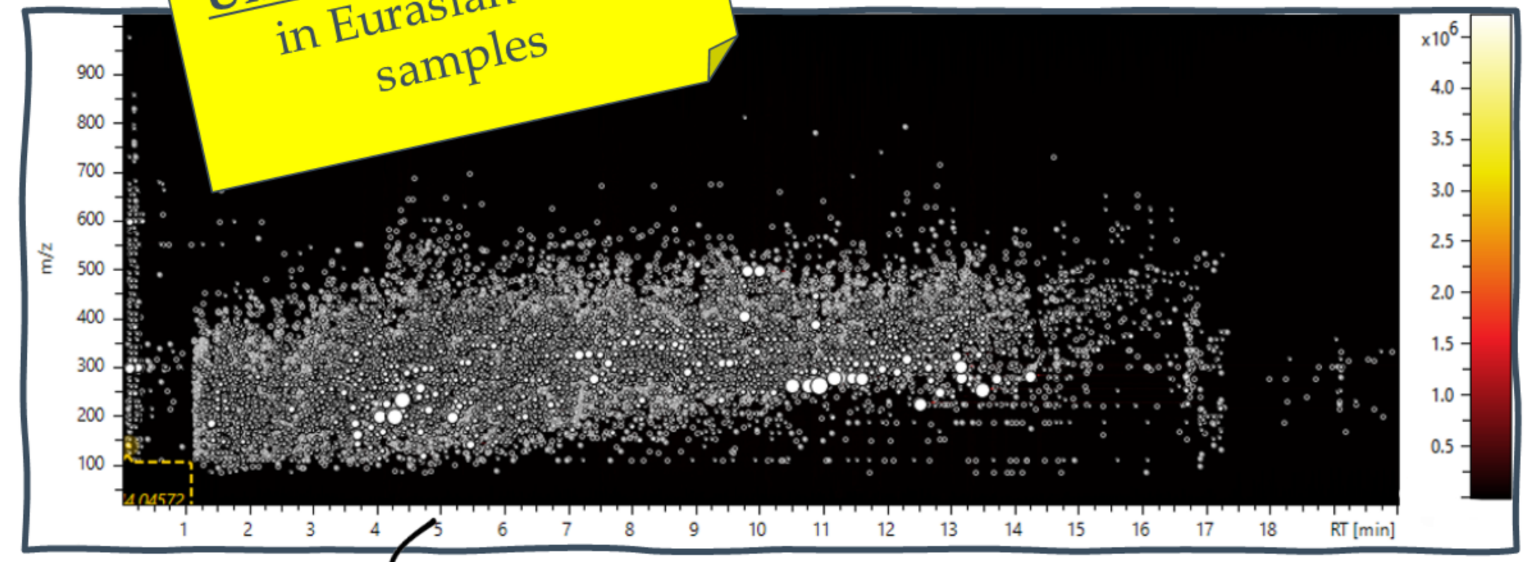




3-times sensitivity enhancement for the most targeted PFAS using VIP-HESI source

Suspect Screening

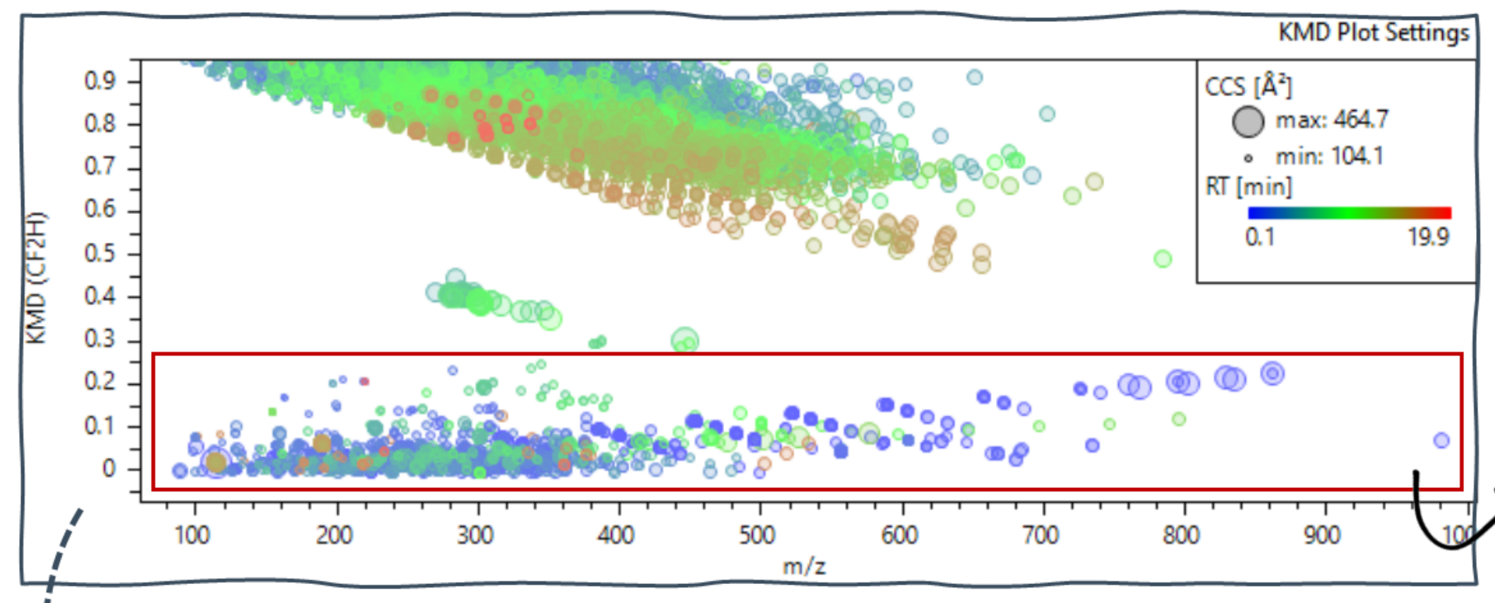
Untargeted workflow in Eurasian otter samples



