Ion Exchange Solid Phase Microextraction coupled to Liquid Chromatography/Laminar Flow Tandem Mass Spectrometry for the Determination of Perfluoroalkyl Substances in Water Samples

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31 **ABSTRACT**

32 Per- and polyfluoroalkyl substances (PFAS) are toxic and bioaccumulative compounds that are

33 persistent in the environment due to their water and heat resistant properties. These compounds

34 have been demonstrated to be ubiquitous in the environment, being found in water, soil, air and

35 various biological matrices. The determination of PFAS at ultra-trace levels is thus critical to

36 assess the extent of contamination in a particular matrix. In this work, solid phase

37 microextraction (SPME) was evaluated as a pre-concentration technique to aid the quantitation

38 of this class of pollutants below the EPA established advisory limits in drinking water at parts-

39 per-trillion levels. Four model PFAS with varying physicochemical properties, namely

40 hexafluoropropylene oxide dimer acid (GenX), perfluoro-1- butanesulfonate (PFBS), perfluoro-

41 n-octanoic acid (PFOA) and perfluoro-1-octanesulfonate (PFOS) were studied as a proof of

42 concept. Analysis was performed with the use of ultra-high pressure liquid chromatography-

43 laminar flow tandem mass spectrometry (UHPLC-MS/MS). This study proposes the use of

44 hydrophilic-lipophilic balance-weak anion-exchange/polyacrylonitrile (HLB-WAX/PAN) as a

45 SPME coating, ideal for all model analytes. A sample volume of 1.5 mL was used for analysis,

46 the optimized protocol including 20 min extraction, 20 min desorption and 6 min LC/MS

47 analysis. This method achieved LOQs of 2.5 ng L^{-1} (PFOS) and 1 ng L^{-1} (GenX, PFBS and

48 PFOA) with satisfactory precision and accuracy values evaluated over a period of 5 days.

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS), also known as perfluorinated chemicals (PFC), are a class of compounds containing a fluorinated hydrophobic alkyl chain and a hydrophilic group, permitting these compounds to be both thermally stable and water repelling. These properties are exploited in the manufacturing of non-adhesive cookware, surface-active agents and stain-resistant carpets, in addition to ingredients in firefighting foams and paints since the 1950s [1–4]. These same properties, unfortunately, result in PFAS being resistant to degradation in the environment. Since their discovery as persistent environmental pollutants, there have been several regulations and bans on the use of longer-chain PFAS, legacy compounds such as perfluoroalkyl carboxylic and sulfonic acids (PFCA and PFSA) [5–7]. The US Environmental Protection Agency (EPA) agreed with fluorochemical manufacturers to phase out these long-chain compounds by bringing an end to their production by 2015 [8]. This has led to the use of PFAS alternatives named "emerging PFAS" (commonly short-chained C ≤ 7) [3,9–11].

In the last decade, most of the research on these chemicals largely focused on the detection of long-chain PFAS, their toxicity and accumulation to humans, and the environment. These compounds were linked to hepatotoxicity, immune and endocrine system disruption, degradation of lipids in cells, neurobehavioral disorders, tumors in multiple organ systems, neonatal toxicity and death amongst others [4,9,12]. It is critical to investigate the effects of the emerging PFAS in the environment to elucidate their fate, transport and partitioning in different environmental compartments and their effect on public health. However, this task cannot be accomplished without an attentive development of analytical methodologies able to detect PFAS

77 at ultra-trace levels and minimize the occurrence of matrix effects. In the literature, it has been

78 proposed by a variety of sources that food intake is the primary pathway for PFAS exposure.

79 However, the consumption of contaminated drinking water has also shown to be of great concern

80 [13]. PFAS contaminating drinking water mainly occurs as a result of traditional drinking water

81 treatment plants failing to remove these substances [14–16].

82   Multiple analytical methods have been developed for the determination of PFAS in water

83 and other environmental matrices (soil, air, house dusts and sediments) [4,17,18]. Owing to the

84 complexity of these matrices and PFAS being present at ultra-trace levels, the need for methods

85 that are sensitive, selective and fast has been on the rise [9,19]. Such methods include fluorine

86 nuclear magnetic resonance (19F NMR) [20,21], attenuated total reflected Fourier transform

87 infrared spectroscopy (ATR-FTIR) [22], capillary zone electrophoresis (CZE) [23,24], gas

88 chromatography mass spectrometry (GC–MS) [25–27], liquid chromatography mass

89 spectrometry (LC-MS), including tandem mass spectrometry (LC–MS/MS) [13,28,29].

90 Although, there are drawbacks involving some of these already developed techniques, for

91 example, CZE combined with indirect UV detector has low sensitivity of about 0.6–2.4 ppm

92 [13,24]. F NMR is a nonspecific method because of the determination of only CF_2 and CF_3

93 moieties in a sample [30]. The use of GC-MS is sensitive with detection limits at ppb levels, but

94 often requires derivatization [31]. Conversely, LC-MS/MS is highly selective and sensitive with

95 detection limits of low ng L^{-1}. However, the technique has been shown to require large sample

96 volumes to achieve the required sensitivity [19,32–37]. The sensitivity required, typically 70 ng

97 L^{-1} for PFOA and PFOS in drinking water [32,38,39], most often is achieved through LC-

98 MS/MS.

99   So far, to detect parts-per-trillion level PFAS, pre-concentration has been accomplished

100   by either solid phase extraction (SPE) or liquid-liquid extraction (LLE) [9,34,40]. These methods

101   have been demonstrated to be time consuming and use large volumes of organic solvents

102   compared to alternative methods like SPME and dilute-and-shoot [15,25,37,28,29,31,38].

