Analytical Method for Quantifying Legacy & Emerging PFAS in Aqueous and Solid Matrices

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**1950s** Incorporation of PFAS into wide variety of consumer and industrial products

**2001** Accumulation of PFOS in most humans, animals and environmental media
(Giesy, 2001; Kannan, 2001; Houde, 2006)

**2002** Voluntary phase out of PFOS in the USA

**2006** USEPA engaged 8 leading PFAS manufacturers 2010/2015 PFOA Stewardship Program
“…..fluorotechnology is essential technology for many aspects of modern life…..”

*Bowman, 2015*

“…..we call on the international community to cooperate in limiting the production and use of PFASs and in developing safer nonfluorinated alternatives.”

*Blum et al., 2015*
**C8 Chemistries**

**PFOS**

- Persistent (UNEP, 2009)
- Mobile (Munoz et al., 2015)
- Bioaccumulative (Conder et al., 2008)
- Toxic (IARC Class 2B Probable Carcinogen)

**PFOA**

**UNEP Stockholm Convention**
- PFOS in 2009
- PFOA under consideration

Replaced with family with varying fluorinated chain length:
- Perfluoroalkylsulfonates (C2-12; PFSA)
- Perfluoroalkylcarboxylic Acids (C4-C22; PFCA)
Sources of PFAS to the Environment

- Industry
- Defense
- Airports
- Domestic Environment
- WWTPs
- Landfill

Other Sources
Potential Contaminated Sites in Australia

- **Queensland**
  - Army Aviation Centre Oakey
  - RAAF Base Townsville
  - RAAF Base Amberley
  - Lavarack Barracks

- **Western Australia**
  - RAAF Base Pearce
  - HMAS Stirling
  - Naval Communication Station Harold E Holt A & B
  - RAAF Base Learmonth
  - Gingin Satellite Airfield

- **New South Wales**
  - RAAF Base Williamtown
  - HMAS Albatross
  - RAAF Base Richmond
  - RAAF Base Wagga
  - Holsworthy Barracks

- **Tasmania**
  - No current PFAS Investigations

- **Victoria**
  - RAAF Base East Sale
  - HMAS Cerberus
  - Bandiana Military Area

- **Jervis Bay Territory**
  - Jervis Bay Range Facility

- **Northern Territory**
  - RAAF Base Darwin
  - RAAF Base Tindal
  - Robertson Barracks

DoD Sites Under Investigation
Airports
PFAS Terminology

Common Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Acronym Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFCA</td>
<td>Perfluoroalkylcarboxylic acid</td>
</tr>
<tr>
<td>PFOA</td>
<td>Perfluorooctanoic acid</td>
</tr>
<tr>
<td>PFAS</td>
<td>Perfluoroalkylsulfonate</td>
</tr>
<tr>
<td>PFOS</td>
<td>Perfluorooctanesulfonate</td>
</tr>
<tr>
<td>PFASI</td>
<td>Perfluoroalkylsulfonate</td>
</tr>
<tr>
<td>FOSA</td>
<td>Perfluorooctanesulfonamide</td>
</tr>
<tr>
<td>FOSAA</td>
<td>Perfluorooctanesulfonamide acetic acid</td>
</tr>
<tr>
<td>FSE</td>
<td>Perfluorooctanesulfonamidoethanol</td>
</tr>
<tr>
<td>FTOH</td>
<td>Fluorinated telomer alcohol (-OH functional group)</td>
</tr>
<tr>
<td>FTA</td>
<td>Fluorinated telomer acid</td>
</tr>
<tr>
<td>FTUA</td>
<td>Fluorinated telomer unsaturated acid</td>
</tr>
<tr>
<td>FTS</td>
<td>Fluorinated telomer sulfonate</td>
</tr>
<tr>
<td>PFAPA</td>
<td>Perfluoroalkylphosphonic acid</td>
</tr>
<tr>
<td>PFPI</td>
<td>Perfluoroalkylphosphinate</td>
</tr>
<tr>
<td>PAP</td>
<td>Mono-substituted polyfluoroalkylphosphate ester</td>
</tr>
<tr>
<td>diPAP</td>
<td>Di-substituted polyfluoroalkylphosphate ester</td>
</tr>
<tr>
<td>PFAI</td>
<td>Perfluoroalkyl iodide</td>
</tr>
<tr>
<td>SFA</td>
<td>Semifluorinated alkane</td>
</tr>
<tr>
<td>FTI</td>
<td>Fluorinated telomer iodide</td>
</tr>
<tr>
<td>FTO</td>
<td>Fluorinated telomer olefin</td>
</tr>
<tr>
<td>FTAC</td>
<td>Fluorinated telomer acrylate</td>
</tr>
</tbody>
</table>

