A Rapid Derivatization for Quantitation of Perfluorinated Carboxylic Acids from Aqueous Matrices by Gas Chromatography-Mass Spectrometry

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ABSTRACT: Ultrashort chain perfluorocarboxylic acids (PFCAs) are receiving more attention due to their ever-increasing presence in the environment. Methods have been established for the analysis of short and long-chain PFCAs, while robust quantitation of ultrashort chain species are scarce. Here we develop a novel derivatization method using diphenyl diazomethane for quantitation of C2-C14 PFCAs in aqueous matrices. The method is highlighted by rapid completion of derivatization (<1 min), and retention and separation of ultrashort chain (C2/C3) PFCA derivatives using H_2 carrier gas (R > 1.5). A weak anion solid phase extraction procedure for analyte recovery from representative aqueous samples was developed and validated by spike and recovery from ultrapure water, synthetic ocean water, and simulated denuder extracts used for collecting gaseous PFCAs. Recoveries for C2-C9 PFCAs ranged from 83-130 % for the majority of analytes and matrices. The instrument detection limits (IDLs) range from 8-220 fg per injection, and method detection limits (MDLs) range from 0.06-14.6 pg/mL for 500 mL aqueous samples, which are within an order of magnitude to conventional LC-MS/MS methods. The method was applied to the analysis of real samples of tap water, rainwater, ocean water, and annular denuder extracts. The overall method provides a cost-effective alternative to conventional LC-MS/MS methods, overcoming the typical GC-MS drawbacks of high detection limits and long sample preparation times, while being able to simultaneously analyze the complete spectrum of environmentally relevant PFCAs.

Introduction

Perfluorocarboxylic acids (PFCAs) are an important subgroup of the large class of chemicals known as poly- and perfluoroalkyl substances (PFAS). PFCAs are fully fluorinated aliphatic compounds with a terminal carboxylate group.[1] Due to strong carbon-fluorine bonds, PFCAs persist near indefinitely in the environment. There are many sources that contribute to an increasing environmental burden of PFCAs. Apart from direct emissions, PFCAs are the final degradation products of many PFAS.[2] Within this, there is recent recognition that some CFC replacements can degrade into ultrashort chain PFCAs (C2-C4).[2,3] Exposure to PFCAs has been linked to immunotoxicity, growth suppression and developmental toxicity across species from algae to mammals.[4] Long chain (C8 and longer) PFCAs are known to be bioaccumulative.[5] While there appears to be a consensus in the PFAS community that short chain (C2-C7) PFCAs do not bioaccumulate,[6,7] there are increasing reports of apparent accumulation of ultrashort and short-chain PFCAs in biota.[8,9] Due to their persistent and mobile nature,[10] all PFCAs are of environmental concern, no matter their carbon chain-length. Given their low pKa values,[6] PFCAs in the environment reside mostly in aqueous media, which is targeted by this work.

Recently, ultrashort chain PFCAs have received greater interest due to their disproportionately high environmental loadings in comparison to other PFCA homologues.[11] The following analytical challenges remain and, as a result, the concentrations of the ultrashort chain compounds are scarcely reported in all environmental matrices.[12] Current methods by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) measure long-chain (>C8) and/or short-chain PFCAs C4 or longer,[13] while recovery and retention issues prevent robust ultrashort chain determinations. Notably, ultrashort chain PFCAs have been analyzed only by mixed mode LC twice. While the initial development of this method demonstrated the ability to simultaneously analyze C2-C18 PFCAs,[14] its application has been limited to C2-C8 PFCAs.[15] To expand future PFAS research and to address global contamination and mobility, there is a need for sensitive, rapid, and easily accessible analytical methods that are inclusive of ultrashort chain and traditionally measured PFCAs in one analysis.

Within existing methodologies, LC-MS/MS is the most popular choice for PFCA analysis, particularly for aqueous matrices because of the option to do direct injection. If sample cleanup and/or preconcentration using weak anion exchange (WAX) solid phase extraction (SPE) is required regardless of instrumentation, GC-MS methods have the potential to be as efficient as LC-MS/MS, with the additional benefit of being more affordable, and therefore having greater reach. Analysis of PFCAs by GC-MS requires a derivatization step to functionalize the highly polar carboxylic acid group and the technique was commonly used before the advent of LC-MS/MS. There are two general themes for existing PFCA derivatizations: alkyl and phenyl. A few examples of alkyl derivatives found in early methods are methyl esters,[16,17] propyl esters,[18] or butyl esters.[19] While liquid injections are employed for methyl esters of long chain PFCAs,[16] the methyl ester of C2 PFCA is extremely volatile, requiring headspace analysis.[17] Products of silyl derivatization, such as trimethyl silyl,[20] are limited by the same volatility issue. While volatility is less of a concern for other alkyl derivatives,[18,19] they still have minimal

intermolecular interactions imparted to facilitate selectivity in chromatographic separation. The more recent approach of phenyl derivatization methods such as benzyl,[21] pentafluorobenzyl[22] or 2,4 difluoroaniline[23] address the selectivity issue by introducing potential for phenyl-phenyl interactions between the derivatives and stationary phases for the otherwise inert fluoroalkyl chains. To date, only the 2,4-difluoroaniline derivatization has demonstrated the ability to separate and quantify C2 and C3 PFCAs, which requires a complex extraction process.[24]