103   Besides, SPE and LLE depend on evaporation and reconstitution for pre-concentration, which

104   often tend to be laborious with significant susceptibility to errors. Another method that has been

105   used in the quantitation of PFAS is dilute-and-shoot [32,33,35,42]. While this method ensures

106   high throughput, it can lead to loss of sensitivity due to the large amounts of dilution often

107   involved, and often require larger sample injection volumes. The approach may lead to increased

108   instrument maintenance time due to clogging issues in the analytical system (e.g., capillary

109   tubing, analytical column). Furthermore, substantial matrix effects can occur from the samples

110   during analysis, making the method not suitable and robust for such complex samples. Solid

111   phase microextraction (SPME), a sampling technology that integrates sampling, cleanup and pre-

112   concentration in one step, has been shown to allow the extraction of organic compounds from

113   both aqueous and biological matrices, sample volumes being much lower than other

114   conventional sample preparation methods [29,43–45]. It has been coupled with different

115   instrumentation techniques for the analysis of a broad group of organic compounds in food

116   [46,47], pharmaceutical [48,49], environmental [50,51] and biological analysis [52,53]. SPME is

117   a technique that consists of an extraction phase embedded on a solid support, which is then

118   exposed to a sample volume for a known period of time. The most widely used is the fiber

119   geometry, which is applied in this work [54]. Biocompatible SPME devices have been developed

120   to enhance reproducibility and fiber efficiency when working with complex samples [55]. These

121   biocompatible properties prevent fouling of the extraction phase and adverse reactions of the

122 system sampled; both important considerations for *in vivo* sampling [55]. The polymeric

123 materials that provide biocompatibility are often not sufficient to ensure adequate extraction of

124 certain compound classes, hence, they are combined with other solid sorbents [56]. Most SPME

125 methods for the determination of PFAS focused more on the development of new materials for

126 analysis rather than quantification and method development [29,41,43,57].

127   Hence, in this study, an SPME-LC-MS/MS method for the determination of perfluoro-n-

128 octanoic acid (PFOA), perfluoro-1-octanesulfonate (PFOS), hexafluoropropylene oxide dimer

129 acid (GenX) and perfluoro-1- butanesulfonate (PFBS) in water samples was developed

130 evaluating various sorbents, including HLB-WAX/PAN, which showed the best extraction

131 efficiency for the targeted analytes. These compounds were chosen due to their diverse

132 chemistries serving a model representation of PFAS. LOQs and linearity of the method achieved

133 exceeded EPA regulatory limits for PFAS in drinking water, matrix effects being evaluated for

134 river, lake, bottled and tap water. This developed protocol not only establishes SPME as a

135 reliable preconcentration method for the ultra-trace analysis of PFAS in aqueous matrices but

136 also lays the groundwork for future studies involving the biomonitoring of PFAS in more

137 complex samples. In addition, this work seeks to verify if SPME can be considered as an

138 alternative procedure to existing methods for the analysis of PFAS, and also understand how

139 selectivity of the extraction process is dependent not only from hydrophobic interaction but also

140 anion exchange mechanisms.

141 **2. Materials and Methods**

142 **2.1 Materials**

143   Standards for PFOA, PFOS, PFBS and GenX were purchased from AccuStandard (New

144   Haven, CT, USA). Isotopically labelled internal standards ($^{13}C_8$-PFOA, $^{13}C_8$-PFOS,$^{13}C_3$-GenX)

145   were obtained from Wellington (Ontario, Canada). LC-MS grade solvents (methanol, water and

146   ammonium formate) and reagent grade additives (ammonium hydroxide and ammonium acetate)

147   and sodium chloride salt were obtained from Fisher Scientific (Waltham, MA, USA). HCl and

148   dimethyl formamide were bought from Honeywell (Charlotte, NC) and Fisher Scientific

149   (Waltham, MA, USA) respectively. Phosphate- buffered saline (PBS) and polyacrilonitrile

150   (PAN) were purchased from Sigma Aldrich (St. Louis, MO, USA). The C_{18}/PAN, mixed-mode

151   (MM) and HLB/PAN fibers were kindly provided by Millipore Sigma (Bellefonte, PA, USA)

152   and HLB-WAX/PAN fibers manufactured according to procedures available in the literature

153   [58–60]. The length of the extraction phases was 1 cm and their thickness are as follows: the

154   C_{18}/PAN average thickness 42 µm, MM/PAN 45 µm, HLB/PAN 37 µm and HLB-WAX/PAN

155   35 µm. Ultra-pure water was obtained from a Milli-Q system (Barnstead, Thermo Fisher

156   Scientific).

157   **2.2 Stock standard preparation**

158   Individual standard stock solutions and primary stock solutions were stored in methanol

159   and kept at -20 °C while working solutions were prepared by diluting the stock solutions with

160   methanol and water (80:20, v:v) to achieve the desired concentrations. For the SPME calibration

161   curve, ultra-pure water was spiked at 5000 ng L^{-1} with the targeted analytes and diluted to give

162   final concentration levels of 0.5, 1, 2.5, 5, 10, 20, 50, 250, 500 and 1000 ng L^{-1} and internal

163   standards were spiked at 100 ng L^{-1}. These values were chosen to have a broad linear dynamic

164   range below the EPA regulatory limits and above what might be found in highly contaminated

165   samples. Each extraction was performed in triplicate and LC-MS analysis of each extract was

166 performed in quintuplicate. Standards for instrument calibration were prepared at 0.01, 0.05,

167 0.125, 0.25, 0.5, 1, 5, 10 and 30 µg L^{-1} concentrations while a 5 µg L^{-1} solution of the analytes

168 was used for instrumental quality control. All standards were prepared in methanol:water (80:20,

169 v:v).