Drinking Water Investigation Levels

Australia

Health Based Guidance Values for PFAS
FOR USE IN SITE INVESTIGATIONS IN AUSTRALIA

In June 2016, the Department of Health conducted national PFAS drinking water surveys and investigations in the ACT, New South Wales, Victoria, South Australia, Western Australia, and Queensland. The Department of Health has released the following PFAS drinking water data: Toxicity reference value

<table>
<thead>
<tr>
<th></th>
<th>PFOS/ PFxS</th>
<th>PFOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerable daily intake (ng or µg / kg bw/day)</td>
<td>20</td>
<td>0.02</td>
</tr>
<tr>
<td>Drinking water quality value (ng or µg / L)</td>
<td>70</td>
<td>0.07</td>
</tr>
<tr>
<td>Recreational water quality value (ng or µg / L)</td>
<td>700</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Note: bw = body weight; ng = nanograms; µg = micrograms

What is a health based guidance value?

Health based guidance values indicate the amount of a chemical or toxic substance that a person can consume over a lifetime without any significant risks. Public health guidance values are based on the results of the risk assessment and are used to protect public health. The threshold for the moiety of the moiety, health based guidance values are usually expressed per day and are considered the maximum level of exposure.

USA

- 49% of Australian drinking water samples (n=34) contained PFAS
- None over investigation level (Thompson, 2011)

Methodology

\[ [PFAS] = TDI \times BW \times RSC \times R (10\%) \times Rate (2L) \]
Municipal Wastewater

<table>
<thead>
<tr>
<th>Exposure scenario</th>
<th>PFOS</th>
<th>PFOA</th>
<th>Exposure scenario</th>
<th>Comments and source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshwater</td>
<td>0.00023 µg/L</td>
<td>19 µg/L</td>
<td>99% species protection — high conservation value systems</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.13 µg/L</td>
<td>220 µg/L</td>
<td>95% species protection — slightly to moderately disturbed systems</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 µg/L</td>
<td>632 µg/L</td>
<td>80% species protection — highly disturbed systems</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31 µg/L</td>
<td>1824 µg/L</td>
<td>80% species protection — highly disturbed systems</td>
<td></td>
</tr>
</tbody>
</table>

Environmental Discharge

Water Recycling Programs
**LCMS Analytical Methodology**

**Standard Methods**
- US EPA Method 537.1
- ISO 25101
- ASTM D7968
- ASTM D7979

**Total PFAS**
- Total Oxidisable Precursor Assay (TOPA)
- Total Organic Fluorine (TOF)

**QA/QC:** Batch of 9 samples includes matrix spike, laboratory control sample (LCS) & blank

**Quantification:** Agilent 6495 LC Triple Quadrupole LC-MS
- **1 μL injection** to meet sensitivity
- **Technique:** Isotope Dilution
- **Reporting Limit:** High 10-50 ng/L; Low <1 ng/L
Extraction Techniques

Solid Phase Extraction (SPE)

- **Sample collection**: 250 mL polypropylene bottle
- **Filtration**: 1 µm glass fibre filter
- **Surrogate spike**: 5 ng isotopically labelled PFAS
- **SPE**: 6 cc, 30 µm particle size, 150 mg weak anion exchange resin (WAX)
- **Elution**: 2 mL methanol, 4 mL methanol (0.1% NH₄OH)
- **Evaporation**: Evaporated to dry under gentle stream of N₂, reconstituted to 500-1000uL of MeOH

Solids Extraction

- **Sample collection**: 250 mL polypropylene bottle or 50 mL polypropylene centrifuge tube
- **Preparation**: Sterilise with 2% w/w sodium azide solution, freeze-dry, powderise
- **Surrogate spike**: 25 ng isotopically labelled PFAS
- **Extraction**: 0.5-1 basic MeOH (10 mM NaOH) neutralised after extraction with glacial acetic acid.
- **Clean-Up**: dSPE with 100 mg C18 and 50 mg PSA
- **Filter**: 0.45 µm PES syringe filter

