



Sample Preparation and LC-MS/MS Method for the Determination of 33 Per- and Polyfluoroalkyl Substances (PFAS) in Food of Animal Origin

Method Report

Version 1.0

09 December 2024



1. Introduction

The method described in this report was developed and validated at CVUA Freiburg/EURL-POPs in 2020. It was tested for 33 per- and polyfluoroalkyl substances (**Table 1**) in food of animal origin. The following matrices were used for the validation studies:

- Fish: trout, pangasius, salmon, hering
- Seafood: prawns
- Meat: lamb, bovine, goose, pork
- Milk (3.5% fat)
- Milk products: feta cheese
- Infant formula
- Eggs (chicken)
- Offal: liver, kidney

2. Target Analytes

Table 1: Target analytes (native standards)

Acronym	Description	CAS-number
Perfluoroalkyl Carboxylic Acids (PFCA)		
PFBA	Perfluorobutanoic acid	375-22-4
PFPeA	Perfluoropentanoic acid	2706-90-3
PFHxA	Perfluorohexanoic acid	307-24-4
PFHpA	Perfluoroheptanoic acid	375-85-9
PFOA	Perfluorooctanoic acid	335-67-1
PFNA	Perfluorononanoic acid	375-95-1
PFDA	Perfluorodecanoic acid	335-76-2
PFUnDA	Perfluoroundecanoic acid	2058-94-8
PFDoDA	Perfluorododecanoic acid	307-55-1
PFTTrDA	Perfluorotridecanoic acid	72629-94-8
PFTeDA	Perfluorotetradecanoic acid	376-06-7
PFHxDA	Perfluoro-n-hexadecanoic acid	67905-19-5
PFODA	Perfluoro-n-octadecanoic acid	16517-11-6

Acronym	Description	CAS-number
Perfluoroalkyl Sulfonic Acids (PFSA)		
PFBS	Perfluorobutane sulfonic acid	375-73-5
PFPeS	Perfluoropentane sulfonic acid	2706-91-4
PFHxS	Perfluorohexane sulfonic acid	355-46-4
PFHpS	Perfluoroheptane sulfonic acid	375-92-8
PFOS	Perfluorooctane sulfonic acid	1763-23-1
PFNS	Perfluorononane sulfonic acid	68259-12-1
PFDS	Perfluorodecane sulfonic acid	335-77-3
PFDoDS	Perfluorododecane sulfonic acid	79780-39-5
Other		
FOSA	Perfluorooctane sulfonamide	754-91-6
4:2 FTS	Sodium 1H,1H,2H,2H-perfluorohexane sulfonate	27619-93-8
6:2 FTS	Sodium 1H,1H,2H,2H-perfluorooctane sulfonate	27619-94-9
8:2 FTS	Sodium 1H,1H,2H,2H-perfluorodecane sulfonate	27619-96-1
N-MeFOSA	N-methylperfluoro-1-octanesulfonamide	31506-32-8
N-EtFOSA	N-ethylperfluoro-1-octanesulfonamide	4151-50-2
N-MeFOSE	2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	24448-09-7
N-EtFOSE	2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	1691-99-2
DONA	Sodium dodecafluoro-3H-4,8-dioxanonanoate	2250081-67-3
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	13252-13-6
9Cl-PF3ONS	Potassium 9-chlorohexadecafluoro-3-oxanonane-1 - sulfonate	73606-19-6
11Cl-PF3OUdS	Potassium 11 -chloroeicosafluoro-3-oxaundecane-1 - sulfonate	83329-89-9