Matrix Complexity

>10,000 features

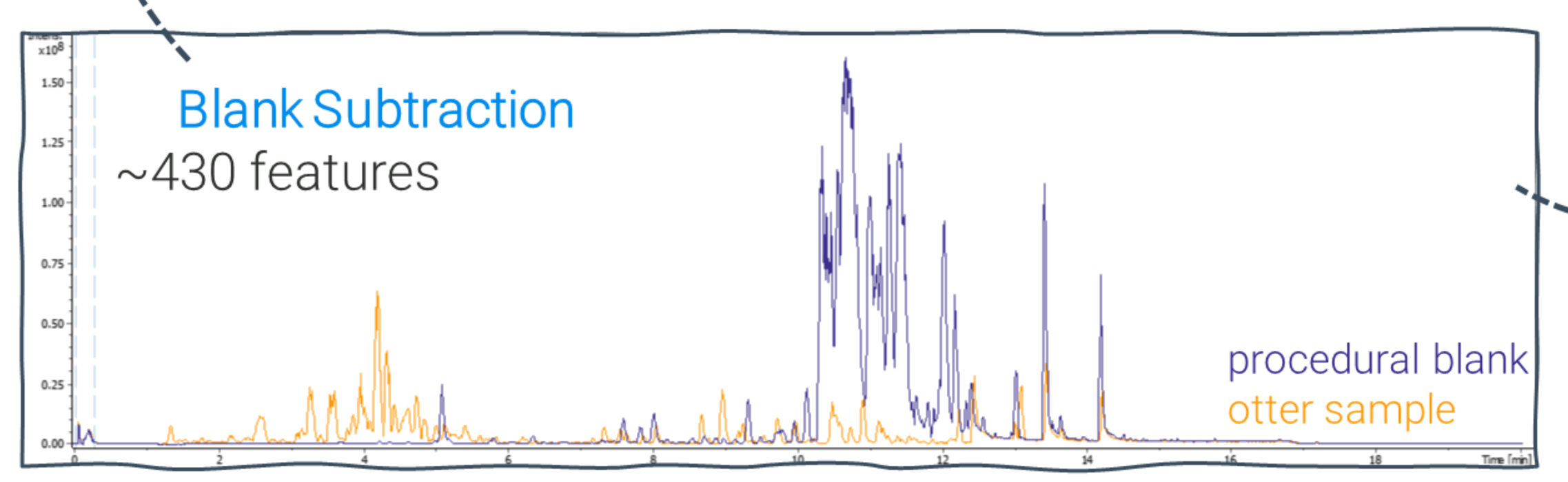
need for efficient untargeted workflows



Kendrick Mass Defect (KMD) plot

repeating unit: CF_2

~1,850 features



Blank Subtraction

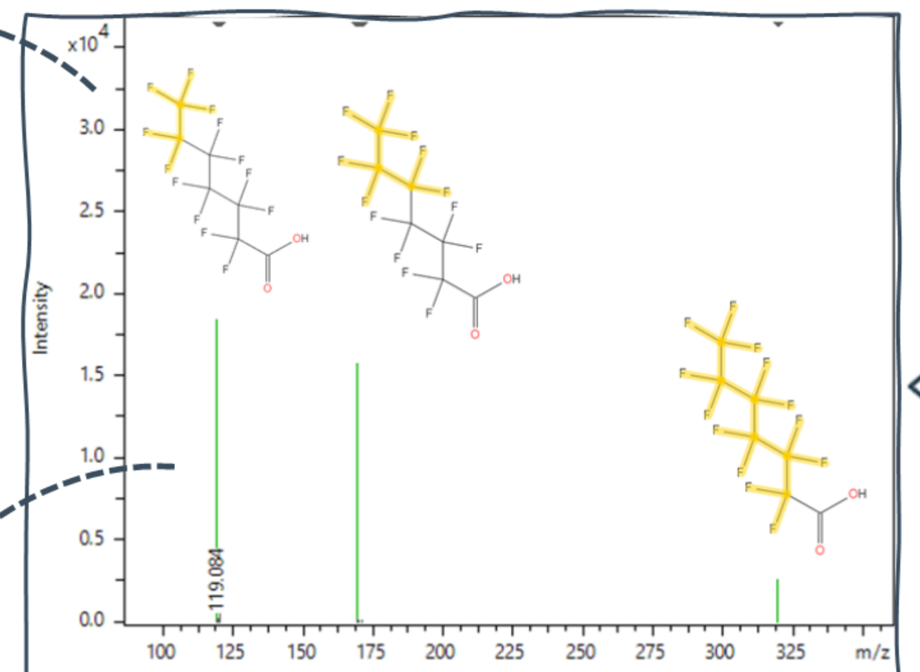
~430 features

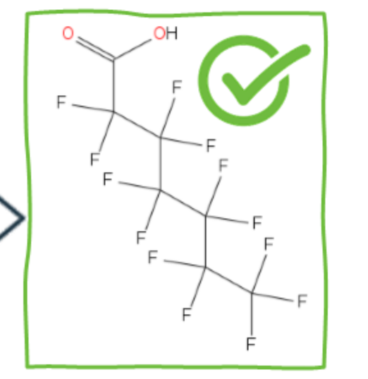
PFAS chemical space

MS/MS spectra annotation

Ion Formula	m/z meas.	Δ m/z [mDa]	Int.	IntCov. [%]
[C2F5]-	118.999	-7.3	18393.838	50.2
[C3F7]-	168.989	-0.5	15737.872	42.9
[C6F13]-	318.978	0.8	2545.803	6.9

Name	Molecul...	Score	# Frag	IntCov. [%]
1,1,1,3,3,3-Hexafluoropropan-2-yl heptafluorobutano...	$C_7HF_{13}O_2$	0.589	2	93.059
Perfluoro-5-(trifluoromethyl)hexanoic acid	$C_7HF_{13}O_2$	0.785	2	49.85
Hexanoic acid, 2,2,3,3,4,4,5,6,6,6-decafluoro-5-(trifluo...	$C_7HF_{13}O_2$	0.785	2	49.85
Perfluoroheptanoate	$C_7HF_{13}O_2$	1.0	3	100.0
Perfluoroheptanoic acid (PFHpA)	$C_7HF_{13}O_2$	1.0	3	100.0





