Application of a Quality Scoring System for Assessing Per- and Polyfluoroalkyl Substances (PFAS) in Organic Solvents for *In Vitro* Toxicokinetic Testing

Marci G. Smeltz, M. Scott Clifton, Larry McMillan, Michael Devito, Barbara A. Wetmore

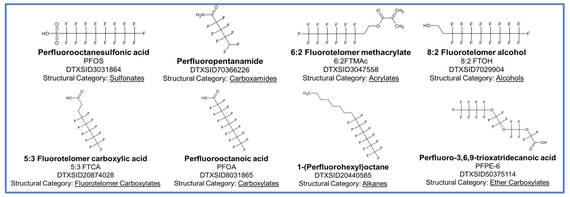
US Environmental Protection Agency, Office of Research and Development, Research Triangle Park, North Carolina

Marci Smeltz | smeltz.marci@epa.gov | 919-541-1064

www.epa.gov

Background

PFAS have become chemicals of concern for human health as more is learned about their widespread presence and persistence in the environment. This chemical class is very diverse, spanning over 4600 unique chemical structures with 1,220 PFAS listed in the Toxic Substances Control Act inventory. Many new approach methodologies are being employed to evaluate this large PFAS landscape. To ensure accurate examination of PFAS by *in vitro* strategies, the quality of the material being utilized needs to be evaluated in the organic solvent used for testing. This poster details an analytical workflow to provide a simple evaluation of each PFAS stock in either dimethyl sulfoxide (DMSO) or ethanol (EtOH) to inform inclusion for assessment in *in vitro* analyses. These scores then were applied in a range of *in vitro* experimental toxicokinetic assays, like determining PFAS bioaccumulative potential.



Key Takeaways

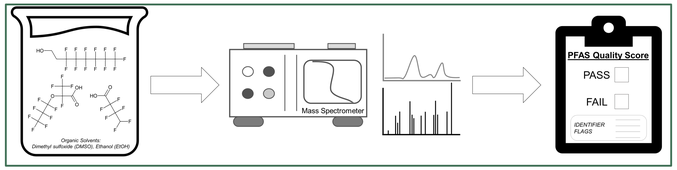
- The quality of PFAS stocks in either DMSO or ethanol was assessed through a simple analytical workflow by mass spectrometry.
  - 201 unique PFAS (a total of 467 PFAS-solvent stocks) were analyzed.
  - A pass or fail score was assigned with informational flags to advise scientists how to proceed with *in vitro* evaluation.
  - Some PFAS-solvent stocks received fail scores due to solvent degradation and/or failure to detect the analyte (i.e., predicted low boiling points).
- One application of this quality scoring workflow was to examine PFAS in DMSO for plasma protein binding, an indicator of bioaccumulative potential.
  - Plasma protein binding was determined through the ultracentrifugation approach.
  - More than 75% of tested PFAS exhibited high protein binding (F_u <0.05).
  - Carboxylate-containing PFAS displayed a large range in binding rates, where chain length influenced the degree of plasma protein binding.

REFERENCES

A. Brockman et al. *Journal of Medicinal Chemistry 58 (2015).*
K. Kieltyka et al. *Journal of Pharmaceutical Sciences 105 (2016).*
National PFAS Testing Strategy: Identification of Candidate Per- and Poly-fluoroalkyl Substances (PFAS) for Testing. USEPA *(2021).*
Patlewicz et al. Environmental Health Perspectives *127 (2019).*
RADAR: Understanding Sample Complexity and Improving Quantitative Data Quality. Waters White Paper *(720005033EN).*

U.S. Environmental Protection Agency
Office of Research and Development

PFAS Stock Quality Scoring System

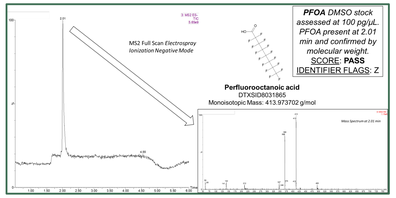


PFAS solubilized in organic solvents (dimethyl sulfoxide=DMSO, ethanol=EtOH) at a concentration between 5 and 30 mM were assessed by either liquid chromatography (LC) or gas chromatography (GC) mass spectrometry (MS) to provide a stock quality score by examining features of the mass spectrum for molecular weight matches, fragmentation patterns, and degradant presence.

201 unique PFAS were examined in either DMSO and/or EtOH.
70 unique PFAS in DMSO receiving passing scores by LC-MS, while 70 unique PFAS in DMSO met criteria to pass by GC-MS.