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172 **2.3 Sample collection, storage and preparation**

173 Tap water, lake water, bottled water and river water samples were evaluated in this study.

174 According to EPA guidelines in Method 533 [38], the lake and river samples were collected

175 onsite using a high-density polypropylene (HDPE) bottle with 1 g L^{-1} ammonium acetate as a

176 preservative. Field reagent blanks also being taken according to EPA guidelines. Prior to sample

177 collection, the ultra-pure water used for the field reagent blanks was transferred from its

178 container to the HDPE bottle containing ammonium acetate. Tap water was collected from the

179 University of Toledo (Toledo, OH, USA); river water was collected from Ottawa River on the

180 campus of the University of Toledo (Toledo, OH, USA); Lake water from Lake Erie collected

181 from Maumee Bay (Maumee, OH, USA); and bottled water was purchased from a local grocery

182 store. All samples were stored at 4°C until analysis and analyzed within 30 days.

183 **2.4 Preparation of SPME fiber coating**

184 Preparation of the HLB-WAX/PAN fiber coating was applied according to the procedure

185 in [58–60]. Briefly, a slurry was made by mixing 5 g of polyacrilonitrile (PAN) and 72.5 mL of

186 dimethyl formamide (DMF) in a beaker. Afterwards, the mixture was then heated for 1 hour at

187 90 °C and 6.3 g of this mixture was mixed with the HLB-WAX particles (Oasis WAX, Waters

188 Corporation, Milford, MA, USA) in a scintillation vial after cooling and was left mixing

189 overnight before the coating process. Nitinol wires were etched with HCl (37%, v:v) before the

190 coating. Each coating layer was applied to 1 cm of the device by dipping in the slurry solution

191 and withdrawing at a speed of 0.5 mm/s. After deposition of each layer the device was cured for

192 1 min at 125 °C, an average of 4 layers were applied to guarantee a homogenous coating.

2.5 SPME procedure

194   All extractions were performed in a 2 mL glass vial with a PTFE cap. Two SPME fibers

195 were simultaneously used for the extraction and were introduced in the vial by piercing the vial

196 cap septum (**Fig. S1**). Prior to extraction, SPME fibers were conditioned in methanol:water (1:1,

197 v:v) for at least 30 min and rinsed briefly in ultra-pure water. Extraction was performed in a 1.5

198 mL sample volume for 20 min using vortex agitation at 1000 rpm. For river and lake water, there

199 was an additional brief rinsing step in ultra-pure water prior to desorption to ensure any

200 particulate from these matrices would not adhere to the fibers. This step did not lead to loss of

201 analyte as it is demonstrated in **Fig. S2**. After extraction, fibers were desorbed in methanol:water

202 (80:20, v:v) adjusted to pH 10 with ammonium hydroxide. Desorption was carried out in 100 µL

203 solution for 20 min using a plastic vial with insert of total capacity 100 µL before LC-MS/MS

204 analysis. The use of the insert allowed to completely submerge the fibers in the desorption

205 solution.

2.6 Liquid chromatography and mass spectrometry conditions

207   Chromatographic separation of PFAS compounds was performed using a QSight LX50®

208 binary UHPLC pump, autosampler and column compartment (PerkinElmer Inc., Waltham, MA,

209 USA), with a Brownlee SPP C18 column, (50 mm x 3 mm, 2.7 µm), at a column temperature of

210 30°C. A delay column, Brownlee SPP C18, (50 x 3 mm, 2.7 µm) was used to trap the system

211 related PFAS for more reliable and accurate quantification. The total run time was 6 min with an

212 injection volume of 10 µL (partial loop injection, total loop size 20 µL). Mobile phases A and B

213 were water and methanol respectively both containing 5 mM ammonium formate. The elution

214 gradient is as follows: 0 – 0.5 min, 95% A, decreasing to 35 % at 1.5 min, then to 0 % A between

215 4 – 4.5 min, and from 4.6 – 6 min held at 95 % A. A triple quadrupole mass spectrometer QSight

216 220® (PerkinElmer Inc. Waltham, MA, USA) with heated electrospray ionization (HESI)

217 operated in the negative mode, was used for analyte detection and quantification. All the analytes

218 and internal standards were monitored in multiple reaction monitoring (MRM) mode. A

219 Parker/Balston nitrogen generator system (Parker Hannifin Corporation, Lancaster, NY, USA)

220 was used to produce the nitrogen gas flow for the ESI source, the laminar flow ion guide, and the

221 collision cell. The MS optimized conditions include ESI voltage -3000 V, drying gas and

222 nebulizer gas 120 and 200 respectively, source and HSID temperature 370 °C and 200 °C

223 respectively. A minimum of 2 MRM transitions were monitored for each analyte with the most

224 intense/reproducible transition being used for quantitation and the other as a qualifier, as

225 described in **Table S1**. Suggested fragmentation patterns for each analyte are shown in **Fig. S3**.