**QA/QC**: Batch of 9 samples includes matrix spike, laboratory control sample (LCS) & blank
**IDLs for a consolidated PFAS method on 6495**

14 different classes of PFAS including PFCAs (dark blue); PFSAs (red); n:2 FTSs (pink); n:2 FTCA, n:3 FTCA, n:2 FTUCA (light blue); PFECA & Cl-PFESA (black); FASA, FASAA, FOSE (maroon); PFPA, diPAP & PFPIA (green); diSAmPAP (orange)

Cogган et al. (2019) Anal Bioanal Chem, 3507-3520
Analysis of >50 PFAS in Water
Method Performance (1 uL injection of extract)

>92% of PFAS tested have RSDs <20% & MDLs <5 ng/L

* Seven replicates at 5 ng/L spiked into 250 mL water samples; followed by SPE and injection of 1 uL onto 6495 LC-MS/MS; ^ based on USEPA 40 CFR Part 136 Appendix B Revision 2
SPE Method accuracy – 5 ng/L spike* (n=7)

Accuracy:
49 of 52 between 70 – 130 %

* n:2 FTCAs and FOSEs spike 20 ng/L

MDL (US EPA 40 CFR part 136, rev 2):
Between 0.28 – 17 ng/L
Target compounds

- PFCAs
- PFSAs
- FTSs
- CI-PFESAAs
- FTCAs/FTUCAs
- HPFO-TA
- FASAAs
- FASEs
- FASAs
- PFPAs
- PFPis
- diPAPs & diSAMPAP

Coggan et al. (2019) Anal Bioanal Chem, 3507-3520
Method Validation - PFOS

Groundwater
*RMIT-Contract Lab 1*

Wastewater
*RMIT-Contract Lab 2*

RSD <10% and no statistically significant difference of mean of PFOS/PFAS between laboratory results in both validation tests.
Project Overview

(1) Analytical Method Development
• SPE Extraction for liquids
• Alkaline digestion for solids
• Validated with two external comparisons
• Quantifying 55-60 PFAS

(2) PFAS Mass Flux at Australian WWTPs
• Mass balance studies at 19 WWTPs (22 PFAS)
• Mass balance studies at 5 WWTPs (55 PFAS)
• Untargeted analysis using Q-TOF
• Australian biosolids survey for PFAS

(3) Environmental and Ecological Impact
• Literature review of ecotoxicology report
• PFOS/PFOA/GenX fish update/depuration study with Australian fish
• Environmental fate in the marine environment from treatment
19 WWTP Frequency of Detection

Coggan et al. 2019
\[ \Sigma_{18} \text{PFAS Concentration in WWTPs} \]

**Influent**
- Mean: 90 ng/L
- Median: 76 ng/L
- Range: 9.3 – 412 ng/L

**Overall**
- Mean: 80 ng/L
- Median: 110 ng/L
- Range: 9.3 – 520 ng/L

**Final Effluent**
- Mean: 140 ng/L
- Median: 140 ng/L
- Range: 34 – 520 ng/L

Coggan et al. *Under Review*
96 - 100% detection frequency

Statistically significant difference between influent and effluent

Coggan et al. *Under Review*
PFSAs: 19 WWTPs Aqueous Samples

Coggan et al. Under Review
PFCAs: 19 WWTPs Solids Samples

Coggan et al. Under Review
PFSAs: 19 WWTPs Solids Samples

Coggan et al. Under Review
Biotransformation – 6:2 FTS in a WWTP

Transformation pathway from Wang et al., *Chemosphere*, 2011
WWTP Effluent Environmental Discharges

**WWTP Mean**: 12 g/day
**Daily Range**: 0.05 to 114 g/day
**Yearly Range**: 0.02 to 41 kg/year
**Estimated Australian WWTP Emissions**: 340 kg/year
Suspect Screening and Untargeted Analysis

- Agilent 6545 LC/Q-TOF
- Custom PFAS database
- MS/MS spectra and retention time data available for a subset of compounds

*Moving Beyond Monitoring Legacy Per and Polyfluoroalkyl Substances (PFAS): Screening Strategies for the Growing List.*