In this study, we developed a novel derivatization method for the gas chromatography-electron capture negative ionization-mass spectrometry (GC-ECNI-MS) analysis of ultra-short through long chain PFCAs (C2-C14) in aqueous matrices relevant to a number of environmental sample types. The novel derivatization agent diphenyl diazomethane (DDM) reacts with PFCAs forming diphenyl methyl PFCA esters, which are extensively optimized for baseline separation and quantification by GC-MS. The method applicability is expanded through optimized matrix-matched spike and recovery of the analytes by WAX-SPE, or standard addition, which were applied to the analysis of real aqueous samples. This method, paired with mass-labeled surrogate and internal standards, is timely in light of recent increased interest in environmental measurements of the ultrashort chain PFCAs.[3,8,11]

Experimental Section

Reagents and Materials.

For nomenclature simplicity, perfluorocarboxylic acids (PFCAs) are referred to by their carbon chain length (Table S1). A detailed description of chemicals and reagents can also be found in Section S1 of the Supporting Information (SI). Briefly, individual C2-C14 PFCAs were obtained from several vendors. C13 could not be purchased due to backorder but was in our commercial mixed standard. Mass labeled $^{13}C_2$-C2 (>97%) was purchased from Toronto Research Chemicals; $^{13}C_3$-C4, $^{13}C_2$-C8, and $^{13}C_2$-C10 were purchased as a mixture in MeOH (2 µg/mL; MPFAC-C-IS) from Wellington Laboratories. These compounds were combined into one methanol (MeOH) solution and used as internal standards (IS).

Gas Chromatograph-Mass Spectrometer

An Agilent 7890A Gas Chromatograph (GC; CA, US) coupled to a 5975c Mass Spectrometer (MS) was used with a CTC Combi PAL autosampler, along with a hydrogen (H_2) generator (Model QL-500A, Shandong Saikesaisi Hydrogen Energy Co. Ltd, Shandong, China). The generator produces high purity H_2 (>99.999%) by electrolyzing deionized water. The autosampler was equipped with a 10 µL syringe to make 1 µL pulsed (25 psig) splitless injections. Three different columns from Agilent with varying dimensions and degrees of phenyl selectivity were explored: a DB-5 (5% phenyl methyl-polysiloxane, 0.25 µm x 0.25 mm x 15 m), a DB-17 (50% phenyl methyl-polysiloxane, 0.25 µm x 0.25 mm x 15 m), and an HP-5MS column (5% phenyl methyl-polysiloxane, 0.18 µm x 0.18 mm x 20 m). Separation temperature programming and mobile phase properties were explored on each column, to reach a final optimized method (see Separation Optimization). The inlet and MS transfer line temperatures were 240 °C and 250 °C, respectively. The MS was operated in ECNI mode with methane as the reagent gas, with a measured mass range up to 1050 amu, and selected ion monitoring (SIM). The source and quadrupole were operated at 150 °C. The remaining MS parameters were calibrated by MSD ChemStation (Version E.02.00.493) using perfluorotributylamine.

Derivatization

The DDM 0.1 M solution was prepared by dissolving solid DDM in dichloromethane (DCM). Ten microliters of the DDM solution were added to each calibration standard or sample and diluted or reconstituted to 1 mL with DCM or 1:1 ethyl acetate (EtAc)/DCM. Four solvents (DCM, Toluene, EtAc, MeOH) for dilution/reconstitution were investigated for solubility limitations and to optimize sensitivity. Derivatization completion time was determined, as well as potential interferents.

Solid Phase Extraction

The pH of aqueous samples (matrix-matched spike and recovery or real samples) was adjusted to 3-4 with 6 M HCl. Strata X-AW cartridges (6 mL x 200 mg x 33 µm; Phenomenex, CA, US) were conditioned with 4 mL of 0.1 M NH_4OH in MeOH, MeOH, and Milli-Q water in succession. The samples were loaded at a rate of 1-2 drops per second at -10 inHg vacuum. The cartridges were washed with 4 mL MeOH and vacuum dried for 1 min. The target PFCAs were eluted with 4 mL 0.1 M NH_4OH in MeOH into a 15 mL round bottom centrifuge tube (Sarstedt, Nümbrecht, Germany), and 10 ng of IS to account for evaporative losses was spiked into this eluate.

The eluates were blown down to dryness with a gentle stream of N_2 controlled at 30 °C with a VWR® standard dry block heater. Immediately, 500 µL each of EtAc and DCM along with 10 µL of 0.1 M DDM were added to the tube and vortexed for 10 seconds. The tube was then sonicated for 15 min at room temperature, and vortexed again for 10 seconds. The sonication and vortexing were repeated a second time, and the resulting extract was decanted into an autosampler vial for analysis.

