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Resolving unknown isomers of emerging per- and polyfluoroalkyl substances (PFASs) in environmental samples using COSMO-RS-derived retention factor and mass fragmentation patterns



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ABSTRACT

Chromatographic retention factors (k) and mass spectral fragmentation patterns of per- and polyfluoroalkyl substances (PFASs) were determined using the optimized parameters in liquid chromatography with tandem high-resolution mass spectrometry (LC-HRMS) analysis. Characteristic fragment ions obtained at various collision energies (MS^2 fragmentation) were used to determine the structures of newly discovered (emerging) PFASs detected from industrial effluent and surface water samples. Moreover, COnductor-like Screening MOdel for Realistic Solvents (COSMO-RS) derived octanol-water partition coefficients (K_{ow}), along with mean isotropic polarizabilities calculated from Density Functional Theory (DFT), of known PFASs were plotted against their experimental k values (k_{exp}) to obtain a multivariable regression model that can be used to predict k values of unknown PFASs. The k values of different structural isomers of unknown PFASs were calculated and compared to k_{exp} . The predicted k value for the isomer that matches the MS^2 fragmentation observed was found to be within 4.2 % of k_{exp} . This study demonstrates the applicability of an approach that combines the observed MS^2 fragmentation patterns and k values, together with computationally-derived log K_{ow} and polarizabilities, in assigning the structures of unknown PFASs at environmentally relevant conditions when no reference standards are available.

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1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a broad class of synthetic chemicals used in industrial and consumer applications. Due to their unique ability to modify surfaces and their high chemical and thermal stability, PFASs are important components of aqueous filmforming foams (AFFFs) used in firefighting, and are major ingredients in textiles, food contact materials, and fluoropolymers (Buck et al., 2011). Because of their widespread use, combined with the extreme stability of the carbon-fluorine bond, complex mixtures of PFASs have become ubiquitous environmental contaminants (Kato et al., 2015; Armitage et al., 2009; Seo et al., 2019). Previously, most studies have concentrated on perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) because of their frequent occurrence and high bioaccumulation in vertebrates (Tartu et al., 2014; Martin et al., 2004). In addition, they have been shown to have harmful biological activities such as immunotoxicity (DeWitt et al., 2009), endocrine disruption (Liu et al., 2007), and disturbances in lipid metabolism (Wang et al., 2014). Hence, PFOS and PFOA were phased out and replaced by their short-chain homologues or other not fully fluorinated alternatives that have been called "emerging" PFASs (Wang et al., 2013). Recent studies have shown occurrence of these emerging PFASs in drinking (Guardian et al., 2020) and groundwater samples (Sun et al., 2016; Kabore et al., 2018). Other polyfluorinated chemicals have also been detected in trout from Lake Michigan (Fakouri Baygi et al., 2016), in AFFFs and AFFF-impacted groundwater (Barzen-Hanson et al., 2017), and in industrially-impacted watershed in North Carolina (McCord and Strynar, 2019). With these discoveries, and worldwide research effort, there are now over 6300 identified fluorinated compounds with Chemical Abstracts Service (CAS) numbers listed in the EPA PFAS Masterlist (USEPA, 2020). With the increasing number of novel PFASs (linear and branched isomers) being discovered (Liu et al., 2019) and the varying degrees of transformations that they undergo in the environment (Li et al., 2019), the identification of unknown PFASs has become a daunting task. Many of the studies that investigate the occurrence of PFASs in the environment perform target analysis using liquid chromatography (LC) with tandem mass spectrometry (MS²), where specific PFASs are selected in advance. However, target analysis will only detect a relatively small portion of these vast number of organic contaminants. For instance, EPA methods 533 (Wendelken and Rosenblum, 2019) and 537.1 (Shoemaker and Tettenhorst, 2018) which have been used for drinking water, only analyze for a total of 29 PFASs. Therefore, due to the preselection of target PFASs, many other emerging PFASs that may have significant ecotoxicological effects remain undetected. In this regard, identification of emerging PFASs in the environment is necessary and requires new analytical approaches to facilitate identification when reference standards are not available.

The octanol-water partition coefficient (Kow) of a molecule is a valuable parameter that has been used as a measure of hydrophobicity. Log Kow values have been used not only in predicting bioaccumulation and environmental mobilities of pollutants (Cohen et al., 1990; Bertelsen et al., 1998), but also in developing analytical methods, such as in selecting appropriate solvents for extraction and stationary phases for chromatographic separations (Szepesi and Valkó, 1991). In reversed-phase LC, linear relationships can be observed between log Kow and retention time or retention factor (k). Therefore, log K_{ow} values have been used to aid in optimizing separations by predicting analyte retention times, which can be accomplished within 5% error (Hanai et al., 1981). In a previous study, the linear relationship between log Kow and retention time was used to augment high resolution mass spectrometry (HRMS) data in the identification of emerging contaminants in water by suspect screening. Using this approach, the number of false positive detections was reduced by limiting the retention time window to ± 2 min of their expected retention time (Bade et al., 2015). The use of log Kow in predicting retention times, combined with HRMS fragmentation patterns, can be a powerful approach in the

identification of emerging PFASs, but have not been demonstrated to date.

Experimentally determined Kow for many PFASs are not available because of the challenges associated with measuring Kow for these surfactant-like compounds and the lack of available reference standards for many emerging PFASs. Hence, traditional experimental methods to determine Kow values, such as shake flask or slow-stir methods, are unsuitable for PFASs. The only experimentally determined Kow values reported for PFASs have been obtained by a voltammetric method (Jing et al., 2009) and by LC method (Xiang et al., 2018; Hammer et al., 2017). Complementary to experimental determination, studies using computational methods have been conducted to estimate physicochemical properties for PFASs. For instance, models have been presented in the literature, such as EPI Suite™, ClogP™, SPARC™ and COSMOtherm[™], to predict the octanol-water partitioning behavior of PFASs (Arp et al., 2006; Wang et al., 2011; Hidalgo and Mora-Diez, 2016). Physicochemical properties estimated using COSMOtherm[™] in a recent study showed good agreement with the experimental data for a limited number of fluorotelomer alcohols (Wang et al., 2011; Arp et al., 2006). COnductor-like Screening MOdel for Realistic Solvents (COSMO-RS) method has been used to predict retention behavior based on partition coefficients in micellar LC (Mehling et al., 2013) and in predicting lipophilicity of solutes using calculated micelle/water partition coefficients (van der Spoel et al., 2019). Moreover, COSMO-RS accurately predicted gas chromatographic retention times of polybrominated diphenyl ethers based on calculated boiling points, which in turn facilitated identification of unknown metabolites without reference standards (Simpson et al., 2015).