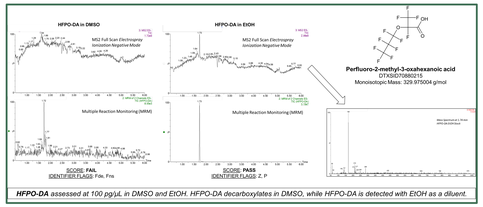
	LC-MS Assessed		GC-MS Assessed		
	PASS	FAIL	PASS	FAIL	*Total*
DMSO	119	6	108	100	*333*
EtOH	48	3	43	40	*134*
Total	*167*	*9*	*151*	*140*	*467*

Examples shown are PFAS compounds in either DMSO or EtOH diluted to 100 pg/µL. Analyses were performed by LC-MS with rapid switching between MRM (MS/MS) mode and MS full scan acquisition to quantify target compounds without losing sensitivity as well as monitoring for any interferences and impurities.

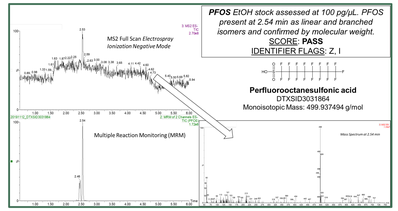


Quality Score	Description
PASS	Chemical detected with utilized analytical instrumentation
FAIL	Chemical NOT detected and/or significant degradation evident

Identifier Flag	Description
E	Parent analyte suspected to be present but cannot be guaranteed
F	Caution: Incorrect molecular weight, biological activity unreliable
Fde	Caution: Degradation of analyte evident
Fns	Caution: No sample detected, biological activity unreliable
I	Isomers (two or more isomers detected)
M	Defined mixture of two or more components
P	Pseudo-parent or adduct monitored; no direct confirmation of parent analyte presence
W	Sample withdrawn
Z	Molecular weight confirmed, no purity information available

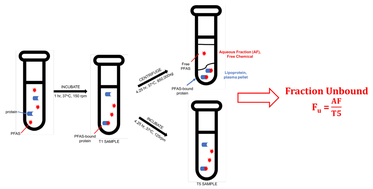


***HFPO-DA** assessed at 100 pg/µL in DMSO and EtOH. HFPO-DA decarboxylates in DMSO, while HFPO-DA is detected with EtOH as a diluent.*

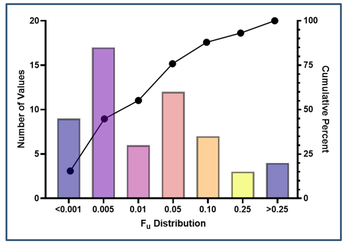


In Vitro Toxicokinetics Application: Plasma Protein Binding

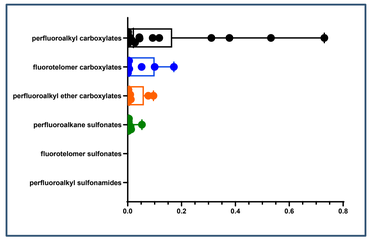
In vitro plasma protein binding (PPB) is a toxicokinetic property used to inform bioaccumulative potential. We assessed PFAS PPB by ultracentrifugation to calculate fraction unbound (F_u) of PFAS in human plasma, determining the free fraction of PFAS within the blood. Mixtures of up to 4 unique PFAS at 10 µM (using DMSO stock) were included within each plasma sample. Only PFAS that provided passing quality scores were pursued for this assessment.



Fraction Unbound Distribution

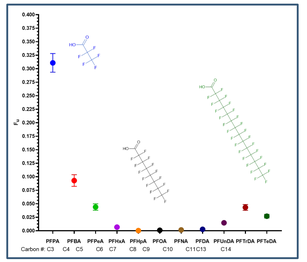


Fraction unbound distribution of PFAS assessed by LC-MS. 58 unique PFAS in DMSO were examined. Nearly 75% of these substances exhibited high plasma protein binding, with F_u values <0.05.

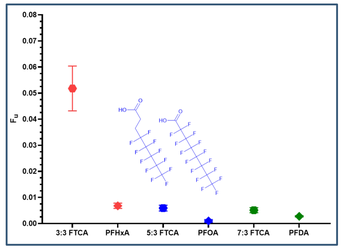


Category-based distribution of fraction unbound values for PFAS assessed by LC-MS. Carboxylate- and sulfonate-containing PFAS were divided into six unique groups based on specific structural features, where all values are shown.

Carboxylate-Containing PFAS Trends



Distribution of fraction unbound values for perfluoroalkyl carboxylate PFAS. Shorter-chain carboxylates (5 carbons or less) had less binding (higher F_u) compared to longer-chain carboxylates (6-10 carbons).



The substitution of hydrogen for fluorine (fluorotelomer carboxylates=FTCA) exhibited less plasma protein binding compared to their perfluoroalkyl carboxylate relatives.

Disclaimer: The views expressed in this poster are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.