Matrix Spike and Recovery

Matrix recoveries were assessed for 50 ng spikes of C2-C14 (excluding C13, see above) into 200 mL Milli-Q water, 200 mL synthetic ocean water (35 g of Instant Ocean® sea salt per 1 L Milli-Q water), and 10 mL simulated denuder extract (0.2 % w/w sodium carbonate, 0.1% w/w glycerol in Milli-Q water).

Real Samples

A detailed description can be found in Section S2. Briefly, tap water was collected in July 2022, from the building water supply in our laboratory. Precipitation was collected from a rooftop in Toronto, ON, Canada from Dec 2019 to Feb 2020. Annular denuders sampled

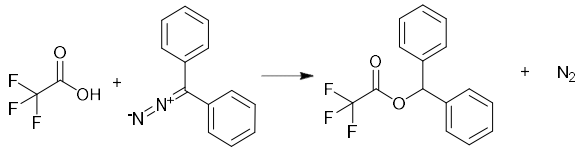
acids in air from the same rooftop lab from Sept to Oct 2021. Denuders were extracted with 10 mL Milli-Q water and stored at 4 °C until analysis. Ocean water samples were collected from the St. Lawrence Estuary in Aug 2020 at Tadoussac, Les Escoumins, and Forestville, QC, Canada. Quantitative analysis on tap water, precipitation, and denuders used spike and recovery correction, while the ocean water utilized standard addition.

Results and Discussion

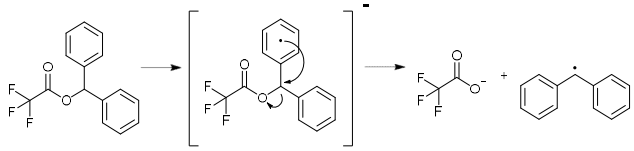
Derivatization

a. Reaction Pathways

Pentafluorophenyl diazoalkane derivatization for GC-MS analysis of carboxylic acids has been proposed and utilized in the past.[25,26] We have taken inspiration and built upon this to develop a novel derivatization product with a diphenyl group for greater selectivity in GC separation generated with reduced reliance on fluorinated reagents. The derivatization reaction (Scheme 1) is very clean, with N_2 being the only product. The ionization of the PFCA derivatives is shown in Scheme 2 with the fragmentation spectrum of a standard solution of C2/C3 presented in Section S3, Figure S1. Briefly, PFCA diphenyl methyl esters are formed, which then undergo dissociative electron capture ionization in the MS, yielding the PFCA carboxylate anions ($C_xF_{2x+1}CO_2^-$). In general, this is the most abundant ion detected for all PFCA derivatives, which is used for quantitation.



Scheme 1. Derivatization reaction: esterification of C2 by DDM



Scheme 2. Dominant electron capture ionization pathway of the C2 diphenyl methyl ester, adapted from Hofmann et al.[26]

Two major side products: benzophenone, and a dimer of DDM, were identified in derivatized samples (Figure S2). Benzophenone is formed in the GC inlet via high temperature oxidation (Scheme S1). The tetraphenyl dimer results from the continual degradation of DDM as it ages, likely due to photodimerization[27] (Scheme S2). This is evident from observing larger peak areas of the dimer when using an older DDM that had not been well protected from light (Figure S2a, S2b). The dimer is strongly retained on all columns, limiting runtime, and is addressed below in Separation Optimization.

b. Dilution Solvents and Derivatization Time Trends

The polar head group of PFCAs, with both hydrogen bond donating and accepting capabilities, means they dissolve well in many polar organic solvents. When the polar head is transformed by derivatization, the resulting molecule (Scheme 1) becomes highly non-polar. Therefore, four solvents (DCM, Toluene, EtAc, MeOH) were tested for the derivatization of ~1000 ng/mL C2-C14 PFCA solutions. The peak area vs time of the C2 PFCA homologue is shown in Figure 1, which is representative of the relative peak area trend for all other PFCAs homologues.

DCM was determined to be the optimal solvent as the signal obtained was >20 % higher than observed for toluene, which was the next best solvent option. To use toluene as an environmentally friendly alternative solvent to DCM, the starting temperature must be raised to 110 °C (Figure S3a) to prevent the condensation of toluene on column, or else it will produce a partial solvent trapping effect (Figure S3b). Peak tailing of C2-C6 was observed in addition to reduced resolution of C2/C3 when using toluene, therefore higher detection limits for C2-C6 would be expected.

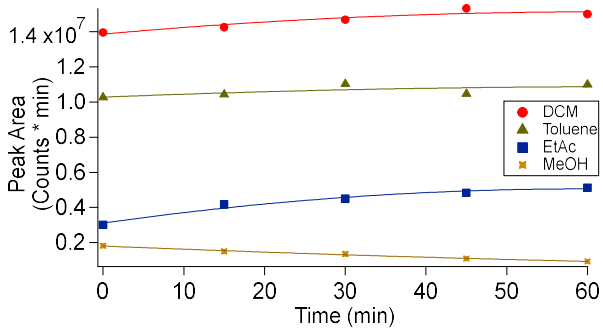


Figure 1. Peak area response measured for detected analytes after derivatization in four dilution solvents at 15-minute intervals during the first hour post-reaction at room temperature. The presented trends are for C2 PFCA at ~1000 ng/mL with symbols denoting the peak area observed in each of the dilution solvents (see Figure S4 for other homologues).