PFASs are classified based on the head group (e.g. carboxylates, sulfonates), the arrangement of the alkyl chain (branched or linear), and the degree of fluorination in the carbon backbone (partial or full fluorinated). These molecular features define the interaction of PFASs with the stationary phase of a chromatographic column; thus, for PFASs in the same class, the order of elution is mainly dictated by the number of carbon atoms in the alkyl chain. Quantum chemistry based COSMO-RS log K_{ow} calculations are dependent on the solute molecule's 3-D polarization charge density that are used to model interactions with the solvent, affecting the calculated partitioning behavior of the solute molecule. Thus, log K_{ow} is a sensitive parameter that can be used to predict retention of fluorinated alkyl substances in a reversed-phase chromatographic column.

The similarity in the structures of PFASs results in characteristic MS² fragmentation patterns that are shared by each class of PFASs (Barzen-Hanson et al., 2017; McCord and Strynar, 2019). The MS² fragmentation led to the development of unique approaches to identify PFASs using HRMS. For instance, monitoring of -CF2 homologues and neutral fragment loss (Place and Field, 2012), and the use of Kendrick mass defect plots (Barzen-Hanson et al., 2017) have been extremely valuable in the identification of PFASs. However, these approaches cannot accurately resolve PFAS isomers (Fakouri Baygi et al., 2016; Barzen-Hanson et al., 2017; McCord and Strynar, 2019). Previous studies have shown differences in the toxicity and bioaccumulation potential between the linear and branched isomers of PFOS and PFOA (Loveless et al., 2006; O'Brien et al., 2011; Greaves and Letcher, 2013) thus, for a more comprehensive risk assessment, it is important to determine which isomeric form is present in the environment. In this regard, the objectives of this study are to: (1) optimize chromatographic separation and MS² collision energies for LC-HRMS analysis to obtain characteristic fragmentation patterns for each type of PFASs, (2) calculate log Kow values of known PFASs using COSMO-RS and mean isotropic polarizability using TURBOMOLE, (3) derive a mathematical relationship between log Kow, mean isotropic polarizability, and k, and (4) demonstrate the complementary use of k and MS^2 fragmentation in resolving isomeric structures of emerging PFASs that have been putatively identified from HRMS suspect screening when reference standards are not available.

2. Materials and methods

2.1. LC – HRMS analysis

Reference standard of perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA): perflouropropanesulfonic acid (PFPrS), perfluorobutanesulfonic acid (PFBS), perfluoropentanesulfonic acid (PFPeS), perfluorohexanesulfonic acid (PFHxs), perfluoroheptanesulfonic acid (PFHpS), perfluorooctanesulfonic acid (PFOS), perfluorononanesulfonic acid (PFNS), perfluorodecanesulfonic acid (PFDS), perfluoroutanesulfonamide (FBSA), perfluorooctanesulfonamide (FOSA), 2-(N-methylperfluorooctanesulfonamido) acetic acid (N-MeFOSAA), 2-(N-ethylperfluorooctanesulfonamido) acetic acid (N-EtFOSAA), 4:2 fluorotelomer sulfonic acid (4:2 FTS), 6:2 fluorotelomer sulfonic acid (6:2 FTS), and 8:2 fluorotelomer sulfonic acid (8:2 FTS), N-methylperfluoro-1-octanesulfonamide (N-MeFOSA), Hexafluoropropylene oxide dimer acid (HFPO-DA), 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdS), 9chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS), Dodecafluoro-3H-4,8-dioxanonoate (ADONA) were obtained from Wellington Laboratories (Guelph, ON, Canada). Ammonium acetate was purchased from J.T. Baker (Phillipsburg, NJ) and LC-MS grade acetonitrile were purchased from MilliporeSigma (Jaffrey, NH).

Chromatographic separation of PFASs was optimized using a Waters X-Bridge[™] C18 column (3.5 µm particle size, 2.1 mm internal diameter, 150 mm length) and a gradient mobile phase consisting of water with ammonium acetate (mobile phase A) and acetonitrile (mobile phase B) at a flow rate of 200 µL min⁻¹. LC – HRMS analysis was performed using Thermo Scientific Q–Exactive Orbitrap Focus[™] with Thermo Scientific UltiMate 3000 UHPLC[™], operated under the negative ion mode during electrospray ionization (–ESI). A full-scan with data-dependent mass fragmentation (full MS – ddMS²) was used with a scan range set to 80 – 1200 m/z. The resolution for full scan was 70,000 and 17,500 for ddMS². MS² fragmentation of PFASs were obtained using three collision energies: 10, 20 and 30 eV.

To demonstrate the applicability of the proposed analytical strategy in real environmental samples, previously characterized water samples that have been shown to contain emerging PFASs were used in this study: an effluent sample from Chemours plant in North Carolina (McCord and Strynar, 2019) and surface water samples from the Philippines and Thailand (Guardian et al., 2020). Details of sample collection, preparation and suspect screening method have been described in the previous publications (McCord and Strynar, 2019; Guardian et al., 2020).

2.2. Log Kow calculations using COSMO-RS

Log K_{ow} values were calculated using either (a) pure octanol (dry) or (b) octanol that has been saturated with water (wet) in order to determine if the small amount of water that dissolves in octanol will have a significant impact on the calculated K_{ow} values (Chen and Siepmann, 2006). For "wet" COSMO-RS calculation of the log K_{ow-wet} a 0.274 mol fraction of water was incorporated into the octanol-phase (Dallos and Liszi, 1995). The following steps were used to calculate log K_{ow} for the electronically neutral and anionic species of each compound:

 Determine the lowest energy conformers using Vconf (Chang and Gilson, 2003). The number of selected conformers was determined based on a reasonable energy cut-off (1–5 kcal/mol) as implemented in Vconf. For most compounds this number did not exceed 100 conformers.

- Optimize the conformer geometries using TURBOMOLE (2020) using the BP86 functional (Perdew, 1986; Becke, 1988) and a def2-TZVPD basis (Rappoport and Furche, 2010; Weigend and Ahlrichs, 2005) for each element. Due to computational expense, only vibrational frequencies for the lowest in energy conformers were calculated to ensure geometries were local minimums.
- Calculate an overall average log K_{ow} for each compound utilizing a weighted Boltzmann distribution (SI part III), as suggested in references (Wang et al., 2011; Klamt, 1995; Buggert et al., 2009).