226 **2.7 Data analysis and method validation**

227 Data acquisition and processing was performed with Simplicity 3Q® software (version

228 3.11142) (PerkinElmer Inc., Waltham, MA, USA). Any additional statistical analysis of the

229 processed data was completed using Excel 2016 (Microsoft Corporation, Albuquerque, NM,

230 USA). Prism 5 (Graphpad Software, La Jolla, CA, USA) was used for the graphical

231 representation of the calibration curves for the comparison between SPME and dilute and shoot

232 methods. For method optimization, the amount of PFAS extracted by the SPME fibers, expressed

233   in ng, was calculated by injecting standard solutions of the model analytes at known

234   concentrations ranging from 0.01 to 30 µg L^{-1}.

235   Method Validation: The validation of this method was performed in accordance with the

236   EPA Method 533 on the determination of PFAS in drinking water in terms of limits of

237   quantification (LOQs), selectivity, linearity, accuracy and precision [38]. Acceptable criteria for

238   accuracy of the mean recovery should be between 70 and 130%. Instrument carryover was also

239   studied by injecting solvent and instrument blanks after a 30 µg L^{-1} analyte mixture injection.

240   Accuracy and precision were evaluated at 15, 70, 750 ng L^{-1}, and 1.5 µg L^{-1} (in triplicate) within

241   5 days. Weighting factors of 1/x were used for all linear regressions. LOQs were accessed based

242   on the lowest point on the calibration curve which exhibited both accuracy (80 – 120 %) and

243   precision (less than 20 % relative standard deviation) in accordance to FDA guidelines [61].

244   **3. Results and Discussion**

245   **3.1. Optimization of extraction conditions**

246   The use of solid sorbents for the extraction or removal of PFAS has been well

247   documented in the literature [62,63]. In the case of SPE techniques, C$_{18}$, HLB and HLB-WAX

248   extraction phases have been successfully used for sample pretreatment and subsequent

249   quantitation [36,37,64,65]. Chemically, PFAS can be characterized by their a) hydrophobic tail,

250   b) hydrophilic head and c) acid/anionic moiety. The model compounds chosen in this study vary

251   based on the length of their hydrophobic tail and the chemical functional group that constitutes

252   the hydrophilic head. PFBS and PFOS containing sulfonic acid moieties whereas GenX and

253   PFOA have carboxylic acid heads (**Table S2,** Supplementary Information). Also, this selection

254   allows the evaluation of two legacy (PFOS and PFOA) and emerging (GenX and PFBS) PFAS.

255   To exploit these shared features of PFAS, extraction phases were chosen based on their potential

256   interaction with the hydrophobic tail and hydrophilic head of these compounds. C_{18}, HLB, HLB-

257   WAX and mixed-mode (MM) were evaluated in this study as sorbents for PAN-based SPME

258   extraction phases, and their structures are presented in **Fig. S4**. As shown in **Fig. 1**, extraction

259   phases that predominantly interact through hydrophobic interactions such as C_{18} favor the

260   recovery of long-chained PFAS while poorly extracting the shorter-chain and more polar PFAS

261   such as GenX and PFBS. The HLB-based extraction phase demonstrated great potential because

262   its chemical moieties interacted better with both the hydrophilic head and the hydrophobic tail of

263   PFAS. HLB demonstrated balanced coverage of all analytes, outperforming C_{18} for the extraction

264   of PFBS. This balanced coverage, however, resulted in lower recoveries of PFOA and PFOS, the

265   two longer-chained PFAS evaluated, compared to the C_{18}/PAN extraction phase. The HLB-

266   WAX/PAN fiber retained the balanced coverage provided by HLB-based extraction phase while

267   increasing all analyte recoveries substantially. These results are in agreement with previous

268   studies [66] that compared HLB and HLB-WAX based extraction phases with SPE and

269   demonstrated that though HLB extracted the longer chained PFAS, WAX moiety was crucial for

270   the extraction of short-chained perfluorocarboxylates. This phenomenon can be explained

271   because WAX- based extraction phases, being cationic at certain pH ranges, allow anion

272   exchange with the negatively charged acidic moieties of PFAS. WAX is preferred to SAX

273   (strong anionic exchange) as its reversible interactions can be easily tuned for quantitative

274   desorption of the analytes [37,67]. Contrarywise, SAX remains positively charged regardless of

275   the pH in the solution. The MM coating in this study consisted of octyl (C_8) and sulfonic acid

276   moieties, the coating demonstrating a poor extraction efficiency as shown in **Fig. 1**. This is most

277   likely due to C_8 having less hydrophobic interaction compared to the C_{18} fiber and the sulfonic

278   acid group being negatively ionized and repelling the negatively charged PFAS. HLB-

279   WAX/PAN was chosen as the optimal extraction phase for the model analytes and was used for

280   further optimization.