The low response from the use of EtAc suggests poor solubility of the PFCA derivative. In general, the derivatization reaction was found to reach completion upon addition of the DDM, four inversions of the container to mix, followed by injection on the GC. The derivative was observed to remain stable for at least 2 days in DCM (Section S3, Figures S4-S5).

This result contrasts with previous findings of Hofmann et al.[26] in their derivatization of docosanoic acid with pentafluorophenyl diazoethane in benzene, which required 10 hours to complete at room temperature. This discrepancy may be due to a higher reactivity of PFCAs from their stronger acidity, or a solubility issue of docosanoic acid in benzene which limits reaction rate. The chain-length of docosanoic acid may also be a rate reducing factor.

c. Solid Phase Extraction Considerations

The low response and decreasing trend with increasing reaction time in MeOH suggests that the derivatives may degrade over time (Figure 1). This loss creates a need in the derivatization methodology to minimize/eliminate MeOH prior to reaction in order to achieve reproducible quantitation. Since MeOH is a very common solvent for commercial PFCA standards that primarily target LC-based methodologies, the inclusion of internal standards (IS; sold as mixtures in methanol) after SPE to track evaporative losses or for preparation of IS-normalized calibrations required additional methodological innovations. If surrogates are used, then SPE would be required to prepare calibration curves.

To include the use of IS for method calibrations, it was spiked into a representative 0.1 M NH_4OH in MeOH SPE sample extract, which was subsequently evaporated to dryness and reconstituted in 1:1 EtAc:DCM. Although this causes the standard preparation to be more laborious, there are significant benefits. The biggest advantage is correction for losses occurring during the N_2 evaporation step (Section S4, Table S2-4), which has been reported across analytical methodologies even when the extract is not evaporated to dryness.[23] In addition, allowing the standards to procedurally mirror more of the sample preparation provides better methodological error control during sample preparation.

Derivatization of the analytes following SPE and N_2 evaporation can be compromised by the presence of interfering compounds that may react with DDM. We have considered the following interferents of concern: MeOH, ammonium salts, and common organic acids. Measures were taken to isolate PFCAs from these interferents. First, MeOH was fully removed during the N_2 evaporation, which precipitates a variety of ammonium salts that vary depending on the sample matrix composition. Second, the ammonium salts include those of the PFCA analytes. As a result, 500 µL each EtAc and DCM were added to the precipitate along with 10 µL DDM to recover and derivatize the PFCAs. The EtAc is necessary to first bring the PFCAs into solution, which are subsequently derivatized, followed by the products partitioning to DCM. The inorganic ammonium salts of other anions were observed to remain as opaque precipitates. No loss of the characteristic pink color of DDM was observed from the derivatized standard or sample for at least 2 days when in contact with these, indicating that the inorganic salts remain inert for the 30 minutes of solution contact before decanting. Last, common organic acids such as formic or acetic acid may be present in the sample at orders of magnitude greater quantities than the target PFCAs.[28] Since environmentally prevalent organic acids are almost entirely weak acids with pKa values of ~4, by adjusting the sample pH to 3-4, 50-90% of the weak acids can be converted to their neutral form, to be subsequently removed by the MeOH wash step during SPE procedure.

d. Comparison to Existing Derivatization Methods

A comprehensive summary of derivatization methods for PFCA analysis is provided in Section S5, Table S5. Most methods require more than one reagent, as well as 1 hr of derivitization at elevated temperatures. In comparison, this work requires one reagent (DDM) and only 30 minutes of sonication to extract PFCAs into solution from an SPE extract solid residue, where the derivatization reaction reaches completion rapidly. Montelone et al.[18] and Liu et al.[19] utilized solid phase microextraction and dispersive liquid-liquid microextraction on small volumes (600 µL and 10 mL) of river water to derivatize C6-C12 and C7-C10 into propyl and butyl esters with detection limits of 0.08-6.6 pg/mL and 37-51 pg/mL. However, the application of the derivatization to shorter chain PFCAs and/or in more complex aqueous matrices was not explored. The pentafluorophenyl diazoethane derivatization reported by Frank et al.[25] is the closest comparison to the method developed in this work. The derivatization reaction should in theory also complete immediately for PFCAs, but was not reported. As a result, the DDM derivatization used here demonstrates superior speed and easy sample preparation for C2-C14, making substantial progress for GC-MS PFCA analysis.

Separation Optimization

A good separation is important to enable reliable quantification when using a non-specific detector. Although not necessary when using mass spectrometry, good separation of all analytes can reduce the impact of potential matrix effects on analyte ionization as much as possible. The extent of separation of two peaks in a chromatogram can be measured by their resolution, which is influenced by key parameters shown in the following equation:

$$R = \frac{1}{4}\sqrt{N}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k'}{1+k'}\right) \quad (1)$$

which describes the relationship of resolution (R) to plate number (N), selectivity factor (α), and retention factor (k'). A resolution of 1.5 between two gaussian peaks of the same dimensions corresponds to peak overlap of 0.15%, which constitutes a ±3σ or 99.7% baseline separation.

