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Short communication

Rapid characterization of perfluoralkyl carboxylate, sulfonate, and sulfonamide isomers by high-performance liquid chromatography–tandem mass spectrometry

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A rapid (<23 min) new HPLC–MS/MS method was developed for simultaneous characterization of 24 per- and polyfluoralkyl compounds in landfill leachate. In addition to isomer-specific analysis of perfluorooctane sulfonate and perfluorooctanoate, branched from linear isomer separation was accomplished for C6 and C10 perfluoralkyl sulfonates, C6, C7 and C9–C11 perfluoralkyl carboxylates, perfluorooctane sulfonamide and, for the first time, 3 perfluorooctane sulfonamidoacetates. The method utilizes a fused-core pentafluorphenylpropyl (PFPP) stationary phase and is approximately 4 times faster than previous comprehensive isomer-specific HPLC–MS/MS methods. This is the first isomer-specific methodology which can be adopted for routine analysis without sacrificing throughput from lengthy run times or limited target lists.

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1. Introduction

Per- and poly fluorinated compounds (collectively ‘PFCs’) encompass a diverse class of substances manufactured for over 60 years for numerous commercial processes and consumer products. Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are among the PFCs garnering international attention due to their widespread global distribution [1], persistence in humans [2] and links to adverse health outcomes in lab animals and humans [3].

Most PFCs have been commercially manufactured as isomeric mixtures by electrochemical fluorination (ECF) and/or as single (typically linear) isomers by telomerization (reviewed elsewhere [4]). Several studies have highlighted the application of isomer-specific analysis for manufacturing [5] and exposure [6] source elucidation, and for potential improvements in analytical accuracy [7,8]. The importance of isomer-specific analysis is further exemplified by evidence of isomer-specific toxicity [9] and pharmacokinetics [10], along with highly variable PFOS isomer profiles in humans [7,11,12] and wildlife [13,14].

Despite the potential utility of isomer-specific methodologies, few are currently available. Isomer-specific gas chromatography–mass spectrometry (GC–MS) methods are limited to perfluoralkyl carboxylates (PFCAs), [15] sulfonates (PFSAs), [16] or certain perfluorooctane sulfonamides (FOSAMs), [4] but not all three classes simultaneously (Table 1). Liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based methods are more common, but these tend to suffer from lengthy run times (95–115 min; Table 1), or are limited to single compounds (typically PFOS; Table 1). Ultra performance liquid chromatography (UPLC) has also shown promise for isomer-specific analysis of PFOS and PFOA [17,18], but the additional costs associated with these systems may be prohibitive for some labs.

The lack of characterized isomeric mixtures (currently only available for PFOS and PFOA) has hampered development of new isomer separation methods. While a few PFCs (e.g. perfluorohexane sulfonate (PFHxS) or perfluorooctane sulfonamide (FOSA)) can be obtained as uncharacterized technical isomeric mixtures, others (e.g. perfluorooctane sulfonamido acetate; FOSAA) are only available as the linear isomer.

The objective of the present work was to develop a rapid, isomer-specific methodology for PFOS and PFOA which also facilitates “total branched” from linear isomer separation for other PFC targets, including PFCs for which branched isomers have not been previously reported. A fused-core, pentafluorophenylpropyl (PFPP) stationary phase was tested based on prior success in resolving PFC isomers using fluorinated stationary phases [19,20]. Technical standards (containing branched and linear isomers) of PFHxS, FOSA, PFOA, and PFOS were utilized for initial method development. To assess the method’s potential for chromatographically
resolving PFC isomers in real samples, and to examine resolution of PFC isomers for which branched isomer standards are not commercially available, we applied the method to landfill leachate samples. PFC isomer profiles in landfill leachate have not previously been examined; however elevated concentrations of ECF–manufactured PFCs have been reported consistently in leachates from Europe and North America, [21,22] increasing the likelihood of branched isomer detection.

