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APPLICATION NOTE

Development of Analytical Methods for Monitoring Degradation Products of 6:2 Fluorotelomer Phosphates in Abiotic Matrices and WWTP Sludge

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INTRODUCTION

- 6:2 polyfluoroalkyl phosphates (6:2 PAPs) are derived from 6:2 fluorotelomer alcohol (6:2 FTOH).
- Biodegradation of 6:2 FTOH in aerobic and anaerobic waste water treatment plant (WWTP) sludge to form environmentally persistent perfluorocarboxylic acids (PFCAs) has been demonstrated in previous studies (Zhao et al, 2013, Zhang et al 2013).
- Reliable quantitative methods for direct measurement of 6:2 PAPs in environmental matrices have not yet been established.
- Monitoring 6:2 FTOH and other potential transformation products (e.g., PFCAs) in biodegradation studies of 6:2 PAPs under controlled abiotic and biotic conditions provides a direct measurement of the extent of PFCA formation from PAPs.

OBJECTIVE

Develop and demonstrate robust analytical methodologies for monitoring 6:2 FTOH and associated transformation products (analytes) following dosing of a 6:2 PAP fluorosurfactant product into abiotic and biotic degradation test systems.

MATERIALS AND METHODS

Internal Standard (IS)	Chemical Structure
M+4 6:2 FTOH	$^{13}C_2{}^{12}C_6D_2H_3F_{13}O$
M+3 PFPeA	$^{13}C_3{}^{12}C_2HF_9O_2$
M+2 PFHxA	$^{13}C_2{}^{12}C_4HF_{11}O_2$
M+4 PFHpA	$^{13}C_4{}^{12}C_3HF_{13}O_2$
M+2 6:2 FTCA	$^{13}C_2{}^{12}C_6H_3F_{13}O_2$
M+2 6:2 FTUCA	$^{13}C_2{}^{12}C_6H_2F_{12}O_2$

SOLVENTS AND REAGENTS

Methyl-t-butyl Ether (MTBE), Ethyl Acetate (EtOAc), Acetonitrile (ACN), Methanol (MeOH), Formic Acid, Hydrochloric Acid (HCl), Reagent Water, HPLC-grade "polished" of trace perfluorinated acids using an Oasis® HLB cartridge (35cc, 6g).

ABIOTIC METHOD

2X MTBE In-Vessel Extraction
- Shake 1st extract @ 240 rpm for 0.2 hr.
- Centrifuge @ 2500 xg for 10 min.
- Freeze @ <-70°C for 20-30 min.
- Decant MTBE extract and repeat
- Combine MTBE extracts

→ **GCMS:** 6:2 FTO, 1H-PFHx

Dilute 2 parts extract with 2:1 v/v MeOH:H2O + IS (M+4 6:2 FTOH and M+2 PFHxA)

→ **LC-MS/MS:** 6:2 FTOH and Acid analytes

Dilute 1 part residual water with 2:2 v/v MTBE:MeOH + IS

→ **LC-MS/MS:** 6:2 FTOH and Acid analytes

EtOAc Septa and Wall Extraction
- Remove residual sample
- Add EtOAc w/ 0.1% v/v formic acid to vessel.
- Manual shake for 60 sec.
- Sonicate for 5 min.
- Shake @ 35°C and 240 rpm for 16 hr.

→ **GCMS:** 6:2 FTO, 1H-PFHx

Dilute 2 parts extract with 2:1 v/v MeOH:H2O + IS

→ **LC-MS/MS:** 6:2 FTOH and Acid analytes

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GCMS

Model 7890A GC w/ 5975C Inert XL MSD

- **GC column:** Agilent GS-GasPro (30.0 m x 0.320 mm)
  Negative CI with methane – SIM mode
  Helium carrier gas
- **Ion Source and MSD Quad Temps**: 150°C
- **Transfer Line Temp:** 260°C
- **Liner:** Agilent 4 mm single taper (Ultra Inert)
- **Injector Temperature:** 140°C
- **Injection Volume:** 1.00 µL splitless

Oven Temperature Profile:

- **Initial temperature:** 120°C
- **Initial hold time:** 2.00 minute

Ramp Rate:		
(°C/minute)	Temperature (°C)	Hold Time (min.)
10	200	5
50	250	4

Run time: 20 minutes	
6:2 FTO	1H-PFHx
m/z 306 quantitation	m/z 250 quantitation
m/z 326 confirmation	m/z 319 confirmation

BIOTIC METHODS

Biotic extraction methods and LCMSMS parameters based on established conditions presented in the literature (e.g. Zhao et al 2013).

