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

Method Report

Version 1.0

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EURL for halogenated POPs in Feed and Food
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# 1. Introduction

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

- leek
- asparagus
- cucumber
- raspberry
- millet
- honey

# 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 |
| PFTrDA | 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 |

| | | |
|---|---|---|
| **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-octansulfonamide | 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

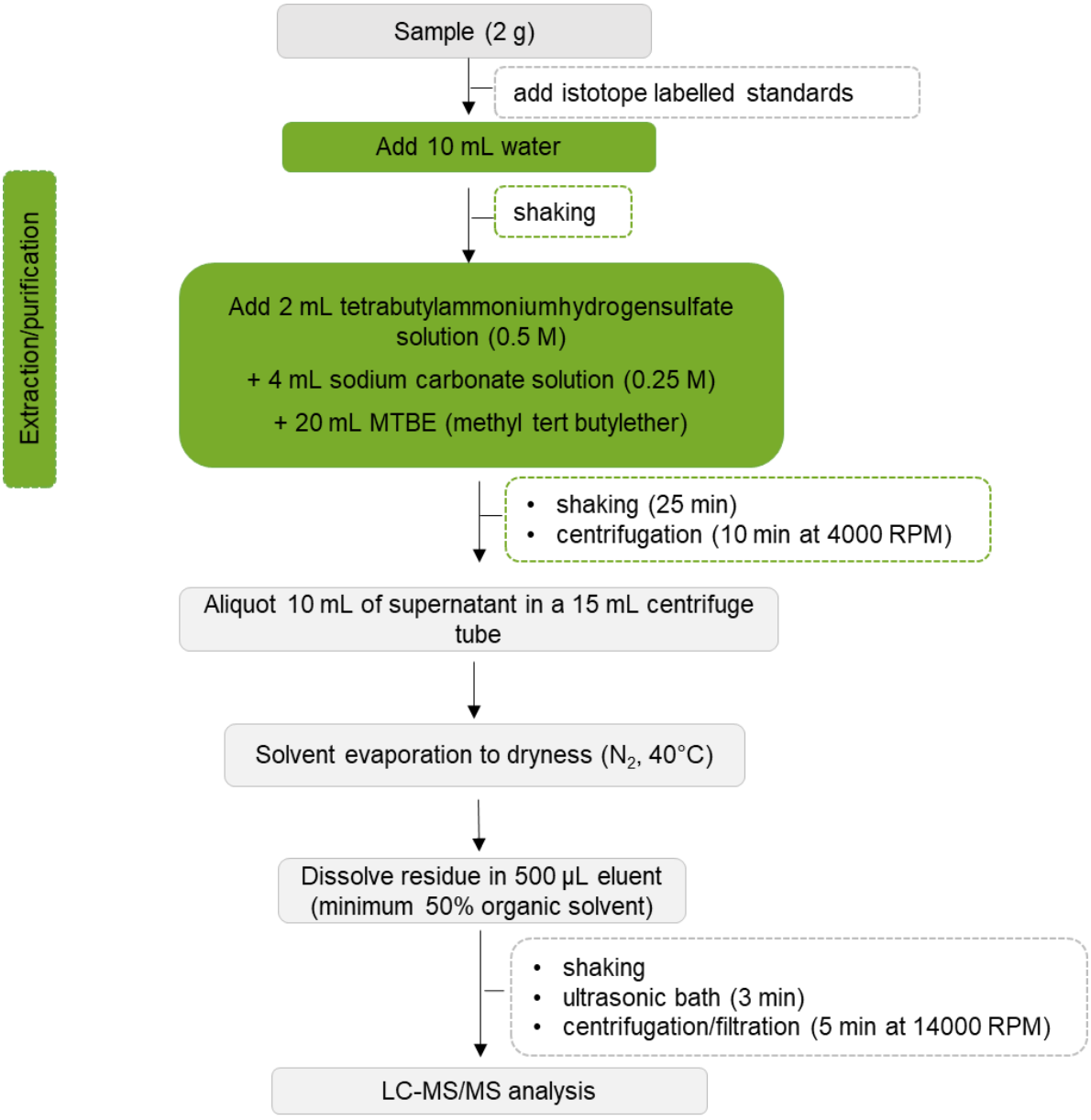


**Figure 1:** Flowchart of the sample preparation method.

## 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 |
| **Ionozation mode** | ESI - |
| **Capillary voltage** | 3000 V |
| **Gas temperature** | 240 °C |
| **Gas flow** | 4 L/min |
| **Sheat 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