EPA Method 533: PFAS in Drinking Water

Scott Krepich
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Per- and polyfluoroalkyl substances (PFAS) are human-made chemicals in aqueous film forming foam (AFFF) which is used to suppress jet fuel fires and is routinely utilized on airbases. They are also useful in a variety of consumer products such as surface treatment for textiles, packaging materials, and non-stick cookware. They are also highly stable, bioaccumulate, referred to today in the media as forever chemicals, and they have become ubiquitous throughout the global environment.

The EPA has established health advisory levels at 70 parts per trillion (ppt) perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in drinking water to account for an expected lifetime of exposure.

While these chemicals have been studied for many decades, PFOA replacement compounds, such as GenX and metabolites, are also spreading throughout the environment while their toxicology studies are still being characterized. The 2019 EPA PFAS Action Plan is committed to establishing requirements, including safe drinking water and characterizing the most commonly known PFAS among the thousands that have been identified.

Overview

EPA Method 533 supports monitoring of applications that include the analysis of multiple short-chain PFAS that cannot be measured by Method 537.1, by utilizing a weak anion-exchange mechanism in the sample extraction and preparation, improving the recovery of the short-chained PFAS that do not retain well in a strictly reversed phase mechanism.

This technical brief outlines the solid phase extraction (SPE) and liquid chromatography (HPLC) parameters included in the isotope dilution anion-exchange SPE liquid chromatography / tandem mass spectrometry (LC-MS/MS) procedure detailed in the EPA Method 533.

Experimental Conditions

Sample Preparation:

A 100-250 mL sample is fortified with isotopically labeled analogues of the method analytes, and then sample is passed through a solid phase extraction (SPE) cartridge with a polystyrene divinylbenzene backbone functionalized with diamino ligands (Strata®-X-AW, part no. 8B-S038-HCH). The cartridge is rinsed with aqueous ammonium acetate followed by methanol, and then the analytes are eluted from the SPE sorbent with ammonium hydroxide in methanol solvent. After drying the eluent with nitrogen in a heated water bath, adjust the final volume to 1 mL with 20 % water in methanol (v/v) before analyzing by LC-MS/MS.

HPLC Parameters

Column: Gemini® 3 µm C18
Dimension: 50 x 2.0 mm
Part No.: 00B-4439-B0
Mobile Phase: A: 20 mM Ammonium Acetate
B: Methanol
Injection Volume: 10 µL
Detection: Electrospray Ionization Tandem Mass Spectrometer (ESI-MS/MS)
Flow Rate: 0.25 mL/min
Gradient:

Time (min)	%A	%B
0	95	5
0.5	95	5
3	60	40
16	20	80
18	20	80
20	5	95
22	5	95
25	95	5
35	95	5

Analytes and Retention Times

Isotopically Labeled Isotope Performance Standards and Retention Times

Isotope Performance Standard	RT (min)
$^{13}C_3$-PFBA	4.14
$^{13}C_2$-PFOA	12.19
$^{13}C_4$-PFOS	13.73

Isotope Dilution Analogues: RTs and Suggested Isotope Performance Standard References

Isotopically Labeled Analyte	RT (min)	Suggested Isotope Performance Standard
$^{13}C^4$-PFBA	4.14	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	6.13	$^{13}C_3$-PFBA
$^{13}C_3$-PFBS	6.62	$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	8.12	$^{13}C_4$-PFOS
$^{13}C_5$-PFHxA	8.35	$^{13}C_2$-PFOA
$^{13}C_3$-HFPO-DA	9.06	$^{13}C_2$-PFOA
$^{13}C_4$-PFHpA	10.34	$^{13}C_2$-PFOA
$^{13}C_3$-PFHxS	10.61	$^{13}C_4$-PFOS
$^{13}C_2$-6:2FTS	12.05	$^{13}C_4$-PFOS
$^{13}C_8$-PFOA	12.19	$^{13}C_2$-PFOA
$^{13}C_9$-PFNA	13.70	$^{13}C_2$-PFOA
$^{13}C_8$-PFOS	13.73	$^{13}C_4$-PFOS
$^{13}C_2$-8:2FTS	14.94	$^{13}C_4$-PFOS
$^{13}C_6$-PFDA	15.00	$^{13}C_2$-PFOA
$^{13}C_7$-PFUnA	16.14	$^{13}C_2$-PFOA
$^{13}C_2$-PFDoA	17.13	$^{13}C_2$-PFOA

Method Analytes, Retention Times and Suggested Isotope Dilution Analogue References

Analyte	RT (min)	Isotope Dilution Analogue
PFBA	4.15	$_{13}C^{4}$-PFBA
PFMPA	4.84	$_{13}C^{4}$-PFBA
PFPeA	6.13	$_{13}C^{5}$-PFPeA
PFBS	6.62	$_{13}C^{3}$-PFBS
PFMBA	6.81	$_{13}C^{5}$-PFPeA
PFEESA	7.53	$_{13}C^{3}$-PFBS
NFDHA	8.01	$_{13}C^{5}$-PFHxA
4:2FTS	8.12	$_{13}C^{2}$-4:2FTS
PFHxA	8.36	$_{13}C^{5}$-PFHxA
PFPeS	8.69	$_{13}C^{3}$-PFHxS
HFPO-DA	9.06	$_{13}C^{3}$-HFPO-DA
PFHpA	10.42	$_{13}C^{4}$-PFHpA
PFHxS	10.62	$_{13}C^{3}$-PFHxS
ADONA	10.73	$_{13}C^{4}$-PFHpA
6:2FTS	12.04	$_{13}C^{2}$-6:2FTS
PFOA	12.19	$_{13}C^{8}$-PFOA
PFHpS	12.28	$_{13}C^{8}$-PFOS
PFNA	13.70	$_{13}C^{9}$-PFNA
PFOS	13.74	$_{13}C^{8}$-PFOS
9Cl-PF3ONS	14.53	$_{13}C^{8}$-PFOS
8:2 FTS	14.94	$_{13}C^{2}$-8:2FTS
PFDA	15.00	$_{13}C^{6}$-PFDA
PFUnA	16.14	$_{13}C^{7}$-PFUnA
11Cl-PF3OUdS	16.70	$_{13}C^{8}$-PFOS
PFDoA	17.13	$_{13}C^{2}$-PFDoA

Reference

EPA Method 533 ‘ Determination of Per- and Polyfluoroalkyl Substances in Drinking Water By Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography / Tandem Mass Spectrometry’ (2019) https://www.epa.gov/sites/production/files/2019-12/documents/method-533-815b19020.pdf

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/LiveChat.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com