#	Annotation Source	Name	Molecular Formula	m/z meas.	RT [min]	CCS (Å²)	AQ	Δm/z [mDa]	MS/MS score	mSigma	ΔCCS [%]
1	NORMAN PRORISKPFAS	Cyclohexanaminium p...	$C_9HF_{17}O_2$	462.96311	9.80	164.5		-0.060	973.8	11.0	-2.8
2	NORMAN PRORISKPFAS	3,3,4,4,5,5,6,6,6-Nonafl...	$C_8HF_{15}O_2$	412.96631	9.00	155.6		-0.071	967.7	8.9	-2.7
3	NORMAN PRORISKPFAS	Sodium 3,3,4,4,5,5,6,6,7,...	$C_8H_5F_{13}O_3S$	426.96814	9.06	167.9		0.113	924.6	16.8	1.9
4	NORMAN PRORISKPFAS	Sodium perfluorodecan...	$C_{10}HF_{19}O_2$	512.95991	10.51	173.6		-0.124	957.7	15.5	-2.1
5	NORMAN PRORISKPFAS	Perfluoroundecanoic a...	$C_{11}HF_{21}O_2$	562.95648	11.11	182.4		-0.139	942.1	10.3	-1.5
6	NORMAN PRORISKPFAS	1H,1H-Perfluoropropyl ...	$C_6H_5F_5O_2$	203.01388	1.67	133.6		0.050	814.0	6.9	1.1
7	NORMAN PRORISKPFAS	Piperidinium perfluoro...	$C_8HF_{17}O_3S$	498.93026	9.78	167.9		0.006	614.2	2.6	-2.5
8	NORMAN PRORISKPFAS	Perfluorooctanesulfona...	$C_8H_2F_{17}NO_2S$	497.94568	11.05	169.3		-0.059	869.1	15.9	-1.9
9	NORMAN PRORISKPFAS	Perfluorooctanesulfonate	$C_8HF_{17}O_3S$	498.92986	10.06	167.8		-0.362	617.1	5.0	-2.6
10	NORMAN PRORISKPFAS	Silane, trimethyl(trideca...	$C_9H_9F_{13}Si$	391.01917	1.79	170.6		-0.146	72.7	24.8	-0.7
11	NORMAN PRORISKPFAS	Silane, trimethyl(trideca...	$C_9H_9F_{13}Si$	391.01912	1.20	170.8		-0.242	27.0	28.4	-0.6
12	NORMAN PRORISKPFAS	(4-Cyclohexylphenyl)(d...	$C_{24}H_{25}S$	344.16081	5.11	183.5		-0.327	78.0	47.1	0.7
13	NORMAN PRORISKPFAS	Cyclopentene, 1,3,3,4,4,...	C_5HF_7	192.98940	5.03	117.5		0.003	1000.0	7.2	-3.2
14	NORMAN PRORISKPFAS	H-substituted Perfluoro...	$C_{12}H_2F_{22}O_2$	594.96278	10.39	171.2		-0.009	1000.0	10.0	-10.5
15	NORMAN PRORISKPFAS	1,1,1,2,2,3,3,4,4,5,5-Und...	C_5HF_{11}	268.98312	7.07	123.8		0.181	1000.0	7.8	-9.1
16	NORMAN PRORISKPFAS	Perfluoroheptanoic aci...	$C_7HF_{13}O_2$	362.96965	8.11	146.8		0.031	953.8	4.9	-3.9

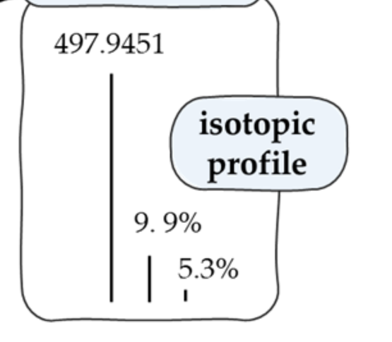
- ✓ Δm/z
- ✓ MS/MS score
- ✓ ΔCCS
- ✓ mSigma

