**SCIENCE HUB DATA**

**An ultra-sensitive method for the analysis of perfluorinated alkyl acids in drinking water using a column switching high-performance liquid chromatography tandem mass spectrometry**

Kavitha Dasua,b*\**, Shoji F. Nakayamac, Mitsuha Yoshikaned, Marc A. Millsb, J. Michael Wrighte, Shelley Ehrlichf

aNational Research Council (NRC), The National Academies of Sciences, Engineering, and Medicine, 500 Fifth Street, NW, Washington, DC 20001

bNational Risk Management Research Laboratory, U.S. Environmental Protection Agency Cincinnati, OH, USA

cCentre for Health and Environmental Risk Research, National Institute for Environmental Studies, Tsukuba, Japan

dEnvironmental Risk Research Center, Institute of Environmental Ecology, IDEA Consultants, Inc., Tokyo, Japan

eNational Center for Environmental Assessment, U.S. Environmental Protection Agency Cincinnati, OH, USA

f Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA

*\**Corresponding author Present address: Pegasus Technical Services, Inc., 26 West, Martin Luther King Drive, Cincinnati, OH 45268, USA. Tel.: +1 513 569 7325; fax: +1 513 569 7620.

E-mail address: dasu.kavitha@epa.gov





**Recoveries in Fortified Laboratory Blanks.** Recoveries of the target and internal standard were calculated using the equation (SI-1).

$Recovery \left(\%\right)= \frac{C\_{measured}}{S\_{conc}}×100$ Eq. (SI-1)

Where Cmeasured is measured concentration in the standards fortified water; Sconc is set concentration (7.5 pg/g).

**Recoveries in Fortified Matrix Samples.** Recoveries of the target chemicals in the fortified matrix samples were calculated using the equation (SI-2).

$Fortified Matrix Recovery \left(\%\right)= \frac{C\_{measured}-C\_{background}}{S\_{conc}}×100$ Eq. (SI-2)

Where Cmeasured is measured concentration in the standards fortified matrix sample; Cbackground is the background concentration in unfortified matrix samples; Sconc is set concentration (7.5 pg/g).

**Surrogate recovery.** Surrogate recovery in the drinking water samples was calculated using the equation (SI-3).

$Surrogate Recovery \left(\%\right)= \frac{A\_{es}×\frac{A\_{ci}}{A\_{ei}}}{A\_{cs}}×100$ Eq. (SI-3)

Where Aes is area of each surrogate standard in the extract; Aei is area of respective internal standard in the extract; Acs is area of each surrogate standard in the calibration solution; and Aci is area of respective internal standard in the calibration solution

**Concentrations in drinking water.** Concentration calculations for each analyte in drinking water are based on the internal standard procedure using the equation (SI-4).

$Concentration in drinking water \left(\frac{pg}{g}\right)=\frac{\left(C\_{ex} × V\_{ex}\right)}{W\_{s}}$ Eq. (SI-4)

Where Cex is the concentration of a compound in the analyte; Vex is extract volume in ml; Ws is sample weight in g.

Further, density of water, 0.9976 g/mL at 73 ºF was used to convert sample weight (g) to volume (ml).