281   Sample pH was next optimized to better investigate the anion-exchange interaction

282   afforded by the WAX moiety. WAX moieties consist of either a primary, secondary or tertiary

283   amine functional group that can be positively charged at lower pH values and at high pH become

284   neutral. The moiety used in this study is a piperazine, with pKa values approximately 6 and 9

285   [68]. As most PFAS are generally negatively charged in aqueous solution, pH optimization was

286   performed to ensure the WAX moiety was cationic to properly interact with the analyte during

287   sampling. pH values of 4, 7 and 10 were evaluated in this study. As demonstrated in **Fig. 2A**, the

288   extraction of PFAS with the HLB-WAX coating was most efficient at a pH value of 7. At pH 10,

289   the weak cation moieties are neutral while at pH 4, they are both protonated. We propose that

290   simultaneous protonation of both amino groups on the WAX moiety does not provide efficient

291   extraction, especially for the bulkier PFAS. This phenomenon could be due to steric hindrance,

292   especially for long chain PFAS such as PFOA and PFOS, which may not efficiently interact with

293   the piperazine moiety of the sorbent when both amino groups are protonated. At pH 7, one N-H

294   moiety is neutral while the other is cationic, allowing balanced extraction of PFAS through the

295   anion-exchange interactions offered by WAX and the hydrophilic/lipophilic interactions

296   provided by HLB. Moreover, the effect of the ionic strength on the extraction process was

297   evaluated, enriching the sample with 0, 5, 10, 15 and 20 (w/v %) sodium chloride. Previous

298   reports have demonstrated minor or deleterious effects as a result of increasing the ionic strength

299   when extracting PFAS using solid sorbents, [29]. Results in **Fig. S5** show that ionic strength

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300   played a very minor role in extraction, thus, to guarantee higher throughput of the sample

301   preparation process, no salt was added in the optimized protocol.

302   In addition to the physicochemical parameters being optimized for SPME extraction

303   efficiency, the amount of extraction phase was also optimized. Previously reported by Godage et

304   al., the simultaneous use of two SPME fibers in one sample for the extraction of nicotine and its

305   metabolites was able to increase the extraction efficiency when used in a matrix such as

306   phosphate-buffered saline solution (PBS) [69]. In this work, dual fiber extraction was evaluated

307   in comparison to the use of a single fiber (**Fig. 2B**), results demonstrating an increase in

308   extraction efficiency. This experiment was performed both at equilibrium (**Fig. 2B**) and pre-

309   equilibrium (**Fig. S6)** conditions. From the results obtained, at pre-equilibrium conditions the

310   response for dual fiber extraction is more than twice the response for single fiber extraction. This

311   is possibly due to the surface area of the extraction phase contributing a larger role in extraction

312   kinetics at pre-equilibrium conditions. Conversely, when extracting at equilibrium conditions,

313   the response will be only related to the total amount of extraction phase and not the surface area,

314   revealing that the dual fiber approach extracted approximately double the amount of analyte than

315   when a single fiber is used. This phenomenon can be explained by Equation 1 [54]:

316                      $$n_{eq} = \frac{K_{es} V_e V_s}{K_{es} V_e + V_s} C_s$$ (1)

317   where n_{eq} is the amount extracted at equilibrium, K_{es} is the distribution constant of analyte

318   between the sample and extraction phase, V_e is the volume of the extraction phase, V_s is the

319   volume of sample and C_s is the initial concentration of analyte. In addition, enrichment factors

320   [70] were calculated (**Fig. S7**) for the dual fiber extraction at equilibrium and values include 94

321   (PFBS and PFOS), 80 GenX and 100 PFOA. In this study, a sample volume of 1.5 mL was

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322 chosen to be well suited for both conventional 2 mL LC vials and 96-well plates. In fact, reports

323 have shown that applying SPME to a 96-well plate format increases throughput dramatically and

324 can be easily automated [71]. When further sensitivity is needed, future work could involve

325 evaluating if increased sample volume will substantially improve the extraction to achieve even

326 lower limits of detection for PFAS. However, large enough sample volumes will eventually not

327 affect extraction efficiency (when $V_s \gg K_{es}V_e$) [54]. Previous studies have utilized large

328 volumes of sample to achieve low ng L^{-1} detection, SPE [28,38] and former SPME studies using

329 20 mL [19,29]. These large volumes not only produce more waste, but also make further

330 development of the sample preparation protocol into an automated high-throughput system more

331 challenging. With the extraction conditions now optimized, the effect of extraction time was

332 evaluated as shown in **Fig. 2C**. The extraction time profile was constructed by using the

333 optimized SPME extraction protocol at time points 10, 20, 30, 45, 60 and 90 min to evaluate the

334 equilibration time of the analytes between the extraction device and the sample. Compromising

335 both sensitivity and throughput, pre-equilibrium extraction using 20 min extraction time was

336 selected, further studies potentially increasing extraction time if sensitivity is an issue.

337 **3.2. Desorption Conditions**

338 Desorption conditions were optimized to ensure quantitative desorption of analytes from

339 the extraction phase while maintaining the throughput of the method. Moreover, as the HLB-

340 WAX/PAN coating was found to be optimal for extraction, it was essential that the pH of the

341 desorption solution was able to neutralize the WAX functional groups to facilitate the

342 quantitative desorption of the model analytes. A desorption solution of methanol:water (80:20,

343 v:v) was chosen according to results from EPA method 533, which uses this solvent

344 composition as the optimal solution for elution of PFAS from SPE cartridges [38]. To evaluate

345 the pH of this desorption solution, pH values of 7, 8, 9 and 10 were chosen. As shown in **Fig. S8**,

346 desorption solution pH seemed to have minor effect on the analytes desorbed from the SPME

347 fiber, within the experimental error obtained. For pH 9 and 10 there is no significant difference

348 with the amount of analytes desorbed while on the other hand, for pH 7 (PFBS and PFOS) and

349 pH 8 (PFBS, PFOA and PFOS) there are minor differences. At a glance, results in **Fig. S8** appear

350 to demonstrate very little correlation between the pH of the desorption solution and the amount

351 desorbed. However, when performing a second desorption to verify exhaustive desorption, it was

352 found that at lower pH values non-negligible amounts of PFAS retained on the fiber (**Fig. S8B**).