The primary rational for the use of DDM in this work was to increase resolving power in the class of phenyl PFCA derivatizations, which is particularly important to target ultrashort chain PFCAs. Hu et al.[24] demonstrated the ability to separate C2/C3 using 2,4-difluoroaniline derivatization with a DB-17 column, indicating the efficacy of phenyl interactions for the two most challenging analytes in our suite. In the new approach using DDM, two phenyls are present in each derivative to further exploit phenyl-phenyl interactions for separation purposes, while not introducing additional fluorine atoms in consideration of environmental impact.

Initial separations were performed on the DB-5 column with He carrier gas at 1 mL/min. With a generic temperature program starting at 80 °C, ramped at 15 °C/min to

125 °C, then 30 °C/min to 260 °C, baseline separation of C4-C14 derivatives was easily achieved. While the C2/C3 derivatives were well-retained, they were also observed to coelute (Figure 2, k' = 7.32). Modifications to the temperature program were explored, such as lowering the initial temperature, and reducing the slope of the temperature ramp. Neither achieved improvement in selectivity (α=1.004-1.008) between this analyte pair.

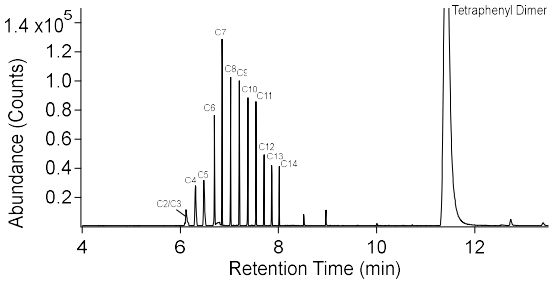


Figure 2. SIM chromatogram of separation with the DB-5 column and He carrier gas with a zoomed-in view of (a) the separations of C2/C3. Program: Begin 80 °C for 0.5 min, 10 °C/min to 120 °C, 30 °C/min to 270 °C, hold 4 min.

From these initial trials, it was apparent that an improved selectivity was needed for C2/C3. In addition, a large matrix peak was observed that eluted well after the analytes (t_r= 11.5 min, 3.5 min after C14 derivative elutes) that limited the overall separation time for the method (i.e., the method must be long enough to fully elute this matrix peak). This matrix peak was identified to be the tetraphenyl dimer described above (Scheme S2). This reaction side product requires elution off the column at a high temperature (270 °C held for 2 min) at the end of each run to prevent on-column accumulation and ensure chemical integrity of the stationary phase.

To increase selectivity, the DB-17 with 50% phenyl content was explored (Section S6, Figure S6) to promote additional phenyl-phenyl interactions, similar to the work from Hu et al..[24] However, the retention of the tetraphenyl dimer on the column was found to be irreversible and thus, this stationary phase was not pursued further.

Altering the carrier gas to H_2 was explored to improve resolution through improved selectivity using an HP-5MS column. The narrower diameter (0.18 mm vs 0.25 mm) and thinner film (0.18 μm vs 0.25 μm) of the column compliments H_2 due to its lower viscosity and higher diffusivity compared to He. It was also anticipated to provide a better separation efficiency by reducing the diffusion and mass transfer induced band broadening of the analytes. The C4-C14 homologues were baseline separated as expected, while C2/C3 required further optimization. To achieve this, a series of pseudo-isothermal holds were employed using a two-variable template: begin at 65 °C and hold 0.5 min, ramp 30 °C/min to *x* °C and hold *y* mins, then ramp 35 °C/min to 270 °C and hold 2.5 mins to complete the run. The H_2 flowrate was kept constant at 1 mL/min (Figure 3).

Each variable was optimized for highest resolution while keeping the other variable constant. The optimal temperature *x* was found to be 130 °C and the optimal hold time y was found to be 3.5 min, with a maximum resolution of 1.75 for the C2/C3 analyte pair. Since a volumetric flow of 1 mL/min corresponds to a linear velocity for H_2 of ~66 cm/s, the separation efficiency could theoretically be improved further by reduction of the flowrate toward the optimal linear velocity, minimizing plate height for H_2 separations (~45 cm/s). Regardless, a resolution of 1.5 is sufficient separation for quantitative analysis and we applied a shorter hold time of 2.5 min in the final temperature program to obtain the shortest total runtime (Figure 4, 11.7 minutes).

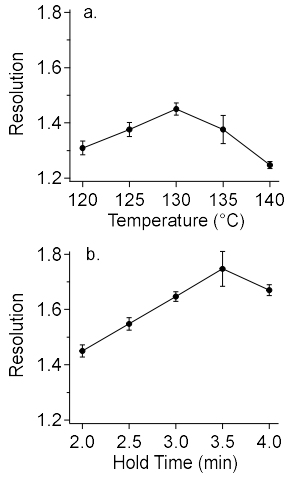


Figure 3. Resolution between C2/C3 derivatives at H_2 flow rates of 1 mL/min through the optimization of (a) holding temperature with a constant hold time of 2 min and (b) hold time at a constant temperature of 130 °C. Error bars are one standard deviation of replicates (n=3).