**Table SI 1.** List of Target PFAAs and mass labelled PFAAs mix and their CAS numbers

|  |  |  |  |
| --- | --- | --- | --- |
| **Compound Names** | **Abbreviation**  | **CAS** | **Purity** |
| ***Target PFAAs mix (PFAC-MXB)*** |  |  |  |
| Perfluoro-n-butanoic acid  | PFBA  | 375-22-4 | > 98% |
| Perfluoro-n-pentanoic acid  | PFPA  | 2706-90-3 | > 98% |
| Perfluoro-n-hexanoic acid  | PFHxA | 307-24-4 | > 98% |
| Perfluoro-n-heptanoic acid  | PFHpA | 375-85-9 | > 98% |
| Perfluoro-n-octanoic acid  | PFOA  | 335-67-1 | > 98% |
| Perfluoro-n-nonanoic acid  | PFNA  | 375-95-1 | > 98% |
| Perfluoro-n-decanoic acid  | PFDA | 335-76-2 | > 98% |
| Perfluoro-n-undecanoic acid  | PFUnA  | 2058-94-8 | > 98% |
| Perfluoro-n-dodecanoic acid  | PFDoA  | 307-55-1 | > 98% |
| Perfluoro-n-tridecanoic acid  | PFTrDA | 72629-94-8 | > 98% |
| Perfluoro-n-tetradecanoic acid  | PFTeDA  | 376-06-7 | > 98% |
| Perfluoro-n-hexadecanoic acid  | PFHxDA | 67905-19-5 | > 98% |
| Perfluoro-n-octadecanoic acid  | PFODA  | 16517-11-6 | > 98% |
| Potassium perfluoro-1-butanesulfonate  | L-PFBS  | 374-73-5 | > 98% |
| Sodium perfluoro-1-hexanesulfonate |  L-PFHxS  | 29420-49-3 | > 98% |
| Sodium perfluoro-1-octanesulfonate  | L-PFOS  | 1763-23-1 | > 98% |
| Sodium perfluoro-1-decanesulfonate  | L-PFDS  | 335-77-3 | > 98% |
| ***Stable isotope labelled PFAAs (MPFAC-MXB) for Surrogates:*** | **Isotopic Purity** | **Purity** |
| Perfluoro-n-[13C4]butanoic acid  | MPFBA | > 99% | > 98% |
| Perfluoro-n-[1,2-13C2]hexanoic acid |  MPFHxA | > 99% | > 98% |
| Perfluoro-n-[1,2,3,4-13C4]octanoic acid |  MPFOA | > 99% | > 98% |
| Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid  | MPFNA | > 99% | > 98% |
| Perfluoro-n-[1,2-13C2]decanoic acid  | MPFDA | > 99% | > 98% |
| Perfluoro-n-[1,2-13C2]undecanoic acid  | MPFUdA | > 99% | > 98% |
| Perfluoro-n-[1,2-13C2]dodecanoic acid  | MPFDoA | > 99% | > 98% |
| Sodium perfluoro-1-hexane[18O2]sulfonate  | MPFHxS | > 94% | > 98% |
| Sodium perfluoro-1-[1,2,3,4-13C4]octane sulfonate  | MPFOS | > 99% | > 98% |
| ***Stable isotope labelled PFAAs for Internal Standards:*** |   | **Isotopic Purity** | **Purity** |
| Perfluoro-n-[13C8] octanoic acid  | M8PFOA | > 99% | > 97.9% |
| Sodium perfluoro-1-[13C8] octanesulfonate  | M8PFOS | > 99% | > 98% |

**Table SI 2.** LC-MS/MS parameters

|  |
| --- |
| **LC: Nexcera (Shimadzu Co. Ltd., Japan)** |
| Mobile Phase | Pump A  | Deionized water |
|  | Pump B  | Methanol |
|  | Pump C  | 0.1% NH4OH in Pre-clean Methanol |
|   | Pump D  | Deionized water |
| Flow rate | Ternary Gradient (A/B/C Pump) | 0.25 mL/min |
|  | Sample Loading (D Pump) | 1 mL/min |
| Column | Scrubber column (Pump A)  | OASIS HLB 2.1 x 20 mm |
|  | Scrubber column (Pump B)  | OASIS WAX 2.1 x 20 mm |
|  | Scrubber column (Pump C)  | Ghost Trap DS 7.6 x 30 mm |
|  | Scrubber column (Pump D)  | OASIS HLB 2.1 x 20 mm |
|  | Pre-concentration column  | OASIS WAX 2.1 x 20 mm |
|   | Analytical column  | Inertsustain C18 2.1 x 50 mm |
| Auto sampler | Injection Volume  | 3 mL |
| **MS: LCMS-8080 (Shimadzu Co. Ltd., Japan)** |
| Interface Parameter | Interface HV  | - 4.5 kV |
|  | Probe Temperature  | 300 °C |
|  | HSID Temperature  | 200 °C |
|  | Nebulizer Gas (Air)  | 2 L/min |
|  | Heating Gas (Air)  | 12 L/min |
|  | Curtain Gas (N2)  | 3 L/min |
|   | Exhaust Gas | ON |

**Table SI 3.** Quality control and acceptance criteria

|  |  |  |
| --- | --- | --- |
| **Prior to analysis** |  | Criteria |
| Initial precision and recovery (IPR) | LFB-NIES (n=7) | <= +/- 30% a |
| Procedure blank | Procedure blank (n=7) | <= LCMRL |
| **For all batches** |  |  |
| Initial Calibration | Calibration Point | >= 5 point |
| r2 | >= 0.99 |
| Deviation at each point | <= +/- 30% |
| Calibration Verification | Cal 5 | <= +/- 20% |
| Blank Analysis for Instrument | LRB-NIES | <= LCMRL |
| Cal 0 | <= LCMRL |
| Blank Analysis for Method | LRB-EPA | <= LCMRL |
| Frequency of Blank analysis in a sequence | After every tenth sample | <=LCMRL |
| Ongoing Precision and recovery standard (OPR) EPA | LFB-EPA Recovery | <= +/- 30% a |
| Quality Control Sample | LFM-EPA | <= +/- 30% a |

a Except for PFPeA, PFHxA and PFHpA for these <= +/- 40%;