**Table 2:** Isotope labelled internal standards

Acronym	Description	CAS-number
Perfluoroalkyl Carboxylic Acids (PFCA)		
M4-PFBA	Perfluoro-n-[¹³ C ₄]butanoic acid	1017281-29-6
M5-PFPeA	Perfluoro-n-[¹³ C ₅]pentanoic acid	2283397-79-3
M5-PFHxA	Perfluoro-n-[1,2,3,4,6- ¹³ C ₅]hexanoic acid	2328024-54-8
M4-PFHpA	Perfluoro-n-[1,2,3,4- ¹³ C ₄]heptanoic acid	2328024-55-9
M8-PFOA	Perfluoro-n-[¹³ C ₈]octanoic acid	1350614-84-4
M9-PFNA	Perfluoro-n-[¹³ C ₉]nonanoic acid	2283397-80-6
M9-PFDA	Perfluoro-n-[¹³ C ₉]decanoic acid	-
M7-PFUnDA	Perfluoro-n-[1,2,3,4,5,6,7- ¹³ C ₇]undecanoic acid	-
M2-PFDoDA	Perfluoro-n-[1,2 - ¹³ C ₂]dodecanoic acid	960315-52-0
M2-PFTeDA	Perfluoro-n-[1,2 - ¹³ C ₂]tetradecanoic acid	-
Perfluoroalkyl Sulfonic Acids (PFSA)		
M3-PFBS	Perfluoro-1-[2,3,4- ¹³ C ₃]butanesulfonate	2708218-84-0
M3-PFHxS	Perfluoro-1-[1,2,3- ¹³ C ₃]hexanesulfonate	2708218-86-2
M8-PFOS	Perfluoro-1-[¹³ C ₈]octanesulfonate	2522762-16-7
Other*		
M8-FOSA	Perfluoro-1-[¹³ C ₈]octanesulfonamide	-
M2-4:2 FTS	Sodium 1H,1H,2H,2H-perfluoro-1-(1,2- ¹³ C ₂)-hexane sulfonate	2708218-88-4
M2-6:2 FTS	Sodium 1H,1H,2H,2H-perfluoro-1-(1,2- ¹³ C ₂)-octane sulfonate	2708218-89-5
M2-8:2 FTS	Sodium 1H,1H,2H,2H-perfluoro-1-(1,2- ¹³ C ₂)-decane sulfonate	2708218-90-8
D3-N-MeFOSA	N-methyl-d ₃ -perfluoro-1-octanesulfonamide	936109-37-4
D5-N-EtFOSA	N-ethyl-d ₅ -perfluoro-1-octanesulfonamide	936109-40-9
D7-N-MeFOSE	2-(N-methyl-d ₃ -perfluoro-1-octanesulfonamido)ethan-d ₄ -ol	1265205-95-5
D9-N-EtFOSE	2-(N-ethyl-d ₅ -perfluoro-1-octanesulfonamido)ethan-d ₄ -ol	1265205-96-6

*Meanwhile an isotope labelled internal standard exists for HFPO-DA (M3-HFPO-DA, CAS: 3030247-97-0)