PRORISKPFAS

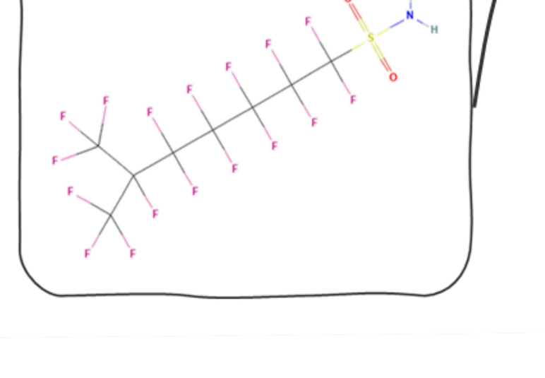
suspect list containing 4,777 PFAS

	Norman SusDat_ID	Name	Formula	CAS_RN	SMILES original / SMILES MS_ready	Monoisotopic mass	StdInChI	StdInChIKey
4429	NS00109557	…tadecafluorooctyl)(methyl)amino]ethyl prop-2-…	C14H10F17NO2	…_RN: 93491-…	…F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)…	547.044008	…,26)10(21,22)…	ZEVGQZNTYAKDNY-UHFFFAOYSA-N
4430	NS00110145	(Perfluorobutyl)-4-bromophenylmethanol	C11H6BrF9O	…_RN: 1228786-…	…=C1)C(F)(F)C(F)c1)C(F)(F)C(F)(F)…	403.9458315	…7(22)8(13,14…	ZEVIVKGHJDBGBM-UHFFFAOYSA-N
4431	NS00108984	…11,11,12,12,13,13,14,14,15,15,15-Pentacosaflu…	C15H6F25IO	…_RN: 80459-…	…)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)…	803.9064178	…4,25)9(26,27)…	ZEYGPNNBXJHOQZ-UHFFFAOYSA-N
4432	NS00018990	Decafluorocyclohexene	C6F10	…S_RN: 355-7…	…(F)C(F)(F)C(F)(F)C(F)C(F)(F)C(F)(F)C(F)…	261.984032	…2(8)4(11,12)6…	ZFFLXJVVPHACEG-UHFFFAOYSA-N
4433	NS00018894	(Perfluorocyclohexyl)methanol	C7H3F11O	…_RN: 28788-…	…)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)…	312.0008249	…10)5(13,14)7…	ZFHKTQUHJNUDNH-UHFFFAOYSA-N
4434	NS00011210	…6,7,7,8,9,9,9-Hexadecafluoro-8-(trifluorometh…	C12H5F19O	…_RN: 41925-…	…(F)F)(F)F)(F)(FF)C(F)(F)C(F)(F)C…	526.0037006	…21)10(24,25)9…	ZFJPGQQBBNLQOX-UHFFFAOYSA-N
4435	NS00110156	Triethylaminium perfluoro-2-propoxypropanoate	C6H15N	…_RN: 165951-…	…)C(F)(OC(F)(F)… CCN(CC)CC	101.1204495	…N/c1-4-7(5-2)…	ZMANZCXQSJIPKH-UHFFFAOYSA-N
4436	NS00109855	…6,7,7,8,8,9,9,10,10,10-hexadecafluorodecane-…	C10H2F16O2	…_RN: 147874-…	…(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C…	457.9799305	…13,14)15)6(1…	ZFNFUONJWJUELL-UHFFFAOYSA-N