2. Experimental methods

2.1. Standards and reagents

Reagents are listed in the Supporting Information (SI). Linear isomers of perfluoropentanoate (PFPA), perfluorohexanoate (PFHxA), perfluorooctanoate (PFOA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnA), perfluorodecaneoate (PFDoA), perfluorotetradecanoate (PFPTA) and perfluorobutanesulfonate (PFBS), purchased from Sigma–Aldrich (Milwaukee, WI), were ≥97% chemical purity. Wellington Laboratories (Guelph, ON, Canada) supplied characterized isomeric mixtures of PFOS and PFOA, isotopically labeled internal standards (ISs; Table S1), linear isomers of perfluorobutanoate (PFBA), perfluorodecanesulfonate (PFDS), N-ethyl FOSAA (NMeFOSAA), N-methyl FOSAA (NMMeFOSAA), and the fluorotelomer acids perfluorohexylmethanoic acid (PFHEA), perfluorooctylmethanoic acid (PFOEA), perfluoroheptyl-2-ethanoic acid (PFHEA), perfluoroctyl-2-ethanoic acid (PFUEA), perfluorodecyl-2-ethanoic acid (PFDEA), and perfluorooctane sulfonamide acetate (FOSAA). Uncharacterized, isomeric mixtures of PFHxS and FOSA were obtained from Sigma–Aldrich and Sygnost Labs (Alachua, FL), respectively.

2.2. Leachate sampling, extraction and treatment

Leachate (4L) was obtained April 13, 2010 from a Municipal Landfill as part of a larger study on PFC occurrence and time trends [23]. The landfill processes ~2000 tonnes/day of solid waste and pumps leachate off-site for treatment. Extractions were performed on unfiltered subsamples (3 × 50 mL) using solid-phase extraction (SPE) [21], details of which are provided in the SI.

2.3. Instrumental analysis

PFC isomer separation and quantification were accomplished by LC–MS/MS using a Dionex HPLC coupled to an API 5000Q triple quadrupole mass spectrometer (Applied Biosystems/Sciex, Concord, ON, Canada) operated under negative ion, multiple reaction monitoring mode (see Tables S1 and S2 for instrument parameters and product ions). Extracts (10 µL) were injected onto an Ascentis Express F5 PFP Column (2.7 µm, 90 Å, 10 cm × 2.1 mm, Sigma–Aldrich) equipped with an Ascentis Express F5 PFP guard column (2.7 µm, 5.0 mm × 2.1 mm), both maintained at 30 °C. Two Waters Xterra columns (each 5 µm, 30 mm × 4.6 mm) connected in series were placed directly upstream of the injector to separate PFCs originating from the LC pump from those injected onto the analytical column. The mobile phase consisted of 100% MeOH (solvent A) and 20 mM ammonium formate/20 mM formic acid in water (solvent B) maintained at a 250 µL/min flow rate. Gradient conditions were: 90% B for 1 min, 40% B by 3 min, 12% B by 14 min, 0% B by 14.5 min, 0% B by 14.5 min, then 90% B and equilibrate for 6.5 min. A diverter valve (VICI Valco Canada, Inc., Brockville, ON, Canada) was placed downstream of the analytical column to divert flow to waste for the first 8 min of the run.
2.4. Assessment of method accuracy, precision and QA/QC

Triplicate spike/recovery experiments were performed utilizing 50 mL leachate spiked with 10 ng of individual PFCs and ISs. Method accuracy was calculated relative to standards spiked directly into MeOH (see SI for details). Method precision was assessed using percent relative standard deviation (% RSD) of spike/recovery experiments. Field blanks (Milli-Q water, n = 3) were transported to the sampling location and exposed to air briefly. Method detection limits (MDLs; Table S3) were calculated for 50 mL leachate samples (1 mL final volume) based on S/N = 3 using a low concentration standard (i.e. 3–10 × MDL).