3. Sample Preparation Method

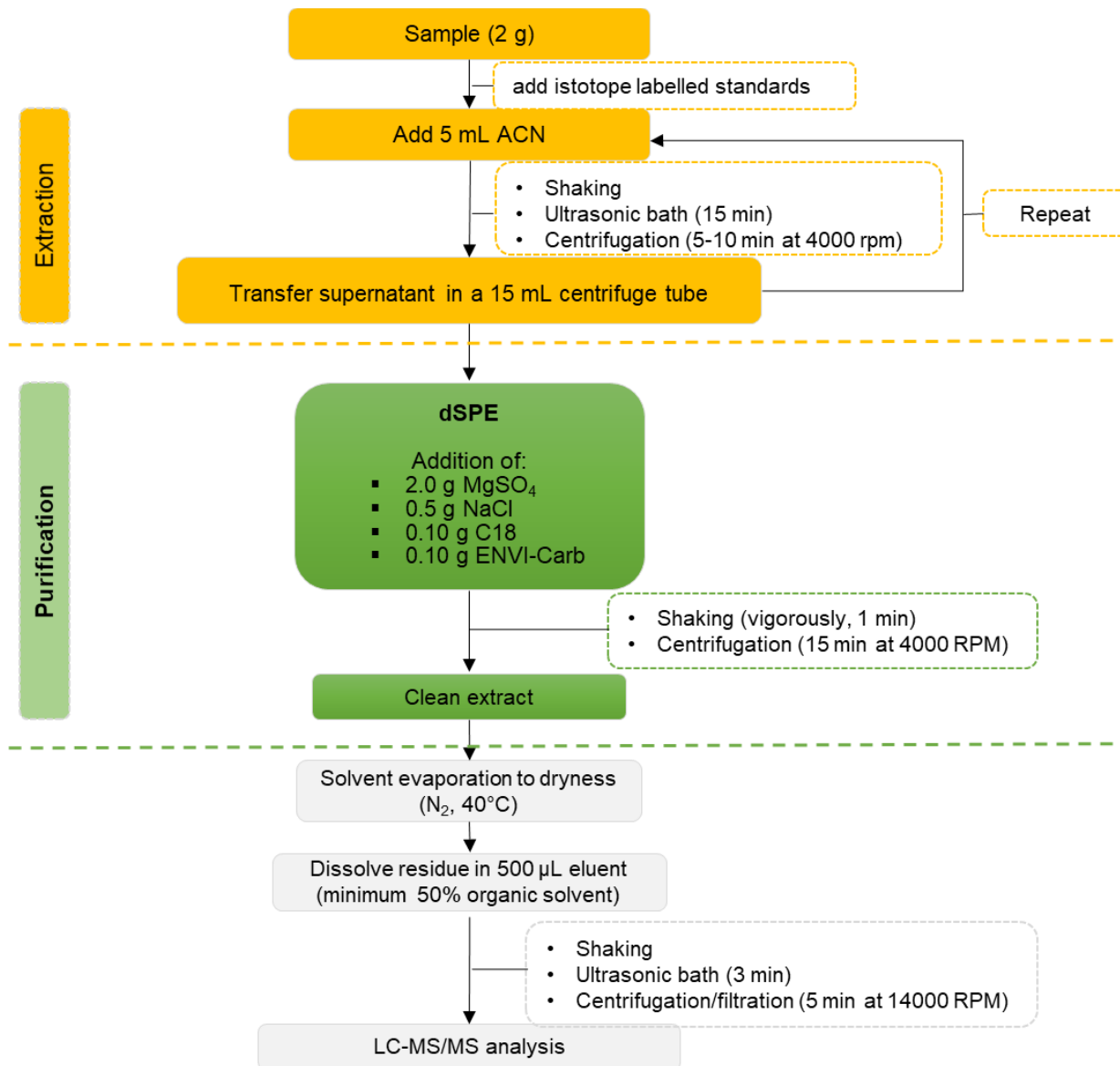


Figure 1: Flowchart of the sample preparation method.



4. LC-MS/MS Method

Table 3: Instrument parameters

Parameter LC-system	
Manufacturer	Agilent Technologies
Type	1260 Infinity II series
Pump (type)	G7112B, binary pump
Autosampler (type)	G7129A
Autosampler (temperature)	15 °C
Column oven (type)	G7116A
Column oven (temperature)	25 °C
Analytical column	Poroshell 120 C ₁₈ 150 x 3.0 mm, 2.7 µm
Trap column	Poroshell 120 C ₁₈ 50 x 3.0 mm, 2.7 µm
Injection volume	10 µL
Parameter mass spectrometer	
Manufacturer	Agilent Technologies
Type	Triple quadrupole 6470
Ionization mode	ESI -
Capillary voltage	3000 V
Gas temperature	240 °C
Gas flow	4 L/min
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Nozzle Voltage	0 V
Nebulizer	25 psi
MS1/MS2 Resolution	Unit/Unit
Scan type	Dynamic multiple reaction monitoring (dMRM)

Table 4: LC gradient program

Time (min)	Mobile phase A (2 mM ammonium acetate in water)	Mobile phase B (2 mM ammonium acetate in methanol)	Flow rate (mL/min)
0.00	75	25	0.3
2.50	40	60	0.3
10.00	20	80	0.3
12.00	10	90	0.3
14.75	10	90	0.3
15.00	0	100	0.3
20.00	0	100	0.3
20.50	75	25	0.3
24.00	75	25	0.3