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**Table SI 4.** Lowest Concentration Method Reporting Level (LCMRL) values and Blank concentrations in the procedural blanks analyzed at extraction laboratory and analytical laboratory

|  |  |  |
| --- | --- | --- |
| **Target Analytes** |  | **Blank Concentration, ng/L** |
| **LCMRL, ng/L** | **NIES** | **EPA** |
| **Batch 1** | **Batch 2** | **Batch 1** | **Batch 2** |
| **Replicate 1**  | **Replicate 2**  | **Replicate 1** | **Replicate 2** | **Replicate 1**  | **Replicate 2**  | **Replicate 1** | **Replicate 2** |
| PFBS | 2.7 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFHxS | 0.77 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFOS | 1.9 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFDS | 1.2 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFPeA | 3.4 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFHxA | 3.3 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | 5.72 | 4.28 | <LCMRL | <LCMRL |
| PFHpA | 0.64 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFOA | 2.5 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFNA | 3.2 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFDA | 1.0 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFUnA | 1.3 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFDoA | 0.59 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFtriDA | 1.4 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |
| PFteDA | 1.8 | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL | <LCMRL |

**Table SI 5.** Percent recoveries of target analytes in laboratory fortified blanks and matrix fortified blanks

|  |  |  |
| --- | --- | --- |
| **Target Analytes** | **Laboratory fortified blanks (n=8)** | **Matrix fortified Samples (n=4)** |
| PFBS | 100±15 | 126±28 |
| PFHxS | 90±16 | 109±30 |
| PFOS | 99±7 | 107±7 |
| PFDS | 74±29 | 56±33 |
| PFPeA | 139±13 | 128±5 |
| PFHxA | 114±10 | 131±17 |
| PFHpA | 110±13 | 110±22 |
| PFOA | 97±12 | 111±5 |
| PFNA | 107±8 | 109±7 |
| PFDA | 108±7 | 110±7 |
| PFUnA | 105±14 | 88±9 |
| PFDoA | 96±8 | 83±13 |
| PFtriDA | 85±17 | 73±14 |
| PFteDA | 87±15 | 73±6 |

**Table SI 6.** Summary of Calibration curve % Relative Standard Deviation (%RSD) of 14 analytes

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Target Compound** | **LCMRL, ng/L** | **MS/MS** | **r2** | **Range** | **% Relative Standard Deviation (n=4)** |
| **Cal1** | **Cal2** | **Cal3** | **Cal4** | **Cal5** | **Cal6** | **Cal7** |
| **0.5** | **2.5** | **5** | **15** | **25** | **50** | **100** |
| PFBS | 2.7 | 298.9>99.0 | 0.999 | C2-C7 | ----- | 5.7 | 3.0 | 1.9 | 5.0 | 3.6 | 0.7 |
| PFHxS | 0.77 | 398.9>99.05 | 0.999 | C1-C7 | 7.5 | 3.6 | 6.8 | 4.2 | 4.8 | 2.6 | 1.8 |
| PFOS | 1.9 | 498.9>99.15 | 0.999 | C1-C7 | 12.7 | 12.1 | 8.3 | 6.4 | 4.5 | 3.0 | 5.0 |
| PFDS | 1.2 | 598.9>99.0 | 0.999 | C1-C7 | 10.7 | 4.8 | 7.6 | 3.0 | 5.2 | 4.8 | 6.6 |
| PFPeA | 3.4 | 262.7>219.0 | 1.000 | C1-C7 | 9.4 | 6.0 | 2.4 | 0.7 | 1.8 | 2.7 | 1.8 |
| PFHxA | 3.3 | 312.9>269.0 | 1.000 | C1-C7 | 7.6 | 1.5 | 2.5 | 4.4 | 4.6 | 1.2 | 2.4 |
| PFHpA | 0.64 | 362.9>169.0 | 0.998 | C1-C7 | 13.2 | 3.7 | 2.1 | 4.4 | 3.0 | 2.0 | 3.5 |
| PFOA | 2.5 | 412.9>169.0 | 1.000 | C1-C7 | 5.5 | 3.9 | 2.6 | 4.6 | 2.1 | 4.3 | 5.3 |
| PFNA | 3.2 | 462.9>419.0 | 0.999 | C1-C7 | 14.6 | 8.0 | 8.2 | 4.2 | 2.7 | 4.8 | 6.2 |
| PFDA | 1.0 | 512.9>468.9 | 1.000 | C1-C7 | 8.1 | 4.8 | 3.8 | 5.6 | 4.2 | 7.2 | 3.5 |
| PFUnA | 1.3 | 562.9>519.0 | 1.000 | C1-C7 | 3.6 | 4.1 | 5.8 | 5.2 | 2.6 | 5.8 | 3.8 |
| PFDoA | 0.59 | 612.9>568.95 | 0.999 | C1-C7 | 7.6 | 6.7 | 9.6 | 2.2 | 4.4 | 4.5 | 4.4 |
| PFtriDA | 1.4 | 662.9>618.9 | 1.000 | C1-C7 | 10.2 | 7.4 | 8.5 | 4.9 | 5.0 | 4.9 | 5.2 |
| PFteDA | 1.8 | 712.9>668.9 | 0.999 | C1-C7 | 17.1 | 5.9 | 7.1 | 5.3 | 4.2 | 5.1 | 5.3 |