4437	NS00083489	…eptadecafluoro-N-(2-hydroxyethyl)octanesulph…	C14H14F17NO3S	…AS_RN: 13283…	…(F)(F)C(F)(F)C(C(F)(F)C(F)(F)C(F…	599.0422934	…16,17)9(18,19…	ZFUCUZACOHLUFM-UHFFFAOYSA-N
4438	NS00112044	…oro(2-[(1-propoxypropan-2-yl)oxy]propanoyl) flu…	C9F18O3	…_RN: 112820-…	…C(F)(F)OC(F)(I)F)C(F)(F)OC(F)(F…	497.9560015	…)29-4(14,7(21…	ZFUIXYCDZABZDB-UHFFFAOYSA-N
4439	NS00110208	1H,1H,2H,2H-Perfluorododecyltrichlorosilane	C12H4Cl3F21Si	…_RN: 102488-…	…C(F)(F)C(F)(F)(F)C(F)(F)C(F)(F)C(F)(C…	679.8812519	…0,21)6(22,23)…	ZFUVZJADECZZMS-UHFFFAOYSA-N
4440	NS00111150	…1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-Heptadecafluoroic…	C20H23F17	…_RN: 124921-…	…F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C…	586.1528301	…15(25,26)16)…	ZFYUUPVIDOJWAX-UHFFFAOYSA-N

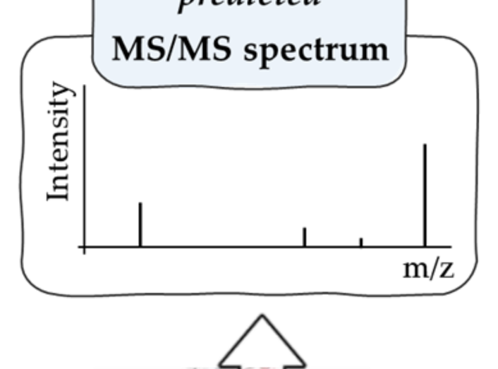
accurate mass



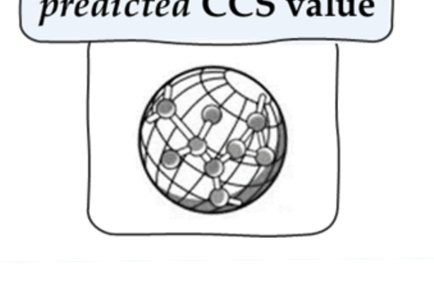
Structure



in-silico tools



predicted CCS value



Conclusions

- ✓ Establishment of an efficient and sensitive **combined targeted and untargeted workflow** for unveiling of **PFAS** in complex environmental matrices, such as **biota**.
- ✓ Ion mobility derived **CCS** values can be used as an **additional identification criterion** in target analysis, resulting to **4D-identification**.
- ✓ The **ion mobility filtration** assists the data processing, leading to **increased reliability** of results and reduced time for data review.
- ✓ Increased sensitivity was observed using **VIP-HESI** source and mobility filtration.
- ✓ An **efficient untargeted workflow**, combining a suspect list with >4,700 PFAS and novel in-silico tools, was established.