2.3. Mean isotropic polarizability calculations using TURBOMOLE

Using the lowest energy conformer determined in the steps described in the previous section, the mean polarizability was calculated. Geometries were optimized using TURBOMOLE (2020) with the BP86 functional (Perdew, 1986; Becke, 1988) and a def2-TZVPD basis (Rappoport and Furche, 2010; Weigend and Ahlrichs, 2005) for each element. As previously described, vibrational frequencies were calculated for these optimized geometries. The mean (isotropic) molecular polarizability was calculated with the same computational methodology used in the geometry optimizations. The isotropic polarizability is defined as ¹/₃ of the trace of the polarizability matrix.

3. Results and discussion

3.1. Optimization of chromatographic separation of PFASs

An important consideration in using COSMO-RS and experimentally measured k (kexp) values for known compounds to establish an accurate regression equation for predicting k (kcalc) of unknown PFASs is to ensure that the PFASs in the training set have sharp and baseline-resolved peaks. Efficient separation of PFASs cannot be achieved by isocratic elution because of the co-elution of several PFASs and broadening of late eluting peaks. Sharp and baseline-resolved peaks were achieved when using the optimized gradient elution program for LC. Two pH values were explored, pH 3.8 and 10.0, in optimizing separation. These pH values (acidic and basic) were based on the pKa of ammonium acetate, the additive used in the mobile phase. It was found that upon changing the gradient program to start at 95 % aqueous phase (mobile phase A: water with 5 mM ammonium acetate), the effect of pH on the kexp values for PFASs becomes negligible (Table S1). Starting the gradient at high aqueous content also allowed the ultra-short chain PFASs $(C_2 - C_4)$ to elute after the LC void volume. Upon assessment of the separation obtained using the four LC conditions tested (Table S1), the following gradient program was selected: starting with 5 % acetonitrile (mobile phase B) held for 1 min, followed by a 29-min linear gradient to $95\ \%$ mobile phase B, which was then held also for 5 min, and then returning to the initial condition (5 % mobile phase B) within 1 min. The column was equilibrated at initial LC condition for 8 min prior to the next injection.

3.2. Determination of MS fragmentation pattern

Mass spectral fragmentation of each PFAS at 10, 20 and 30 eV were obtained (SI Part II). Varying collision energies produced unique fragmentation patterns for each class of PFASs (Table 1). Knowledge of these common fragments and specific fragmentation pattern is highly valuable in determining the structure of emerging PFASs. Moreover, MS^2 characteristic fragments could also be applied to determine the number of PFASs present in the sample. For example, one can extract m/z = 118.9925 (corresponding to $CF_3CF_2^-$, a common fragment shared by all PFCAs) and m/z = 79.9573 (corresponding to SO_3^- the common fragment ion shared by all PFSAs) to determine how many PFCAs or PFSAs are present in the sample (Figure cc, SI part II). The number of peaks in the chromatogram corresponds to the number of PFASs in the sample sharing these common ions. Further investigation

Table 1

Summar	y of c	common fra	agments	(m/z)	shared	by	PFASs	in t	ne same	class	and	observed	trends	in th	eir fr	agmentation	patterns
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PFASs class	common fragment mass (m/z)	Formula of fragment	Fragmentation pattern trend
PFCA	118.9925	$C_2F_5^-$	 most abundant fragment at 10 eV corresponds to [M-CO₂-H]⁻ after neutral loss of CO₂ there is an increasing ratio of [M-H]⁻/[M-CO₂-H]⁻ at 10 eV with the increase in chain length fragmentation pattern is characterized by [M-H]⁻, [M-CO₂-H]⁻, followed by
PFSA	98.9557 79.9573	FSO ₃ ⁻ SO ₃ ⁻	 [M-3C+2-C0₂-H] and subsequent loss of C+2 until the terminal chain of C₂+5 most abundant ion at 10, 20 and 30 eV corresponds to [M-H]⁻ only these two common ions will be observed upon MS² fragmentation with increasing relative abundance to [M-H]⁻ as the chain length decreases
perfluoroalkyl sulfonamides	77.9655	NSO_2^-	 most abundant ion at 10 and 20 eV corresponds to [M-H]⁻ relative abundance of NO₂S⁻/[M-H]⁻ at 20 and 30 eV increases with increasing chain length
Perfluorooctane-sulfonamidoacetic acids	82.9608	FSO ₂ ⁻	 presence of fragment corresponding to neutral loss of acetic acid further fragmentation corresponding to loss of the sulfonamide group and subsequent CF₂ loss
fluorotelomer sulfonic acids	80.9655	HSO ₃ ⁻	 presence of fragment corresponding to neutral loss of HF
ether – PFSA	98.9557	FSO ₃ ⁻	• the number of ether functional groups can be determined based on fragments
	82.9608	FSO ₂ ⁻	• the number of ether functional groups can be determined based on fragments
ether -PFCA	-	-	 fragmentation will occur at the C-O-C bond of the ether functional group the number of ether functional groups can be determined based on fragments

of the fragmentation patterns can reveal the structure of the unknown PFAS, as described in Table 1 and SI part II. While the common fragments for legacy PFASs have been reported in previous studies (McCord and Strynar, 2019; Barzen-Hanson et al., 2017), this present work reports the differences in the fragmentation patterns of these legacy PFASs at varying voltages. Moreover, fragmentation patterns for fluorinated alkyl ether acids, fluorotelomer sulfonic acids and other PFASs class are also reported in this study (Table 1) to aid in future identification of emerging PFASs that are being introduced in the environment.

3.3. Calculation of log Kow values

The log K_{ow} values of the neutral and anionic forms of PFASs were calculated using COSMO-RS. While the main purpose of the log $K_{\rm ow}$ calculation is for the prediction of k values, log Kow values can also advance our understanding of the sorption behavior and mobility of PFASs in the environment. Many of the PFAS species have pKa value of < 1 (Goss, 2008) which implies that the neutral form of these compounds has negligible contribution to the speciation of PFASs in water, and that the anionic form dominates under most environmentally relevant conditions (Higgins and Luthy, 2006). The results of the log Kow values calculated (wet and dry) for all PFASs considered in this study are listed in Table S2. As expected, the calculated log Kow value increases as the number of fluorinated carbon increases (Wang et al., 2011). A negative value for log $K_{\rm ow}$ of the compound indicates preferred partitioning into the aqueous phase, while a positive value indicates higher partitioning to the octanol phase. However, for ionizable compounds like PFASs, interpretation of the log Kow must consider similar species (charged or neutral) in both solvents (Sangster, 1997). It is notable in Table S2 that the log $K_{\rm ow}$ values for the neutral species are significantly different from the log K_{ow} calculated for the corresponding anionic species of PFASs. These results suggest that the pH of the mobile phase used in LC analysis is an important factor when predicting k values of PFASs.