2.5. PFC isomer identification and quantification

PFOS and PFOA isomer structures and nomenclature are provided in Figs. S1 and S2. With the exception of FOSA (which produces one major product ion, m/z 498/78), isomer peaks were distinguished from interferences by multiple product ions eluting within 4 min of the linear isomer peak. When multiple isomers were present for a given PFC, both linear and $\sum$ linear + branched isomer concentrations were determined. Characterized isomer mixtures were only commercially available for PFOS and PFOA, and for these compounds, quantification was performed using calibration curves for (a) individual isomers present in the mixture, and (b) the linear isomer and $\sum$ branched isomer peaks [7]. For PFHxS and FOSA (the only other standards containing measurable branched content), calibration curves were based on $\sum$ linear + branched isomer peaks. The remaining PFCs were quantified using linear isomer standards. Six-point calibration curves (linear, $r^2 > 0.99, 1/x$ weighting) were constructed using analyte response relative to an IS (Table S2). The accuracy of individual calibration points was 70–130%.

3. Results and discussion

3.1. Analytical method development and QA/QC

Several mobile phases were examined (combinations of ACN, MeOH, ammonium formate, formic acid, acetic acid, and ammonium acetate) and 100% MeOH and 20 mM ammonium formate/20 mM formic acid in water was found to be most effective in terms of speed, sensitivity, and isomer resolution, consistent with others [20]. Figs. 1 and 2 show optimized separation of PFOS and PFOA isomers in standards (discussed later). Structural elucidation of individual PFOS and PFOA isomers was accomplished using collision-induced dissociation patterns from Refs. [4,19,20] and product information sheets from Wellington Labs.

Following optimization of PFHxS, PFOS, PFOA, and FOSA isomer separation using technical standards, the method was applied to landfill leachates. Method accuracy averaged 119% for target PFCs and 104% for ISs. Precision ranged from 4 to 19% RSD for targets and 2 to 7% RSD for ISs (Table S3). Method accuracy and precision are expected to improve with the use of additional ISs, in particular for FOSA mixtures, which utilized $^{13}$C-PFOS as an IS in the present work. Dilution may also reduce matrix effects, but was not pursued because...
this raised branched isomer MDLs. PFC concentrations in blanks were typically over an order of magnitude lower than concentrations in samples (Table S3).

3.2. Resolution of PFOS, PFOA, PFHxS, and FOSA isomers in standards and leachate

Five PFOA isomer peaks were observed using m/z 413/369, which were further resolved to 8 individual isomers (1-, 6-, 5-, 4-, and 3-PFOA along with 3 dimethyl branches; see Fig. S2 for structures/nomenclature) using multiple product ions. The resolution of PFOA isomers here is comparable to a previous study but was accomplished in 15 min versus 45 min, respectively [5]. Of the 8 isomers observed in our standard, 1-, 6-, 5-, 4-, and 3-PFOA were observable in leachate (Fig. 1). Branched content was lower in leachate (10% by wt) compared to our standard (21%), which cannot be explained by the absence of dimethyl branches, since these isomers account for <1% of isomers in commercial ECF PFOA. Alternatively, contributions from telomer-manufactured PFOA or PFOA-precursors (i.e. linear isomers) may decrease observed branched content. Branched content calculated using individual PFOA isomer calibration curves compared to \( \sum \) branched isomer calibration produced consistent results (11 versus 10% branched, respectively; Table S4).

Six branched PFOA isomer peaks were distinguishable using m/z 499/80 (Fig. 2), and these were further resolved to 10 isomer peaks (1-, 6-, 5-, 4-, 3-, 1-, dm1-, dm2-, dm3- and dm4-PFOA, see Fig. S1 for structures and nomenclature) using multiple ions (Table S1). Leachate contained higher branched PFOA than our technical standard (40 versus 21% by wt, respectively). Isomer-specific analysis of leachate (Table S5) produced similar branched content to that determined using a curve based on \( \sum \) branched isomer peaks (39 versus 40%, respectively). The higher branched content in leachate relative to 3 M PFOA (~30% branched [4]) may reflect isomer-specific fractionation processes [10,13].

Resolution of PFHxS isomers was comparable to previous work, but was accomplished in < 12 min versus 27 min reported elsewhere [20]. In leachate and standards, linear PFHxS and 3 branched isomer peaks were observed using m/z 399/80 (Fig. 3). An additional peak was observable using m/z 399/99 for a total of 5 isomers. The relative contribution of branched PFHxS isomers in leachate was greater than in standards (24 versus 8%, respectively, using the average of branched and linear response factors (RFs)). The weight % branched content could not be calculated in leachate because characterized technical PFHxS standards are unavailable.