As a result, the optimized separation conditions for the PFCA-DDM derivatives are: H_2 flowrate of 1 mL/min, temperature program: begin at 65 °C and hold 0.5 min, ramp 30 °C/min to 130 °C and hold 2.5 mins, then ramp 35 °C/min to 270 °C and hold 2 mins before returning to initial conditions. The total run time is 11.7 min, or ~15.5 min from injection to injection with the autosampler used in this work. The SIM chromatograms of a denuder matrix spike and a real denuder sample in comparison to a standard (Figure 4) show clean analyte separations without interference from the sample matrix, showcasing the selectivity of the diphenyl group. For comparison, separation was also optimized using He carrier gas with the DB-5 column, which is more commonly available. The optimal resolution achieved between C2/C3 was 1.15. A detailed investigation of the optimization through parameters of temperature, flow rate, and hold times were systematically explored (Figure S7), yet baseline separation was not possible.

This work is the second chromatographic method that demonstrates the baseline separation of ultrashort chain C2/C3 (derivatives) in under 10 min, and the first

GC-MS method to simultaneously separate and analyze the full suite (C2-C14) of PFCAs. The strong retention of C2/C3 minimizes matrix effects from early eluting components, a common issue for even C4 in LC-MS/MS methods.[29] In comparison, a greater separation of C2/C3 2,4-difluoroanilide derivatives with DB-17 is possible (R>2.5)[24]. However, the derivatization procedure is arduous, and the DB-17 stationary phase may have a shorter lifetime from the irreversible retention of phenyl matrix components, such as unexpected side products like those reported here (Figure S2).

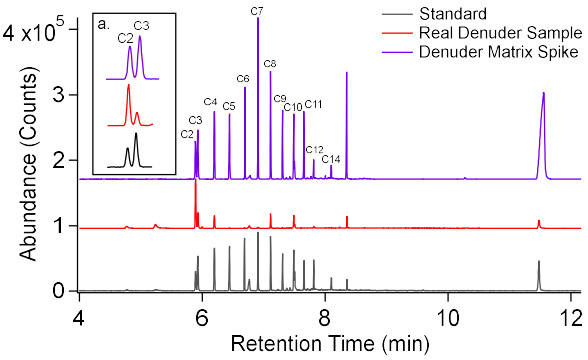


Figure 4. Optimized SIM chromatograms with offset baselines for the separation of a standard solution (black, signal x 5), a real denuder sample (red) and a denuder matrix spike (purple) for C2-C14 PFCA derivatives (no C13). The inset (a) shows the separation performance for C2 and C3.

We argue that a 3σ (R = 1.5) separation of this work is sufficient for quantitative analysis and has substantial increase in throughput via the simplified sample preparation.

Quality Control

A summary of quality control parameters is reported in Table 1, and a detailed description can be found under Section S7 in the SI. The instrumental detection limits (IDLs) of C2-C14 range from 8-220 fg on column, with good repeatability of 1.2-4.1%. Method detection limits (MDL) range from 0.06-0.84 pg/mL for C4-C14, while MDL for C2/C3 are 14.6 and 1.11 pg/mL respectively. The higher MDLs of C2/C3 here are a result of high levels of systematic contamination caused by use of these chemicals for experiments in our laboratory and may be lower in other laboratories. For comparison, MDLs estimated from the calibration curve for C2/C3 are 0.04 and 0.03 pg/mL. Despite this, C2/C3 MDLs are still well below typical concentrations in aqueous environmental samples. Good method precision of 1.1-13% was obtained from the spike and recovery of Milli-Q water, except C6 whose standard deviation was 21%. The cause of this is discussed further under matrix spike and recovery.

The IDL of 55 fg for TFA is high in comparison to some exemplary phenyl derivatization GC methods. Martin et al.[30] reported 5.1 fg for TFA using 2,4-difluoroaniline derivatization with GC-MS/MS detection, while Frank et al.[25] reported 0.18 fg for TFA using pentafluorophenyl diazoethane derivatization with GC-ECNI-MS detection. This was an expected result due to DDM being fluorine free, therefore sacrificing some of the ECNI-MS detection sensitivity. Ji et al.[22] employed pentafluorobenzyl derivatization and reported GC-MS MDLs of 0.1-0.28 pg/mL for C6-C12 in 500 mL tap water samples concentrated to 1 mL, similar to values reported in this work. However, their derivatization procedure requires two reagents, pentafluorobenzyl bromide and K_2CO_3, and takes 1 hour of heating at 55 °C to complete. Accounting for the 2 times greater concentration factor, MDLs in this work are similar, or within an order of magnitude. The same is true in comparison to many other LC-MS/MS methodologies, demonstrating the broad utility this new method is capable of while being far more accessible to the global environmental chemistry community.