The number of conformers that were included into the Boltzmann distribution were determined based upon log K_{ow} calculations for 4:2 fluorotelomer alcohol (4:2 FTOH) as this is one of the few PFAS molecules that has an experimentally determined log K_{ow} (Wang et al., 2011). Our calculated values were determined to be 2.820 and 2.787 for log K_{ow-wet} and log K_{ow-dry} , respectively, whereas the experimental log K_{ow} for 4:2 FTOH was determined to be 3.30 \pm 0.04 (Wang et al., 2011; Carmosini and Lee, 2008). It can be seen that the log K_{ow} values essentially converge beyond 15 conformers (SI part III). However, it

was determined that for larger molecules, convergence requires more conformers to be included into the Boltzmann average (Mokrushina et al., 2012). As demonstrated with our calculations for 4:2 FTOH, we can assume that our certainty is within ± 1 logarithmic unit. While the error for 4:2 FTOH is less than 1 log unit, others (Wang et al., 2011; Carmosini and Lee, 2008) have determined this $\pm 1 \log$ unit window to generally be the error in COSMOtherm predictions looking at a large number of PFASs. However, the huge number of inconsistent Kow measurements for PFASs and other compounds makes this uncertainty estimate large. The influences on the predictions most likely have an impact as the majority of the predicted retention factors are lower than what is observed in experiment. The trends of PFASs that have their Kow measured contained in our training set follow the same trend as our predicted values. Several papers have shown that parameters calculated via COSMO-RS can be different than the experimental value, but the trends appear to be consistent with experimental observation. Our work is consistent with previously published study with this estimation of error for log K_{ow} values for several FTOHs (Wang et al., 2011). It is possible that the calculated log Kow values are lower than the experimental Kow values based upon our previously mentioned calculations for 4:2 FTOH, and values others have calculated for various PFASs (Wang et al., 2011) and for polyaromatic hydrocarbons (Schroder et al., 2010). Studies determining the $\log K_{ow}$ of these compounds have been shown to be influenced by pH. For example, Xiang et al., determined log Kow values of neutral species of PFCAs and found huge deviations in results compared to literature (i.e. PFOA result = 4.67 while literature values are 1.76-7.75) (Xiang et al., 2018). The well-known tendency of COSMO-RS to perform better for systems with delocalized charges may also contribute to deviations in calculated log $K_{\rm ow}$ values for the anionic species (Klamt et al., 2010). This is due to the neglect of long-range ionion interactions. It is also worth noting that there have been a large number of reports of disagreements of experimentally determined log Kow values for even the most well-studied compounds (Renner, 2002). Depending on what methodology is used to obtain log $K_{\rm ow}$, researchers can get drastically different values (Hermens et al., 2013). Therefore, for consistency, log Kow values for each PFAS is calculated using the same method.

3.4. Determination of PFASs structure

HRMS has established its usefulness in identifying environmental contaminants via accurate prediction of the molecular formula and structural elucidation based on fragmentation and accurate mass measurements (Schymanski et al., 2015; Knolhoff and Croley, 2016;

Table 2

List of possible structures for unknown PFASs detected in water samples and their corresponding calculated log K_{ow}, polarizability, and retention factor values.

Unknown	Structure of possible isomer	log K _{ow} wet-neutral	log K _{ow} - _{wet-} anion	Polarizabilit- y, a.u.	Calculated Retention factor (k _{calc})	Experimental Retention factor (k _{exp})	% Error (peak a)	% Error (peak b)
Unknown 1. C ₅ H ₅ OF ₇ Observed mass (m/z): 213.0156 mass error	1 F ₂ CH CH ₃	2.5230	-4.1322	78.0094	4.999	5.1345	-2.63	NA
(ppm): 0.46	$\begin{array}{c} F_2\\ OH\\ 2F_3C\\ CH\\ F_2\\ F_2\\ CF_3\\ GF_3\\ GF_3\\ GH\\ F_2\\ CH\\ CH\\ CH\\$	2.5100	-4.6862	77.4958	5.271		2.67	NA
		2.3695	-4.6057	77.3323	5.107		-0.54	NA
	но с _{F2} сн ₃	2.2321	- 5.7025	78.1597	5.633		9.70	NA
Unknown 2. $C_5HO_4SF_{11}$	H_{3C} C C F_{2} F_{2} F_{2} F_{3} F_{3}	4.7836	-3.1602	114.9719	7.845	7.5297	4.19	NA
364.9353 mass error (ppm): 1.61	$F_{3}C'$ F_{2} F_{2} F_{3} F_{3} F_{2} F_{3} $F_$	4.7926	- 3.249	122.6671	8.225		9.23	NA
	$\begin{array}{c} F_2 \\ F_2 \\ F_3 \\ F_3 \\ F_2 \\$	4.8339	- 3.2626	115.0415	7.945		5.52	NA
	$4_{F_3C} \xrightarrow{F_2} \xrightarrow{F_2} \xrightarrow{F_2} \xrightarrow{F_2} \xrightarrow{F_2} \xrightarrow{F_2} \xrightarrow{F_3O_3H}$	4.8841	- 3.2779	114.9738	7.991		6.13	NA
Unknown 3. C ₆ H ₂ O ₄ SF ₁₂ Observed mass (m/z): 396.9412 mass error	1 F ₃ C C C C C SO ₃ H	4.4069	-3.0314	126.5586	7.957	7.6438 (3a) 8.3256 (3b)	4.10	-4.43
(ppm): 0.65	$\begin{array}{c} F_2 \\ F_3 \\ F_2 \\ F_2 \\ F_3 \\ F_2 \\ F_3 \\ F_2 \\ F_3 \\$	4.8582	-3.2330	128.8589	8.529		11.58	2.44
	F_3C C F_2 F_2 F_2 3 F_3C C C F_2 F_2 C C F_2	4.8280	-3.4421	127.9886	8.583		12.28	3.09
	F_2 F F_2 H F_3C CF O C F_2 CF O C F_2 CF O F_3 F	4.5693	-3.2206	128.3394	8.267		8.15	-0.71
	$\vec{F}_2 = \vec{F}_2 = \vec{F}_2 = \frac{1}{2}$ $\vec{F}_2 = \vec{F}_2$ $\vec{F}_2 = \vec{F}_2 = \vec{F}_2$ $\vec{F}_2 = \vec{F}_2 = \vec{F}_2$	4.9869	-2.6315	127.4975	8.246		7.87	-0.96
	$F_{3}C$ F_{2} $F_{$	4.7318	-3.2057	126.9077	8.330		8.97	0.05
		4.4986	- 3.0158	128.4147	8.100		5.97	-2.70
		4.5041	-1.5514	128.4283	7.302		-4.48	-12.30
		4.8117	- 3.6438	128.8560	8.717		14.04	4.70
	$F_{3}C$ $C = C$ C C C C C C C C C	4.7296	- 3.7130	128.5868	8.677		13.52	4.22
	$10 F_{2}HC$ $7 F_{2}$ 7	4.3958	- 3.3794	128.0727	8.203		7.31	-1.48
		4 8808	- 2 7714	128 2207	8 274		8 25	-0.62
		1.3090	<i></i>	120.2207	0.27 1		0.20	0.02
	F₂ 513 SO3H CF3 F2C I	4.7728	-2.7871	127.4598	8.156		6.71	-2.03
	$\begin{array}{c c} 13 & & \\ H & CF & CF \\ H & CF & CF_2 \\ \hline \end{array} \begin{array}{c} CF \\ F_2 \end{array}$							