353 As stated earlier, at pH 10 both ionizable WAX functional groups are neutral, thus desorption is

354 facilitated and the occurrence of carryover avoided. With the pH of the methanol:water (80:20,

355 v:v) desorption solution optimized at pH 10, the effect of desorption volume on the method's

356 sensitivity was also evaluated to ensure maximum sensitivity and preconcentration. Samples

357 spiked near the limit of quantitation (5 ng L^{-1}) were desorbed in 320 and 100 µL of desorption

358 solution. Results shown in **Fig. S9,** revealed that 100 µL desorption volume provides optimal

359 pre-concentration to detect PFAS at low ng L^{-1}. Moreover, an evaluation of the stability of PFAS

360 in desorption solution was performed to check the analyte shelf life when stored in glass and

361 plastic vials. From the results obtained (**Fig. S10**) the stability test was investigated over a period

362 of 28 days. It was observed that the analytes, particularly the long-chained PFAS (PFOS and

363 PFOA), show similar stability in both glass and plastic vials. One important consideration during

364 analysis is to regularly vortex the solution mixtures, as the compounds can adhere to the wall of

365 the vials or settle at the bottom as observed by Prakash *et al*.,[72]. A desorption time profile was

366 constructed from 10 to 90 min using the optimized desorption solution (**Fig. S11**), 20 min being

367 sufficient to desorb all analytes with no observed carryover. **Fig. S11B** shows the desorption

368 time profile performed in a solution of 100% methanol which was compared to that of the

369 methanol:water (80:20, v:v). The amount desorbed from the fiber was higher with the

370 methanol:water (80:20, v:v) and was used for further studies in this work.

3.3. LC-MS optimization

372 **Fig. 3A** demonstrates the chromatogram obtained under optimized conditions in this

373 study. It should be noted that the standard utilized in this study for PFOS also contains another

374 constituent that elutes earlier, presumably its branched isomer, which was not integrated or

375 optimized for in this study [72]. Representative chromatograms of PFOA and PFOS are shown at

376 their respective LOQs (1 and 2.5 ng L^{-1}) in **Fig. 3B**, the developed LC-MS method

377 demonstrating very low levels of background or interferences (other compounds represented in

378 **Fig. S12**). This reduction in interferences is particularly important for PFAS as they are

379 ubiquitous in most LC systems, and as such a delay column is necessary to discriminate these

380 PFAS from the ones found in the sample. Injection volume was evaluated at 5, 10 and 20 µL, as

381 demonstrated in **Fig. S13**, to determine both the best sensitivity and chromatography. Results

382 show the lowest sensitivity with 5 µL and the poorest chromatographic performance with 20 µL,

383 thus 10 µL injection volume was selected as the best compromise. With the injection volume of

384 20 µL, the peaks for the early eluting analytes (PFBS and GenX) were highly distorted, possibly

385 due to the increased volume of organic solvent in the injection. This correlates to the significant

386 difference in the composition of the starting mobile phase conditions and the injection solvent,

387 resulting in solvent mismatch. If larger volumes of injection are needed in further applications of

388 the method, the composition of the desorption solution would need to be re-optimized,

389 compromising between desorption efficiency and optimal chromatography.

3.4. Method Validation

391   The linearity of the method was evaluated in ultra-pure water samples within a

392   concentration range of 0.5 ng L^{-1} – 5000 ng L^{-1}. Calibration curves of all model analytes can be

393   found in **Fig. 4**, the optimized method showing good linearity for all target analytes ranging from

394   1 ng L^{-1} (except for PFOS with a LOQ of 2.5 ng L^{-1}) to 5000 ng L^{-1}. Method LOQs and other

395   figures of merit are shown in **Table 1,** with obtained results below the regulatory limits achieved

396   with the EPA methods [38,39]. Method accuracy determined at 15, 70, 750 ng L^{-1} and 1.5 µg L^{-1}

397   were also analyzed within 5 days to assess the inter-day reproducibility of the method (**Table**

398   **S3**). The figures of merit of this study were compared to other methods found in the literature in

399   **Table 2**. This work was able to reliably quantitate each analyte below the recommended limits of

400   PFAS in drinking water (70 ng L^{-1}). Moreover, in comparison to previous reports found in the

401   literature, that report 75 to 65 min of workflow our sample preparation protocol took a total of 40

402   min for each sample [29,43], with the potential of further reducing the total analysis time using

403   simultaneous automated extraction of multiple samples. With the potential of automating this

404   method into an automated 96-well plate format, sample throughput would be less than a minute

405   per sample. In addition, the developed method uses far lower sample volume (1.5 mL) compared

406   to other studies (up to 250 mL in the case of SPE extraction) [38,39]. It is worth mentioning that

407   contamination of samples can arise when performing SPE due to the use of various consumables,

408   each potentially able to leach PFAS. Compared to methods that require very fast sample

409   processing such as dilute and shoot, our method provides higher preconcentration and limits

410   occurrence of matrix effects.