**Table 5:** Retention times (RT), MS/MS transitions and optimised mass spectrometer parameters

Analyte	RT (min)	Precursor ion (m/z)	Fragment ion (m/z)	Fragmentor (V)	Collision energy (eV)
PFBA	7.7	213	169	75	5
PFPeA	9.3	263	219	80	5
PFBS	9.4	299	80	145	34
		299	99	145	28
PFHxA	10.6	313	269	80	5
		313	119	80	21
4:2 FTS	10.6	327	307	150	20
		327	81	150	36
PFPeS	10.7	349	80	160	41
		349	99	160	35
HFPO-DA	11.2	329	285	40	5
		285	169	65	5
PFHxS	11.9	399	80	175	39
		399	99	175	45
PFHpA	12.3	363	319	80	7
		363	169	80	15
DONA	12.4	377	251	70	9
		377	85	70	33
6:2 FTS	13.2	427	407	150	30
		427	81	150	32
PFHpS	13.3	449	80	190	48
		449	99	190	44
PFOA	13.3	413	369	85	7
		413	169	85	17
PFOS (linear)	14.2	499	80	190	47
		499	99	190	51
		499	169	190	44
PFNA	14.6	463	419	95	7
		463	219	95	15
9Cl-PF3ONS	14.7	531	351	150	29
		531	83	150	31
PFNS	15.1	549	80	200	55
		549	99	200	53
PFDA	15.2	513	469	90	7
		513	269	90	17
8:2 FTS	15.2	527	507	200	30
		527	81	200	46
PFUnDA	15.8	563	519	100	9
		563	269	100	17
11Cl-PF3OUdS	15.8	631	451	180	31
		631	83	180	37
FOSA	16.0	498	78	165	39
PFDS	16.0	599	99	215	54
		599	80	215	56



Analyte	RT (min)	Precursor ion (m/z)	Fragment ion (m/z)	Fragmentor (V)	Collision energy (eV)
PFDoDA	16.0	613	569	100	9
		613	319	100	21
N-MeFOSA	16.7	512	219	155	29
		512	169	155	29
N-MeFOSE	16.7	616	59	115	11
PFDoDS	17.0	699	99	215	61
		699	80	215	61
PFTrDA	17.0	663	619	105	19
		663	169	105	19
N-EtFOSA	17.3	526	219	160	27
		526	169	160	31
N-EtFOSE	17.3	630	59	110	15
PFTeDA	17.6	713	669	115	11
		713	169	115	29
¹³ C ₄ -PFBA	7.7	217	172	75	5
¹³ C ₅ -PFPeA	9.3	268	223	80	5
¹³ C ₃ -PFBS	9.4	302	80	145	38
¹³ C ₆ -PFHxA	10.6	319	274	80	5
¹³ C ₂ -4:2 FTS	10.6	329	309	150	24
¹³ C ₃ -PFHxS	11.9	402	99	175	39
¹³ C ₄ -PFHpA	12.3	367	322	80	7
¹³ C ₂ -6:2 FTS	13.2	429	409	150	28
¹³ C ₈ -PFOA	13.3	421	376	85	7
¹³ C ₈ -PFOS	14.2	507	99	190	47
¹³ C ₉ -PFNA	14.6	472	427	95	7
¹³ C ₂ -8:2 FTS	15.2	529	509	200	28
¹³ C ₉ -PFDA	15.2	522	477	90	7
¹³ C ₉ -PFUnDA	15.8	572	528	100	9
¹³ C ₂ -PFDoDA	16.0	615	570	100	9
¹³ C ₈ -FOSA	16.0	506	78	165	39
d ₃ -N-MeFOSA	16.7	515	169	155	29
d ₇ -N-MeFOSE	16.7	623	59	115	11
d ₅ -N-EtFOSA	17.3	531	169	160	31
d ₉ -N-EtFOSE	17.3	639	59	110	15
¹³ C ₂ -PFTeDA	17.6	715	670	115	11



5. Validation Data

Validation data only available at request: eurl-pops@cvuafr.bwl.de