(continued on next page)

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Table 2 (continued)

Unknown	Structure of possible isomer	log K _{ow} wet-neutral	log K _{ow} - _{wet-} anion	Polarizabilit- y, a.u.	Calculated Retention factor (k _{calc})	Experimental Retention factor (k _{exp})	% Error (peak a)	% Error (peak b)
	14 F_2 C CF CF_3	4.4884	- 3.4546	127.7307	8.304		8.64	-0.26
	$F_{2}HC$ CF_{3} F_{2} F	4.5829	- 3.8908	128.3117	8.644		13.09	3.83
	$\begin{array}{c} F_2 \\ F_2 \\ F_3 \\ F_4 \\ F_2 \\$	4.6468	-3.8182	128.6672	8.671		13.44	4.15
	$17 \begin{array}{c} F_2 HC \\ F_3 C \\ F_2 \\ F_2$	4.4592	- 3.4222	129.1044	8.321		8.85	-0.06
	SO ₃ H 18 _{F3} C	4.9647	- 2.9285	128.8142	8.446		10.49	1.45
	$19 \begin{array}{c} F_2 \\ F_3 \\ F_2 \\ F_3 \\ F_2 \\ F_3 \\ F_$	4.5137	- 2.4369	128.4507	7.796		2.00	-6.36
	$20 \begin{array}{c} F_2 & F_2 \\ F_3 & C & C \\ F_3 & C & C \\ F_5 & F_2 & C \\ F_5 & C & C \\ F_5 & F_2 & F_2 \\ F_5 & F_2 & C \\ F_5 & F_2 & F_2 \\ F_5 $	4.5488	- 3.5350	128.5855	8.433		10.33	1.29
	$21_{F_3C} \bigvee_{OH}^{F_2} \bigvee_{F_2}^{F_2} \bigvee_{F_2} \bigvee_{F_2}^{F_2} \bigvee_{F_2}^{F_2} \bigvee_{F_2} \bigvee_{$	4.5895 4.6909	- 4.1378 - 3.9401	129.2074 129.8515	8.823 8.823		15.43 15.43	5.98 5.98
	$2 \sim c_{F_2} \sim c_{F_2} \sim c_{F_2} \sim s_{O_3H}$ $F_2HC \sim c_{F_2} \sim c_{F_2} \sim s_{O_3H}$	5.0038	-2.3027	131.8955	8.264		4.95	0.63
	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	4.9090	- 3.0655	132.4447	8.629		9.58	5.08
	$F_{3}C$ F_{2} F_{2} F_{2} F_{2} F_{2} F_{2} $F_{3}H$ H $F_{3}C$ C C C C C C C C C	5.3336	-2.0942	132.3816	8.436		7.14	2.74
	F_2 F_2 F_2 F_2 F_2 F_2 F_2 F_2 F_3 F_2 F_2 F_3 F	5.0714	- 3.1197	132.5748	8.795		11.70	7.11
Unknown 4. C ₆ H ₂ O ₅ SF ₁₂ Observed	$5 \frac{F_2}{F_2} $	5.0660	-2.9149	132.4057	8.671	7.8741 (4a)	10.12	5.60
mass (m/z): 412.9364 mass error (ppm): 1.32	6 F2 F2 F2 S03H	5.0624	-3.0510	133.2977	8.781	8.2114 (4b)	11.51	6.93
	$7 \xrightarrow{F_2}_{F_3C} \xrightarrow{F_2}_{C} \xrightarrow{F_2}_{C} \xrightarrow{F_2}_{C} \xrightarrow{F_2}_{C} \xrightarrow{F_2}_{SO_3H}$	4.9566	-2.9721	132.1684	8.604		9.27	4.78
	$8 F_3 C \underbrace{F_2}_{O} \underbrace{F_2}_{C} \underbrace{H}_{F_2} \underbrace{F_2}_{F_2} \underbrace{H}_{F_2} \underbrace{F_2}_{F_2} \underbrace{SO_3H}_{F_2}$	5.1196	-3.1378	132.7994	8.853		12.44	7.82
	$9 \underset{F_3C}{\overset{F_7}{\underset{O}{\overset{O}{\overset{O}{\overset{O}{\overset{O}{\overset{O}{\overset{O}{O$	H		9.45	4.95			
	$10 \begin{array}{c} & & \\ F_2 \\ F_3 \\ \hline \\ F_2 \\ \hline \hline \\ F_2 \\ \hline \\ F_2 \\ \hline \hline \hline \\ F_2 \\ \hline \hline \\ F_2 \\ \hline \hline \hline \\ F_2 \\ \hline \hline \hline \hline \\ F_2 \\ \hline \hline \hline \hline \hline \\ F_2 \\ \hline \hline \hline \\ F_2 \\ \hline \hline \hline \hline \hline \hline \hline \hline \hline \\ \hline \hline \hline \hline \hline \hline \hline \hline $	4.0405 5.0426	- 3 0240	132.9380	8 741		5.40	6.45
	$11 \operatorname{F_3C} \circ \operatorname{C} \operatorname{C} \operatorname{C} \operatorname{C} \operatorname{C} \operatorname{C} \operatorname{C} \operatorname{C}$	5.0720	5.0240	100.0900	0./11		11.01	0.73
	$12 \begin{array}{c} F_2 \\ F_3 \\ F_3 \\ F_4 \\ F_5 \\ F_5 \\ F_5 \\ F_5 \\ F_5 \\ F_2 \\ F_$	5.0897	-3.1861	132.7610	8.854		12.45	7.83

(continued on next page)

Table 2 (continued)