Table 1. Instrument and method performance and quality control.

PFCA	Quantitation Ion (*m/z*)	Instrumental Repeatability (RSD%)[a]	Instrumental Detection Limit (fg on column)[a]	Calibration Linearity (R^2)[b]	Method Precision (RSD%)[c]	Method Detection Limit (pg/mL)[a, d]
C2	113	1.8	55	0.9969	4.4	14.60
C3	163	1.3	156	0.9983	6.4	1.11
C4	213	1.2	9	0.9997	2.5	0.21
C5	263	1.6	15	0.9997	8.7	0.10
C6	313	4.1	10	0.9997	21	0.06
C7	363	2.3	8	0.9969	3.9	0.20
C8	413	2.6	14	0.9964	1.3	0.18
C9	463	2.2	10	0.9955	4.0	0.32
C10	513	3.2	17	0.9959	8.4	0.47
C11	563	3.0	18	0.9921	6.8	0.84
C12	613	2.9	14	0.9952	5.3	0.30
C14	713	3.6	220	0.9940	13	0.12

[a] See section S7 for detailed descriptions. [b] Method calibration range of 0-1000 pg/mL. [c] Determined from the spike level of 100 pg/mL in Milli-Q water. [d] Based on 500 mL aqueous samples

Matrix Spike and Recovery

Recoveries of C2-C9 from the 3 matrices were generally quantitative (80-120%; Figure 5) except C6 from Milli-Q water, and C2/C3 from ocean water. While the exact reason is unknown, the 177 ± 21% recovery of C6 is likely a result of matrix enhancement related to the close elution of C6 with the benzophenone peak in this simpler matrix (Section S8, Figure S8). This can be accounted for by including mass labeled C6 in the IS mix. The poor recoveries of C2/C3 from ocean water (19%, 54%) were likely due to ion competition on the anion exchange sites, driven by the high concentration of chloride and other anions in the sample matrix. Prior studies have reported similarly poor recoveries of C2/C3. Janda et al.[15] explored recoveries of C2-C8 from natural spring water for pH values of 3-6. They found that recoveries of C4-C8 were unaffected, yet recoveries of C2/C3 were quantitative only at lower pH levels from 3-4 for the Strata X-AW cartridge. Wujcik et al.[31] has explored the effect of ionic strength on WAX SPE using Empore anion-exchange SR disks. Losses of C2 on WAX SPE were observed for extractions of 300 mL aqueous salt solutions with NaCl concentrations as low as 400 mg/L. To address this, they opted to introduce an additional liquid-liquid extraction cleanup for samples of high salinity. Here, we have opted for standard addition to account for losses in high ionic strength ocean water sample matrices instead of surrogates, while the recoveries from the Milli-Q water and denuder matrix spike experiments were used to correct for SPE losses in tap water, precipitation, and denuder samples. When applying this SPE method to real samples, addition of a second set of mass-labeled surrogate standards prior to extraction, could also be used to account for SPE losses in each individual sample.[32]

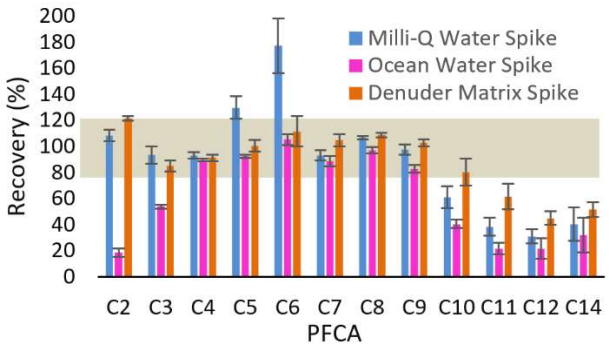


Figure 5. Matrix recoveries of 50 ng spikes of C2-C14 PFCAs in Milli-Q water, synthetic ocean water, and simulated denuder extract matrix by WAX-SPE. Error bars show standard deviation of replicates (n = 3). The shaded area represents the region of quantitative recovery (80%-120%).

The poor recoveries of C10-C14 were caused by their lack of retention on the Strata X-AW cartridge during sample loading (see Section S4). This agrees with the findings of Montes et al.,[33] where lower recoveries were observed for long chain perfluoroalkyl acids (PFAAs) using Strata X-AW, while recoveries using Oasis WAX were quantitative. This could be explained by the hydrophobic backbone of Oasis WAX compared to Strata X-AW sorbents, which results in better retention of the long chain PFAAs (Figure S9). Therefore, these findings indicate that Strata X-AW should not be used for the SPE of C10-C14 and also that WAX SPE is likely not appropriate for the quantitative extraction of C2/C3 from most high salinity samples.

Real Sample Analysis

Because of the high levels of C2/C3 observed in Milli-Q water, Strata X-AW pre-cleaned Milli-Q water was used as a method blank. This was used for analytical determinations of the PFCA analyte suite in tap water, precipitation, ocean water, and denuder samples. Due to the SPE losses of C2/C3 seen in ocean water spike and recovery, the real ocean water sample was quantified by standard addition with 0, 50, and 100 ng of C2-C14.