411   To investigate the applicability of the developed SPME protocol to environmental

412   samples, lake, river, tap and bottled water were analyzed and their matrix effects evaluated.

413   Results shown in **Fig. S14**, reveal that none of the model PFAS were detected in the analyzed

414   matrices. The occurrence of matrix effects was also evaluated by testing, for each matrix

415   collected, the accuracy of samples spiked at concentration levels of 15, 50 and 250 ng L^{-1}, using

416   the calibration curve equation obtained from the ultra-pure water calibration. As demonstrated in

417   **Table S4**, all points fall within the established line of best fit for the calibration performed in

418   ultra-pure water, accuracy being between 70 and 130 % for all the spiked samples and RSD

419   being less than 10% for most analytes. PFOA and PFOS had accuracy values lower or higher

420   than the acceptable limits for some water samples. For PFOA, values at 15 ng L^{-1} for bottled,

421   lake and tap water while at 50 ng L^{-1} for bottled, lake and river water samples and that of PFOS

422   was lake water at 15 ng L^{-1}. This can be as a result of matrix effect occurrence during analysis.

423   Additionally, the effects of dilution and pre-concentration were also investigated to compare the

424   developed method to dilute-and-shoot methods (EPA 8327 method) [32]. This was evaluated by

425   comparing the response and sensitivity of the SPME method with a classical dilute-and-shoot

426   method at various concentration levels that bracket the EPA regulatory limit of 70 ng L^{-1} of

427   PFAS in drinking water (**Fig. S15**). From the results demonstrated, SPME showed higher

428   sensitivity for all analytes with the slope of the calibration curve being up to 10 times higher than

429   the dilute-and-shoot method. This increased sensitivity as an effect of the pre-concentration

430   provided by our method.

431   **4. Conclusion**

432   An efficient and convenient SPME method was developed for PFAS in aqueous matrices

433   and applied to river, lake, tap and bottled water samples. HLB-WAX/PAN SPME extraction

434   phase was highly selective for PFAS and thus has greater potential for use in further studies of

435   more complex samples in light of its biocompatibility. The EPA health advisory level for PFOA

436   and PFOS is 70 ng L^{-1} while GenX and PFBS are currently still being evaluated by EPA and

437   other environmental agencies. This developed protocol achieved LOQs of $1 - 2.5$ ng L^{-1} for the

438   model analytes with a sample volume of only 1.5 mL, demonstrating the efficacy of pre-

439   concentration offered by SPME for PFAS. Furthermore, the pre-concentration effects augmented

440   method sensitivity far greater than dilute-and-shoot methods, which is widely employed by

441   regulatory agencies for PFAS analysis. No substantial matrix effects from water samples were

442   observed for PFBS and GenX. Through the use of the developed protocol, a highly sensitive

443   quantitative analysis can be performed for PFAS of varying physicochemical properties even

444   below current EPA guidelines and methods. Further work could allow the investigation of this

445   method into a larger range of PFAS and similar contaminants. An adaption of this method to a

446   96-well plate format would also permit ultra-high throughput analysis of PFAS in aqueous

447   samples, it perhaps being modified for biological sampling as well.

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458   **References**

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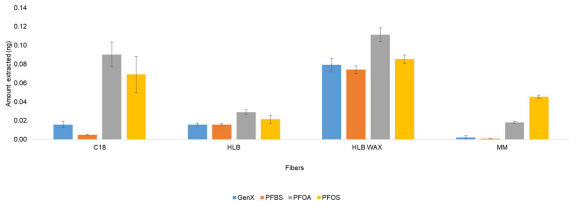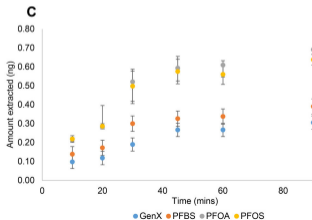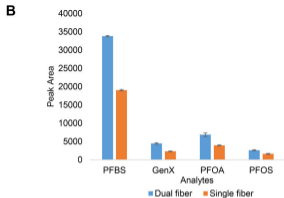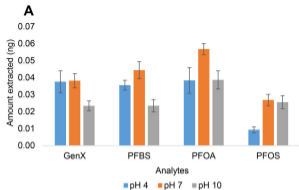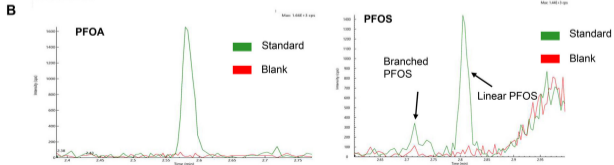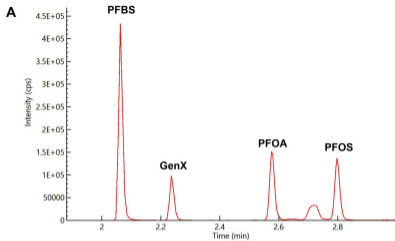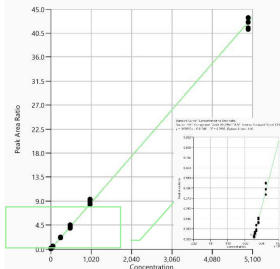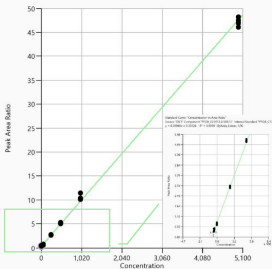
A Standard Curve: "Concentration vs Area Ratio"
Source "ESI" Component "PFBS_01 (298.8/79.9)" Internal Standard "GenX_C13"
y = 0.09332x + 0.19513   R² = 0.9957 (ByArea, Linear, 1/X)
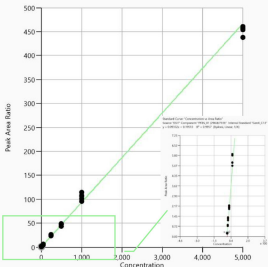
B Standard Curve: "Concentration vs Area Ratio"
Source "ESI" Component "GenX_01 (285/118.9)" Internal Standard "GenX_C13"
y = 0.00852x + 0.01340   R² = 0.9983 (ByArea, Linear, 1/X)