Unknown	Structure of possible isomer	log K _{ow} wet-neutral	log K _{ow} -wet- anion	Polarizabilit- y, a.u.	Calculated Retention factor (k _{calc})	Experimental Retention factor (k _{exp})	% Error (peak a)	% Error (peak b)
	$\begin{array}{c c} & F_3C & & \\ & & & \\ 13 & F_3C & F_2 & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & $	4.6959	- 2.9236	131.8645	8.354		6.10	1.74
	14 F3C CF3 F2 SO3H	4.6841	- 3.2964	131.8947	8.550		8.59	4.13
	F_3C F_3C F_3C F_3C F_3C CF_3 F_3C CHF_2 $16 F_3C$ CHF_2 CHF_2 CHF_2 CHF_2 CHF_3 CHF_2 CHF_3	4.6306	-2.9734	131.8871	8.329		5.78	1.44
		4.7740	- 2.9922	131.8938	8.456		7.39	2.98
	F ₂ F ₂ F ₃ C CF ₃ 17 F ₂ HC C SO ₃ H	4.8873	- 2.9595	131.9456	8.532		8.35	3.90
Unknown 5. C ₉ H ₂ O ₂ F ₁₆ Observed mass (m/z): 444 9722 mass error	F_2 F_2 H 1 F_2 F_2 F_2 C C C C C	5.2592	-1.5514	141.4055	8.457	8.6433	-2.16	NA
(ppm): -1.02	F_3C C F_2 F_2 $COOH$ COOH 2 F_3C C C CH CH CH	5.4610	-1.1919	138.8875	8.317		-3.77	NA
		5.5702	-0.9425	138.5908	8.256		-4.48	NA
	$\begin{array}{c} F_2 \\ F_2 \\ COOH \\ F_3C \\ F_2 \\ F_2$	5.6030	-0.8562	138.6274	8.237		-4.70	NA

McCord and Strynar, 2019; Barzen-Hanson et al., 2017). For most studies (McCord and Strynar, 2019; Barzen-Hanson et al., 2017; Newton et al., 2017), discovery of new PFASs from samples collected around fluorochemical manufacturing or contaminated sites were communicated using the Schymanski classification (Schymanski et al., 2014). Identification can be classified as level 1 when proposed compound is confirmed via comparison of MS, MS² and retention time to a reference standard, and as level 2a if a probable structure matches to a library spectrum of known PFAS or 2b if there is diagnostic evidence of MS and MS² spectrum when no reference standard is available. A tentative candidate (level 3) based on diagnostic fragments and similarity to identified compounds can also be assigned.

To demonstrate the applicability of using both unique and common fragmentation patterns (listed in Table 1) in proposing structures of newly identified PFASs, we analyzed an effluent collected from Chemours plant in North Carolina (McCord and Strynar, 2019), and surface water samples collected from Thailand and Philippines (Guardian et al., 2020) using the optimized LC-HRMS method. Five unknown PFASs found in these samples (see Table 2) were previously reported with confidence level 3 (unknown 1) and 2b (unknown 5) (Guardian et al., 2020) and level 3 (unknowns 2, 3, 4) (McCord and Strynar, 2019). The putative structures of these unknown PFASs were evaluated using the relationship between COSMO-RS derived log Kow and k. The experimental MS² fragmentation, ion assignment and the proposed structures for these compounds are presented in the supplementary material part IV. The mass error for all precursor ions assigned to the experimental m/z observed is within \pm 1.32 ppm, while for MS² fragments the mass error ranged from 0.01 to 16.49 ppm (see SI, part IV). While mass error of \pm 5 ppm has been considered generally acceptable for precursor ions, the mass error tolerance for MS² fragments are higher, as has been reported in recent studies involving nontargeted analysis of PFASs in environmental matrices (McCord and Strynar, 2019; Wang et al., 2020).

For unknown 1, which contains an –OH functional group, only a loss of --CH₃ was evident in the MS² spectra (SI part IV). For unknowns 2-4, the observed fragmentation pattern (SI part IV) was similar to the fragmentation pattern of the fluorinated alkyl ether acid standards analyzed (SI part II figure aa and bb), wherein presence of fragments corresponding to FSO_2^{-} (*m*/*z* = 82.9613) and FSO_3^{-} (*m*/*z* = 98.9567) confirms sulfonate as head group. The fragmentation occurring at the ether (C - O - C) bond was evident based on the fragment ions observed for each compound and the neutral loss corresponding to HF observed for polyfluorinated alkyl ether acids. For unknown 5 (SI part IV), the neutral loss of CO₂ confirms the presence of a carboxylate as head group in the proposed polyflouroalkyl carboxylate structure. The loss of mass corresponding to $-CF_2$ (m/z = 49.9971) and the presence of $C_2F_5^{-}(m/z = 118.9934)$, a common fragment for carboxylates, were also observed, further supporting the proposed structure for unknown 5. The earliest discovery of fluorinated alkyl alcohol and H-substituted PFCAs in the environment were reported by Wang and co-workers (Wang et al., 2018) from a wastewater treatment plant of a PFASs manufacturing facility in China, while ether-PFSAs were first reported by Liu and co-workers (Liu et al., 2018) in fish samples collected from Yangtze River (China) that is impacted by a PFASs manufacturing company.

Two chromatographic peaks for unknowns 3 and 4 were observed. The MS² spectra for each peak showed differences in the MS² fragments



Fig. 1. Extracted ion chromatograms (EIC) and MS^2 spectra of two unknown PFASs, each with two isomers, detected in real environmental (water) samples. 1A - EIC of unknown 3a and 3b; 1B - EIC of unknown 4a and 4b; 1C - MS^2 spectra and the corresponding fragmentation of unknown 3A; 1D - MS^2 spectra and the corresponding fragmentation of unknown 4A; 1 F - MS^2 spectra and the corresponding fragmentation of unknown 4B.

(Fig. 1), indicating presence of isomeric forms (i.e. different substitution patterns that vary based upon the position of a hydrogen or oxygen atom). While the environmental risk and toxicity of the isomeric forms of PFASs are not yet fully understood, a recent study has shown that branched PFOA isomer in human serum is positively correlated to uric acid levels. It appears that higher levels of uric acid occur for each logunit increase in branched PFOA, and that likelihood of hyperuricemia increases linearly with increasing branched PFOA (Zeng et al., 2019), highlighting the importance of distinguishing between PFAS isomers.

3.5. Confirmation of retention factor

Reversed - phase LC has become a common technique for the indirect determination of log $K_{\rm ow}$ values for new chemicals using a calibration plot that establishes the relationship between known log $K_{\rm ow}$

values and experimentally determined LC retention times or k values for structurally similar compounds (OECD, 1989). A regression equation from the calibration plot can be used to predict k values of unknown compounds with similar structures, as has been reported in the literature that used COSMO-RS derived physicochemical properties or other theoretical data to predict k of unknown compounds (Paschke et al., 2001; Simpson et al., 2015; Izydorczak et al., 2020).