**Table SI 7.** Surrogate recoveries (%) in all the 25 drinking water samples

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample ID | 18O2-PFHxS | 13C4-PFOS | 13C2-PFHxA | 13C4-PFOA | 13C5-PFNA | 13C2-PFDA | 13C2-PFUnA | 13C2-PFDoA |
| Sample 1 | 70 | 78 | 69 | 110 | 95 | 102 | 92 | 107 |
| Sample 2 | 99 | 95 | 46 | 92 | 85 | 64 | 41 | 43 |
| Sample 3 | 76 | 102 | 46 | 79 | 109 | 102 | 80 | 69 |
| Sample 4 | 89 | 94 | - | 82 | 82 | 75 | 60 | 54 |
| Sample 5 | 97 | 90 | 53 | 90 | 101 | 108 | 101 | 81 |
| Sample 6 | 75 | 87 | 47 | 69 | 86 | 96 | 84 | 70 |
| Sample 7 | 82 | 84 | 70 | 100 | 110 | 118 | 98 | 102 |
| Sample 8 | 75 | 86 | 48 | 92 | 100 | 91 | 83 | 76 |
| Sample 9 | 73 | 96 | 50 | 81 | 91 | 114 | 93 | 102 |
| Sample 10 | 87 | 98 | 114 | 118 | 168 | 154 | 111 | 115 |
| Sample 11 | 102 | 95 | 49 | 92 | 84 | 91 | 83 | 81 |
| Sample 12 | 117 | 106 | 50 | 90 | 100 | 111 | 109 | 85 |
| Sample 13 | 85 | 89 | 56 | 82 | 93 | 89 | 76 | 65 |
| Sample 14 | 82 | 88 | 58 | 82 | 90 | 92 | 71 | 51 |
| Sample 15 | 90 | 89 | 64 | 108 | 120 | 99 | 60 | 34 |
| Sample 16 | 75 | 76 | 59 | 84 | 91 | 74 | 57 | 47 |
| Sample 17 | 92 | 92 | 37 | 91 | 102 | 84 | 69 | 57 |
| Sample 18 | 87 | 97 | 55 | 87 | 90 | 82 | 57 | 48 |
| Sample 19 | 92 | 85 | 51 | 94 | 102 | 86 | 73 | 60 |
| Sample 20 | 96 | 104 | 48 | 79 | 93 | 72 | 54 | 44 |
| Sample 21 | 94 | 71 | 45 | 91 | 89 | 71 | 49 | 43 |
| Sample 22 | 84 | 104 | 49 | 94 | 113 | 106 | 78 | 57 |
| Sample 23 | 121 | 90 | 53 | 90 | 68 | 19 | 51 | 57 |
| Sample 24 | 98 | 105 | 57 | 82 | 97 | 92 | 79 | 99 |
| Sample 25 | 45 | 37 | 28 | 53 | 47 | 39 | 34 | 36 |

**Table SI 8.** Stability testing data showing the ratio of recoveries of analytes from the cartridges between day 0 and day 28; and the p-values of Kruskal-Wallis followed by Scheffe's test showing no significant differences

|  |  |  |
| --- | --- | --- |
| **Analyte** | **Ratio (day 0/day 28)** | **p-value** |
| PFBS | 0.96 | 0.9271 |
| PFHxS | 0.90 | 0.6281 |
| PFOS | 0.91 | 0.4432 |
| PFDS | 0.93 | 0.9994 |
| PFPeA | 1.22 | 0.0429 |
| PFHxA | 0.86 | 0.0779 |
| PFHpA | 0.82 | 0.0938 |
| PFOA | 0.86 | 0.0643 |
| PFNA | 0.93 | 0.6127 |
| PFDA | 0.94 | 0.8950 |
| PFUnA | 0.88 | 0.4136 |
| PFDoA | 0.86 | 0.6433 |
| PFTriDA | 1.02 | 0.9998 |
| PFTeDA | 1.04 | 0.9960 |