Five FOSA isomer peaks were observed in both standards and leachate using m/z 498/78; further resolution of FOSA isomers was hindered by poor sensitivity among secondary ions. The actual
Fig. 3. Chromatograms of (A) PFSA isomers and (B) FOSAM isomers in landfill leachate. ‘L’ denotes the linear isomer (assumed based on relative intensity and late elution) and ‘B’ denotes branched isomers. Branched peaks were confirmed using a technical standard (PFOS, PFHxS, FOSA) or multiple product ions (PFDS, FOSAA, NMeFOSAA, NEtFOSAA). No branched isomers were observed for PFBS.

Fig. 4. PFCA isomers in landfill leachate. Branched peaks were confirmed using a technical standard (PFOA) or multiple product ions (PFHxA, PFHpA, PFNA, PFDA, PFUnA).
number of isomers present is likely closer to that of PFOS (i.e. 10 isomers), since both are derived from perfluorooctane sulfonetyl fluoride \((C_8F_{17}SO_2F)\) \([4,6]\). Branched content in our standard accounted for slightly less of the total FOSA concentration than in leachate (35% versus 47%, respectively), consistent with observations for PFOS and PFHxS.

### 3.3. PFC isomer identification in the absence of standards

For PFCs in which isomeric mixtures were unavailable we relied on a combination of multiple product ions and retention times within 4 min of the linear isomer peak for confirmation of branched isomers in leachate. For PFBS, PFBA, PFPA and fluorotelomer acids, only linear isomers were observed. This is not surprising for PFBS since C4 perfluoroalkyl chains are too short to undergo rearrangement during ECF \([24]\). The remaining PFCs are likely produced by telomerization, thus branched isomers are not expected.

For PFHxA, PFHpA, PFDA, and PFUnA, one branched isomer peak was observed per compound (in addition to linear), while for PFNA, 2 branched isomer peaks were observed (Fig. 4). Among the PFCs, branched isomers accounted for <6% of total concentrations in leachate (estimated using linear isomer RFs), except for PFUnA, in which a single branched isomer made up 40% of the total PFUnA in leachate. Multiple branched chain, non-C8 impurities can arise during ECF \([4]\), but a single major branched isomer is unexpected. Possible explanations for this isomer include formation during polymer fluorination \([25]\) or intentional manufacture by telomerization \([15]\). Further work is necessary to elucidate the environmental prevalence and origin of this isomer.

For PFDS, 9 branched isomer peaks were observed in m/z 599/80, accounting for 83% of the total PFDS concentration. While these were chromatographically resolved from the linear isomer, partial separation was achieved among individual branched isomers. Little is known about the origin of PFDS, but it may arise as an impurity during ECF manufacturing of PFOS \([4]\).

Linear FOSA was distinguishable from 6 branched isomer peaks using m/z 556/498 (Fig. 3), and combined with m/z 556/419 a total of 9 isomer peaks were observed. Branched content made up 44% of the total FOSA concentration (using m/z 446/498). In comparison, 5 isomer peaks (using m/z 570/419) accounted for 38% of the total NEFOSA concentration, while 7 isomer peaks (using m/z 584/419) accounted for 47% of the total NEFOSA concentration. Using primary and secondary ions, 7 NEFOSA isomer peaks and 10 NEFOSA isomer peaks were observable. Characterized isomer standards are unavailable for perfluorooctane sulfonamidoacetates therefore the accuracy of reported branched content remains unclear. Nonetheless, to our knowledge this is the first report of branched perfluorooctane sulfonamidoacetate isomers in any environmental sample.

### 3.4. Conclusions

The present study utilized a novel fused-core PFP stationary phase and achieved comparable chromatographic separation to a previous comprehensive isomer-specific method but in nearly ¼ the time (i.e. <23 min versus 95 min \([5]\)) and with 3 new perfluorooctane sulfonamidoacetate targets. This method provides a means of separating the major PFSA, PFCA, and FOSAM isomers without compromising run times or target lists.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2012.05.077.

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