In this study, COSMO-RS calculated log K_{ow} values of 32 PFASs (deemed the training set), along with their mean isotropic polarizability plotted against their corresponding k_{exp} were used to construct a regression line. While it is possible to use only the log $K_{ow-neutral}$, the sensitivity of the model was greatly improved with the inclusion of log $K_{ow-anionic}$. Thus, 3 of the compounds (FOSA, FBSA, N-MeFOSA) were removed from the regression because they do not have an easily accessible anionic form at the experimental conditions used in this study.

Note that our regression does not account for the specific intermolecular attraction forces of the analyte with the stationary phase of the column, which obviously contribute to the differences between k_{exp} and k_{calc} . Inclusion of a parameter that considers this analyte-column interaction may improve our predictions in future studies (Bidlingmeyer et al., 1982; Guo et al., 1986). It is also noted that this model is specific to this experimental methodology but the theoretical results reported herein can be used by other scientist to produce their own multivariable regression to calculate retention factors. We have recently determined that polarizability is a good predictor for the retention times of polybrominated diphenyl ethers (Izydorczak et al., 2020), thus, we included it in our regression. The equation of the regression using the log K_{ow-wet-neutral}, log K_{ow-wet-anion} and polarizability values was determined to be:

 $k_{calc} = 0.80823 \cdot log K_{ow-wet-neutral} - 0.54887 \cdot log K_{ow-wet-anion} + 0.04200 \cdot polarizability - 2.5842 (1)$

with a coefficient of determination (R²) of 0.983 and a residual standard error (RSE) of 0.365. All statistics and regressions were determined using the R environment (R Core Team, 2020). It was determined that there is a significant relationship between the variables and k base on *p*-values, f-tests, and other factors (see the SI - part V for more details) for log K_{ow-wet-neutrab} log K_{ow-wet-anion}, and polarizability (6.90 × 10⁻¹⁰, 1.93 × 10⁻⁶, 2.88 × 10⁻¹⁴, respectively) at the 0.05 significance level (α).

In order to evaluate the performance of this 3-variable regression, a new modified regression was constructed by removing 5 of the compounds from the training set and treating them as "unknowns". These compounds were PFPeA, PFPeS, PFHxS, N-EtFOSAA, and 8:2 FTS which were chosen randomly. It was determined that 3-variable (log K_{ow-wet-neutral}, log K_{ow-wet-anion}, and polarizability) regression performed approximately the same as a 5-variable (log K_{ow-wet-neutral}, log K_{ow-wet-anion}, log K_{ow-dry-neutral}, log K_{ow-wet-anion}, mean isotropic polarizability), and a 6-variable (log K_{ow-wet-neutral}, log K_{ow-wet-neutral}, log K_{ow-wet-neutral}, log K_{ow-wet-anion}, log K_{ow-dry-neutral}, log K_{ow-dry-neutral}, log K_{ow-wet-anion}, log K_{ow-dry-neutral}, log K_{ow-dry-neutra}, log K_{ow-dry-neutra}, log K_{ow-dry-neutra}, log K_{ow-dry-neutra}, log K_{ow-dry-neutra}, log

3.6. Differentiating PFAS isomers

In a previous experimental study on the separation of PFOS and PFOA isomers using high-resolution differential ion mobility tandem mass spectrometry (Ahmed et al., 2019), it was found that isomers that differ by the position of a single perfluoromethyl group can be resolved. As shown in Fig. 1, two of the unknowns (UNK3 and UNK4) each have two isomers that are baseline separated in the chromatogram, which prompted us to investigate if we can accurately determine the isomeric forms present in the sample. Table 2 shows the summary of the different possible isomers for each unknown and the corresponding % error in $k_{calc}.$ For the first unknown (C_5H_5OF_7), 4 isomers were compared and %error in k_{calc} ranged from 0.54 % to 9.70 %, which are equivalent to retention time shifts ranging from 0.04 to 0.85 min. An error above 5% is high, considering the MAE of the standards used in the test set (maximum error obtained was 3.41 %) and the observed drift in the retention time of \pm 0.4 min (maximum drift observed after 1 month of analysis of standards spiked in matrix for method quality control). Hence, for unknown 1, the predicted structure 4 that has a large error in k_{calc} (9.70 %) is unlikely to be accurate. On the other hand, isomers 1, 2 or 3 are all possible structures for unknown 1 based on the kcalc % error being all below 5%. However, due to the limited MS^2 fragments for unknown 1 (see SI part IV - a) it is not possible to determine which of these three isomers is the most probable structure without a reference standard. In contrast, for unknown 2, isomer 1 has the lowest % error in k_{calc} (4.19 %), and shows the presence of m/z = 184.9851 (C₃F₇O -) in the MS² spectra (see SI part IV - b) corresponding to the fragment occurring at the C-O bond of the proposed structure of isomer 1. Therefore, isomer 1 is the most probable structure for unknown 2.

Unknowns 3 (m/z = 396.9412) and 4 (m/z = 412.9364) each have two isomers present, as shown in the two separated peaks in Fig. 1A (UNK 3a and 3b) and Fig. 1B (UNK 4a and 4b), respectively. We compared k_{calc} for each isomer to the k_{exp} of the two peaks in unknowns 3 and 4. For unknown 3a, among the 22 isomers calculated for unknown 3, isomers 9–16 have the tendency to produce a $C_2F_4HO - (m/z)$ = 116.9969) that could loss HF resulting to $m/z = 96.9906 (C_2F_3O -)$. However, k_{calc} % error for these isomers are too high (6.71–14.04%) to be considered probable structure. Only 3 out of the 22 isomers (isomer 1, 8, 19) have an error of < 5% for k_{calc} with respect to unknown 3a. Based on the structure, isomer 1 is expected to produce m/z =184.9842 ($F_3CCF_2CF_2O-$) while isomer 8 is expected to produce m/z= 134.9874 (F_3CCF_2O –), both of which were not observed in the MS² spectra for unknown 3a (Fig. 1C). The lack of the common fragment for ether-PFSA (m/z = 98.9557 (FSO₃⁻) and m/z = 82.9608 (FSO₂⁻)) in MS² spectra of unknown 3a suggests that there could be other functional groups present in the molecule. Isomers 17-22 contain both the OH and SO₃H functional groups in the molecule. Of these isomers, only isomer 19 (where -OH is at C₄) has the lowest k_{calc} % error (2.00 %). Therefore, we designated isomer 19 to be the most probable structure for unknown 3a. We suspect that upon breaking of the C₆–SO₃ bond, the presence of -OH in the fluorinated alkyl fragment could lead to rearrangement reactions forming $C_2F_4HO^-$ moiety (m/z = 116.9979).