*James Pyke, Tuesday 11:00 AM*
Continuing work

Known knowns
Expanded targeted list (~50 compounds)

Known unknowns
Expanded PFAS database

Continually refining targeted method

6:2 FTS
Mass: 427.97458
Mass diff: -1.41 ppm
Score: 98.11

Unknown unknowns
Adding identified compounds to database list
PFAS in Biosolids

Mean: $\sum_{42}^{PFAS}$ 250 ng/g ($\pm$ 210)
Range: $\sum_{42}^{PFAS}$ 35 to 840 ng/g

Moodie et al. *in prep*
PFAS in Biosolids

Mean Concentration ng/g

Frequency of Detection

Moodie et al. *in prep*
PFAS in a WWTP Solid Sample

PreFAS: 5:3 Acid 7:3 Acid Et FOSAA Me FOSAA 6:2 diPAP 8:2 diPAP 6:2/8:2 diPAP

Will breakdown to form PFOA and other PFCAs
PFASs in WWTP Impacted Groundwater

79%  82%  86%  96%

Concentration (ng/L)

Year  
2017  2018

Compound
A single analytical method for the determination of 53 legacy and emerging per- and polyfluoroalkyl substances (PFAS) in aqueous matrices

Timothy L. Coggan1, Tarun Anumol1, James Pyke2, Jeff Shimeta1, Bradley O. Clarke1

Received 30 November 2018; Revised 37 February 2019; Accepted 3 April 2019

Abstract
A quantitative method for the determination of per- and polyfluoroalkyl substances (PFAS) using liquid chromatography (LC) tandem mass spectrometry (MS/MS) was developed and applied to aqueous wastewater, surface water, and drinking water samples. Fifty-three PFAS from 14 compound classes (including many contaminants of emerging concern) were measured using a single analytical method. After solid phase extraction using weak anion exchange cartridge, method detection limits in water ranged from 0.28 to 18 ng/L and method quantitation limits ranged from 0.53 to 26 ng/L. Method accuracy ranged from 70 to 125% for 69 of the 53 extracted PFAS, with the remaining four between 60 and 138%. Method precision ranged from 2% to 21% RSD, with 49 out of the 53 PFAS lying below <20%. In addition to quantifying >50 PFAS, many of which are currently unregulated in the environment and not included in typical analytical lists, this method has efficiency advantages over other similar methods as it utilizes a single chromatographic separation with a shorter runtime (14 min), while maintaining method accuracy and stability and the separation of branched and linear PFAS isomers. The method was applied to wastewater influent and effluent, surface water from a river, wetland, and lake; and drinking water samples to survey PFAS contamination in Australian aquatic matrices. The compound classes F'TCAs, PFOAAs, PFs and BaPeOs were detected for the first time in Australian WWTPs, and the method was used to quantify PFAS concentrations from 60 to 190 ng/L. The range of compound classes detected and different PFAS signatures between sample locations demonstrate the need for expanded quantitation in future investigations of PFAS, especially newer classes in aquatic environmental samples.

Keywords PFAS - Wastewater - Surface water - Drinking water - LC-MS/MS

Analysis of >50 Legacy and Emerging PFAS in Water Using the Agilent 6495B Triple Quadrupole LC/MS

Timothy L. Coggan, Jeff Shimeta, and Bradley O. Clarke
RMIT University, Melbourne, VIC, Australia
Tarun Anumol and James Pyke
Agilent Technologies, Inc.

Abstract
The contamination of the environment with per- and polyfluoroalkyl substances (PFAS) is a serious concern to regulators, scientists, and the public worldwide; due to their ubiquitous presence, persistence, and toxicity. Robust analytical techniques that can accurately and precisely quantify these pollutants at trace levels are necessary for understanding their environmental fate, ecological impacts, and impacts on public health. Appropriate analytical techniques and the fundamental data they generate allow scientists and regulators to make informed assessments of PFAS use in modern society.

This Application Note describes a sensitive and reliable method for the simultaneous quantitation of 53 legacy and emerging PFAS from 14 compounds classes. The method uses isotope dilution on an Agilent 1200 Infinity II LC coupled to an Agilent 6495B triple quadrupole LC/MS.
Thank you

For more information please contact Dr Bradley Clarke on brad.clarke@unimelb.edu.au