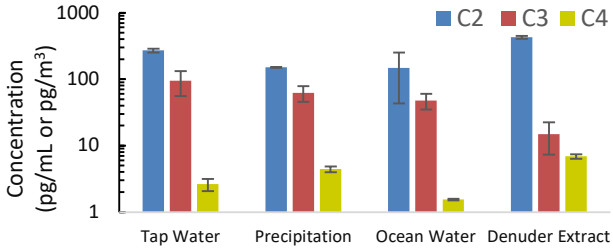


Figure 6. Analyzed concentrations of C2-C4 in four environmental samples reported in pg/mL, except the denuder extract which is reported in pg/m³. Error bars show standard deviation of replicate measurements (n = 3).

Concentrations of all analyzed PFCAs are shown in Section S9, Table S6. C2-C4 were detected in all 4 samples (Figure 6), with C2 detected at concentrations at least an order of magnitude greater than other homologues. Although within the same order of magnitude, the concentrations of C2 and C3 in tap water (270.9 and 94.5 pg/mL) are greater than both precipitation (150.4, 62.3 pg/mL) and ocean water (148.1, 47.8 pg/mL). This may suggest the creation of these compounds through precursor degradation during the disinfection process, similar to observations from wastewater treatment.[34] The 150 pg/mL C2 concentration in the precipitation sample is within the range of rainwater concentrations of 87-270 pg/mL reported by Scott et al.[35] in north Toronto during 2003-2004. However, C3 and C4 concentrations of 62 and 4.4 pg/mL are greater than the reported values of 0.8-2.4 and 0.1-2.1 pg/mL for C3 and C4 in the same study. This increase agrees with the overall increasing trend of C2-C4 PFCA fluxes observed in the environment since 1990.[3] The analyzed 426 pg/m³ gaseous atmospheric mass loading of C2 from the denuder sample is similar to previously reported

concentrations of ~250 pg/m^3 to ~5 ng/m^3 in Toronto during Jun-Dec 2000.[30] C5-C8 were detected in atmospheric abundances of 0.44-0.56 pg/m^3. In contrast, all of C5-C14 (C13 not analyzed) were detected in precipitation at concentrations of 0.43-5.4 pg/mL. These results indicate that C2-C8 can reside in the atmospheric gas phase long enough to be sampled, consistent with previous modelling work[36] on the gas-particle partitioning of PFCAs, which predicts PFCAs to predominantly reside in the gas phase under atmospherically relevant conditions. The relative abundance of atmospheric gas phase C2-C4 is also consistent with precipitation and other aqueous samples, supporting atmospheric formation and transport of PFCAs as a dominant source and/or process.

Conclusion

The derivatization method was demonstrated to be reproducible, easy to perform, and the reaction completed immediately upon mixing the reagents. Derivatization and separation of C2-C14 PFCAs was achieved in a single GC-MS method for the first time. Integrating the derivatization to an existing solid phase extraction procedure using surrogate standards requires only an additional 30 minutes for sonication. A sample handling robot could be used to automate the entire process. With detection limits comparable to typical LC-MS/MS methodologies, this GC-MS method overcomes the typical drawbacks of time-consuming derivatizations and high detection limits. The diphenyl derivatization represents a substantial improvement to that of existing derivatizations both in function and ease of the procedure. The overall methodology was applied to 4 different aqueous environmental sample matrices where C2-C4 PFCAs were detected in all samples, highlighting the importance of including ultrashort chain PFCAs for PFAS analysis. We believe this novel derivatization GC-MS methodology provides a globally cost-effective and needed analytical method for the analysis of all relevant PFCAs in various environmental matrices, inclusive of the ultrashort chain homologues.

ASSOCIATED CONTENT

Supporting Information.
The Supporting Information is available at http:/pubs.acs.org

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Author Contributions

The manuscript was written through contributions of all authors. All authors approved the final manuscript.

Notes

The authors declare no conflict of interest.

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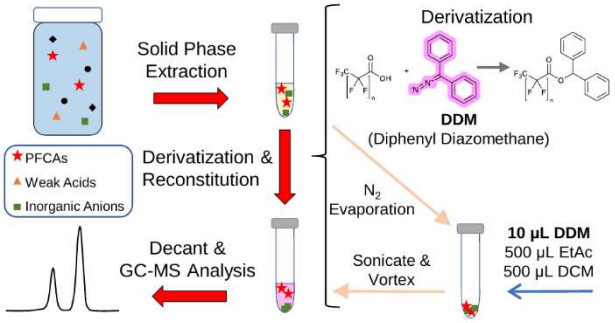
Solid Phase
Extraction
PFCAs
Weak Acids
Inorganic Anions
Derivatization &
Reconstitution
Decant &
GC-MS Analysis
Derivatization
DDM
(Diphenyl Diazomethane)
N2
Evaporation
10 µL DDM
500 µL EtAc
500 µL DCM
Sonicate &
Vortex