For unknown 3b, several structures (1–7, 10–18 and 20) resulted to k_{calc} error of < 5%. However, the MS² fragments, specifically m/z = 216.9910 (corresponding to HF₂CCF₂CF₂CF₂O – moiety from to O–C₅ fragmentation (Fig. 1D)) limits the possibilities to structures 1–6 only. Neutral loss of HF from the m/z = 216.9910 resulting to m/z = 196.9845 (Fig. 1D), further reduce the possibilities to structures 3–6 where H resides on the 4th, 3rd, 2nd and 1st carbon atom, respectively. The low % error in k_{calc} values for these isomers showed that any of the proposed structures 4 (-0.71 %), 5 (-0.96 %), or 6 (0.05 %) is possible for unknown 3b.

Similar analysis was done for unknown 4; the structure that accurately fits unknown 4a is structure 1 and for unknown 4b is structure 10 (Table 2, Fig. 1B). Structure 1 has the lowest % error in k_{calc} relative to kexp of unknown 4a, and the observed MS² fragmentation can be explained by the position of the C-O bonds in the molecule (Fig. 1E). To locate the likely position of H in the alkyl chain, the % error in k_{calc} for isomers 1-4 were evaluated; based on the low % error (4.95 %) of structure 1 it is predicted that this is the most appropriate structure for unknown 4a. We also varied the position of oxygen in the molecule to predict if it is possible to separate these isomers experimentally. As shown in the k_{calc} for isomers 5–12, some of them are expected to coelute (e.g. isomer 7 and 9 and isomer 8 and 12) while others are likely to be baseline separated (e.g. isomers 5, 6, 7 and 8). For unknown 4b, several structures resulted in a % error for k_{calc} below 5% (e.g. isomer 1, 3, 7, 9 and 10, 13–17), however only isomer 10 can result to the MS^2 fragmentation observed. This can be explained by the presence of m/z= 246.9815 corresponding to C_5F_9O - as a result of C_5 - O bond breaking and a subsequent neutral loss of HF (Fig. 1F). Isomers 13-17 are possible branched isomers of unknown 4b. While error with respect to unknown 4b are all below 5%, MS² fragmentation observed does not support possibility of a branched isomer. These branched isomers are expected to have $CF_3O - (m/z = 84.991)$ and $-CF_3 (m/z = 68.996)$ as fragments. These results highlight the importance of combining MS² fragmentation and k values to distinguish between the isomers of PFASs.

Lastly, for unknown 5 in which all of the proposed isomers had k_{calc} with errors below 5%, only isomers 1 and 2 can be explained by the MS^2 fragmentation with m/z = 380.9766 resulting from the simultaneous neutral loss of CO_2 and HF from the precursor ion (SI, part IV - e). With this, isomer 1 could be the most probable structure; having the H and F reside on the same carbon (C_8) will likely have the highest

probability to loose HF as compared to structure 2 where H and F reside on different C atoms. The combination of lower k_{calc} error (-2.16 %) and MS² fragments support structure 1 to be the most probable structure for unknown 5.

To ensure that we get an accurate prediction for the regression, the $k_{\rm exp}$ values used to build the regression line were the average values of 3 different runs. For unknowns 1 and 5, which were detected in the water samples from the Philippines and Thailand (Guardian et al., 2020), we used an average $k_{\rm exp}$ from five samples, while for unknowns 2, 3 and 4 that were detected in the Chemours effluent sample, 3 separate runs were performed at varying voltages and the average $k_{\rm exp}$ from these runs was used.

As mentioned earlier, these unknowns were reported in previous studies (Guardian et al., 2020; McCord and Strynar, 2019) with a confidence level of 2b and 3, based on Schymanski classification (Schymanski et al., 2014). In this present work, level 2b can be assigned to the possible isomers identified for unknown 1 (structures 1-3) and 3b (structures 4-6). Level 2a can be assigned to the most probable structure assigned for unknown 2 (structure 1), 3a (structure 19), 4a (structure 1), 4b (structure 10) and 5 (structure 1), which is an improvement in the classification level previously reported for these compounds. This level assignment considers the fact that HRMS data were obtained using the same acquisition method applied to PFASs standards and MS² analysis was based on the fragmentation pattern observed for PFASs standard. Moreover, the k_{exp} of these unknowns matched k_{calc} (within ± 4.2 % error) as predicted using the relationship between COSMO-RS-derived Log Kow and k values established using PFASs standards.

3.7. Potential caveats

One major downside to this methodology is that our regression requires to have compounds that can form anions. In the future, we plan to build a regression that can be applied to molecules that form cations using the neutral and cationic log K_{ow} values to predict the k values of these compounds. To achieve this, more neutral and cationic PFAS analytical standards are needed. Another caveat is that due to the limited fragmentation of some emerging PFASs, determining plausible isomers based on the fragmentation pattern can be taxing because of the large number of possibilities for structures. However, as the number of available analytical standards in the training set increase, the predictive capability of the combined COSMO-RS based regressions and MS^2 fragmentation data for unknown identification should improve.

4. Conclusions

In conclusion, we have calculated the log K_{ow} values for both the neutral and the anionic forms of several legacy and emerging PFASs using COSMO-RS. We have also demonstrated that using the established relationship between calculated log K_{ow} values, calculated mean isotropic polarizability values, and k_{exp} values for 32 PFASs in our training set, we are able to obtain the k_{calc} values for 5 emerging PFASs within 4.2 % error through a simple multivariable regression. Moreover, the systematic study on HRMS fragmentation patterns of PFASs standards allowed elucidation of the structures of newly identified PFASs and facilitated proposal of putative chemical structures that are supported by characteristic fragment ions. The combination of accurately predicted k_{calc} values, accurate mass measurements, and characteristic MS² fragmentation patterns con facilitate the identification of the largely unknown structures of emerging PFASs that are continuously being unraveled in the environment.

CRediT authorship contribution statement

Mary Grace E. Guardian: Conceptualization, Methodology, Investigation, Writing - original draft. Jonathan P. Antle: Methodology, Investigation. Paul A. Vexelman: Methodology, Investigation. Diana S. Aga: Conceptualization, Writing - original draft, Supervision, Funding acquisition. Scott M. Simpson: Conceptualization, Methodology, Investigation, Writing - original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the NSF.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.123478.

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