C Standard Curve: "Concentration vs Area Ratio"
Source "ESI" Component "PFOA_02 (413.2/369.1)" Internal Standard "PFOA_C13"
y = 0.00948x + 0.33026   R² = 0.9939 (ByArea, Linear, 1/X)

D Standard Curve: "Concentration vs Area Ratio"
Source "ESI" Component "PFOS_02 (499.1/99)" Internal Standard "PFOS_C13"
y = 0.00198x + 0.02315   R² = 0.9965 (ByArea, Linear, 1/X)
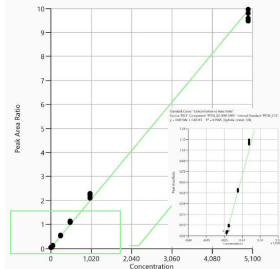
Table 1: Figures of merit for the optimized method for the quantitation of PFAS by SPME-LC-MS/MS.

Analyte	LDR (ng L^{-1})	R^2	Equation	LOQ (ng L^{-1})	S/N	Weight
PFBS	1 – 5000	0.9957	y = 0.09332x + 0.19513	1	16	1/x
GenX	1 – 5000	0.9983	y = 0.00852x + 0.01340	1	3	1/x
PFOA	1 – 5000	0.9939	y = 0.00948x + 0.33026	1	38	1/x
PFOS	2.5 – 5000	0.9965	y = 0.00198x + 0.02315	2.5	9	1/x

LDR; linear dynamic range, LOQ; limit of quantitation (described in section 3.2), S/N; signal-to-noise ratio (at LOQ)

Table 2: SPME and EPA methods for the determination of PFAS in water matrices.

Sample	Sample Preparation	Extraction Phase	Analytes	Instrumentation	LOQ	LDR	Ref.
Surface water	SPME	MM	PFOS, PFOA	LC-ESI-MS	2.5 – 7.5 ng L^{-1}*	10 – 10000 ng L^{-1}	[29]
Tap water, river water, pond water and water eluate from frying pan by heating	In-tube SPME	Amine functionalized PLOT column	PFOS, PFOA	LC-MS	1.5 – 3.2 ng L^{-1}*	50 – 5000 ng L^{-1}	[41]
Tap water, rain water and sea water	SPME	MOF	PFOA	nESI-MS	11.0 ng L^{-1}*	N/A	[15]
River water	SPME	PIL-POSS	PFOA, PFOS,PFHA, PFDA,	HPLC-ESI-MS/MS	5 – 80 ng L^{-1}*	100 – 50000 ng L^{-1}	[43]

			PFDoA,				
			PFTA				
			PFOS,				
			PFHxA,				
Lake water and			PFHpA,		0.21 – 1.98 ng	0.5 – 100	
	SPME	Wooden tip	PFOA, PFNA,	SPME-AMS	L^{-1}	ng L^{-1}	[57]
river water			PFDA,				
			PFUnDA,				
			PFDoDA				
Drinking water						500 –	
(EPA method	SPE	PS2	25 PFAS	LC-ESI-MS/MS	3.4 - 4.4 ng L^{-1} **	25000 ng	[38]
533)			compounds			L^{-1} ****	
Drinking water						40 – 160	
(EPA method	SPE	SDVB	18 PFAS	LC-ESI-MS/MS	0.82 – 6.3 ng L^{-1} **	ng L^{-1}****	[39]
537.1)			compunds				
Drinking water						10 – 400	
(EPA method	Dilute and Shoot	N/A	24 PFAS	LC-ESI-MS/MS	10 – 50 ng L^{-1}***	ng L^{-1}****	[32]
8327)			compounds				

River, lake, bottled and tap water	SPME	HLB-WAX/PAN	PFBS, GenX, PFOA, PFOS	LC-ESI-MS/MS	$1 – 2.5$ ng L^{-1}	$1 – 5000$ ng L^{-1}	This work

MM; Mix mode, **MOF;** metal organic framework, **PIL-POSS;** polymeric ionic liquid- polyhedral oligomeric silsesquioxane, **PS2;** polystyrene divinylbenzene with a positively charged diamino ligand, **SDVB;** polystyrenedivinylbenzene, **LDR**; linear dynamic range, **LOQ;** limit of quantitation,

*LOD; limit of detection.

** LCMRL; lowest concentration minimum reporting level.

*** LLOQ; lower limit of quantitation.

**** Calibration range.