- ACN acidified w/ 50 mM HCl 1:1 v/v with sludge.
- Shake @ 35°C and 240 rpm for 16 hr.
- Centrifuge and filter.
- All six IS introduced in dilution solvent.
- C18 headspace trapping medium extracted with ACN, IS = M+4 6:2 FTOH.

RESULTS AND DISCUSSION

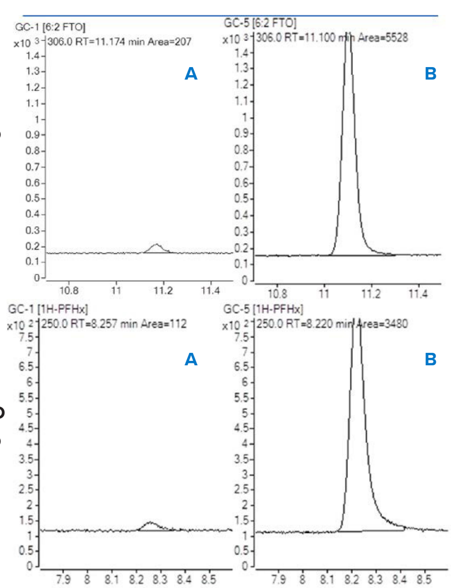
R^2

6:2 FTO STD
A – 2.00 ppb
B – 50.0 ppb

0.9994

1H-PFHx STD
A – 2.00 ppb
B – 50.0 ppb

0.9988



Abiotic (Hydrolysis, US EPA OPPTS 835. 2130)			
Analyte	Nominal Fortified Mass (ng a.i.)	Nominal Fortified Concentration (ng a.i./mL)	Mean Percent Recovery (± SD)
6:2 FTOH $C_6F_{13}C_2H_4OH$	600 6.00E+03 8.40E+03	33 328 459	126 ± 14 96.6 ± 3.3 92.9 ± 4.5
PFHxA $C_5F_{11}COOH$	16.0 160 336	0.87 8.7 18	94.5 ± 7.5 87.0 ± 3.1 84.4 ± 4.7
PFHpA $C_6F_{13}COOH$	16.0 160 336	0.87 8.7 18	82.3 ± 4.2 77.2 ± 5.5 83.3 ± 3.4
6:2 FTCA $C_6F_{13}CH_2COOH$	8.00 80.0 168	0.44 4.4 9.2	99.0 ± 9.0 76.0 ± 3.3 76.8 ± 3.3
6:2 FTUCA $C_5F_{11}CF{=}CHCOOH$	4.00 40.0 84.0	0.22 2.2 4.6	106 ± 6 81.0 ± 5.0 88.7 ± 3.1
6:2 FTO $C_6F_{13}CH{=}CH_2$	150 750 1.80E+03	8.2 41 98	107 ± 5 98.9 ± 3.0 106 ± 6

Biotic (OECD 311)		
Analyte	Nominal Fortified Concentration (ng a.i./mL)	Mean Percent Recovery (± SD)
6:2 FTOH $C_6F_{13}C_2H_4OH$	49.5 178 400	96.1 ± 5.5 83.0 ± 3.7 78.5 ± 5.9
PFPeA C_4F_9COOH	0.990 4.46 29.7	114 ± 9 110 ± 8 98.9 ± 6.4
PFHxA $C_5F_{11}COOH$	0.990 4.46 29.7	107 ± 3 103 ± 2 93.0 ± 4.0
PFHpA $C_6F_{13}COOH$	0.990 4.46 29.7	98.0 ± 0.9 97.1 ± 3.4 85.9 ± 4.9
6:2 FTCA $C_6F_{13}CH_2COOH$	0.495 2.23 14.9	98.4 ± 5.4 97.9 ± 6.4 97.9 ± 10.6
6:2 FTUCA $C_5F_{11}CF{=}CHCOOH$	5.20 10.0 37.1	96.0 ± 2.4 97.5 ± 5.8 96.4 ± 5.1
5:2 sFTOH $C_5F_{11}CH(OH)CH_3$	49.5 178 990	85.3 ± 5.5 80.5 ± 2.6 102 ± 2
5:3 Acid $C_5F_{11}C_2H_4COOH$	39.9 72.5 245	93.9 ± 5.2 95.5 ± 5.3 95.8 ± 6.9

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CONCLUSIONS

- Methods have been verified for monitoring transient and persistent transformation products of 6:2 PAPs in both abiotic and biotic environmental matrices.
- Novel analytical methodology has been developed to enable trace level determination of 6:2 FTO and 1H-PFHx in aqueous matrices.
- The hydrophobic, volatile and adsorptive properties of 6:2 FTO and 1H-PFHx require unique strategies for containment and quantitative analysis.

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