Identification of an Analytical Method Interference for Perfluorobutanoic Acid in Biological Samples

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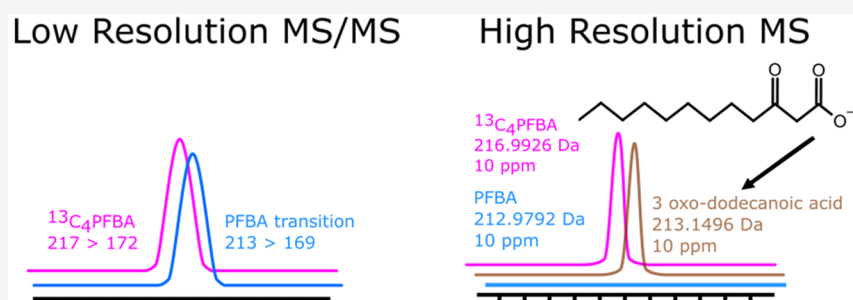Cite This: *Environ. Sci. Technol. Lett.* 2021, 8, 1085−1090

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ABSTRACT: The investigation of per- and polyfluorinated alkyl substances (PFAS) in environmental and biological samples relies on both high- and low-resolution mass spectrometry (MS) techniques. While high-resolution MS (HRMS) can be used for identification and quantification of novel compounds, low-resolution MS is the more commonly used and affordable approach for studies examining previously identified PFAS. Of note, perfluorobutanoic acid (PFBA) is one of the smaller PFAS observed in biological and environmental samples and has only one major MS/MS transition, preventing the use of qualitative transitions for verification. Recently, our laboratories undertook a targeted investigation of PFAS in the human placenta from high-risk pregnancies utilizing low-resolution, targeted MS/MS. Examination of placental samples revealed a widespread ($n = 93/122$ (76%)) chemical interferent in the quantitative ion channel for PFBA (213 → 169). PFBA concentrations were influenced by up to ∼3 ng/g. Therefore, additional chromatographic and HRMS/MS instrumentation was utilized to investigate the suspect peak and putatively assign the identity of the interfering compound as the saturated oxo-fatty acid (SOFA) 3-oxo-dodecanoic acid.

INTRODUCTION

Per- and polyfluoroalkyl acids (PFAS) are a class of thousands of unique chemicals containing carbon−fluorine bonds.[1] Production of the first PFAS began in the late 1940s, and since their genesis, usage has expanded to over 200 categories, from industrial applications to consumer products.[2] Because of their decades-long, widespread usage, PFAS are detectable in environmental media, wildlife, and humans across the globe.[3,4] The most investigated subclass of PFAS (often termed legacy PFAS) are the perfluoroalkyl acids (PFAAs).[5] Of the PFAAs, the two most studied to date are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS); however, in recent decades, many applications of PFAS have transitioned to using shorter chain replacement PFAS, many of which have been quantified in both abiotic (e.g., drinking water)[6] and biotic matrices (e.g., plasma and tissue).[7]

The investigation of PFAS in environmental and biological samples relies on both high- and low-resolution mass spectrometry (MS) techniques. While high-resolution MS (HRMS) can be used for identification and quantification of novel compounds, low-resolution MS is the more widespread and affordable approach for studies examining previously identified PFAS. Low-resolution, targeted tandem mass spectrometry (MS/MS) quantification experiments are regarded as highly accurate and precise for chemical measurements but still possess the potential for unresolved chemical interference. Investigations of PFAS in biological matrices (e.g., blood, serum, muscle, brain) has identified several instances where compounds interfere with quantitation of PFAS at low resolution. For example, taurodeoxycholate (a common bile acid) has been observed to mimic the primary perfluorooctanesulfonic acid (PFOS) MS/MS transition (499 → 80),[8] while endogenous steroid sulfates have been observed to mimic two of the perfluorohexanesulfonic acid (PFHxS) MS/MS transitions (399 → 80 and 399 → 99).[9] In the case where such interference is anticipated, qualifying ion MS/MS

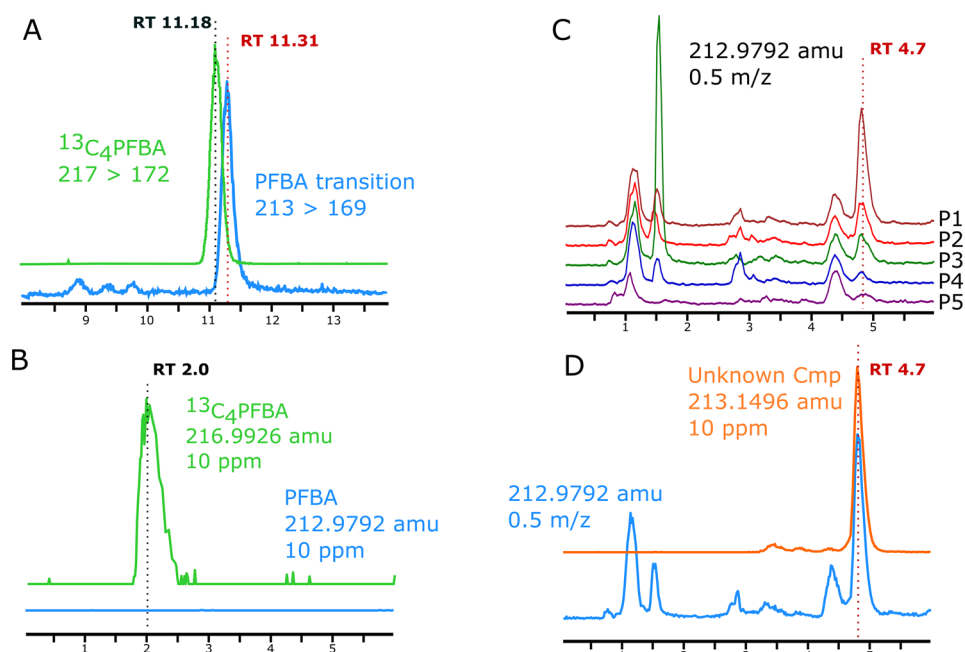**Figure 1.** (A) Low-resolution MS/MS analysis of a placental sample for PFBA internal standard ($^{13}C_4$−PFBA) and PFBA mass transition ion channels (precursor > product ion). (B) High-resolution MS analysis of a placental sample for $^{13}C_4$PFBA precursor ion and native PFBA precursor ion. (C) On the high-resolution MS, a simulated low-resolution mass window (± 0.5 m/z) was set around the mass of the PFBA precursor ion in placentas with high level of chemical interferent (P1), midlevel of chemical interferent (P2 and P3), and levels below detection for the low-resolution method (P4 and P5). (D) Identified interfering compound at mass 213.1496 m/z. All extracted ion chromatograms in each window are normalized to the largest peak to maintain scale across grouped traces.

transitions, along with enhanced chemical separation, can be used to ensure chemical specificity. As a result, the use of qualifying ion MS/MS transitions are suggested to verify the chemical identity for PFOS and PFHxS; however, the degree of historical over-reporting of PFOS and PFHxS from biological matrices in the literature as a result of these interferences is not fully known.

Recently, studies undertaken by the FDA and CDC have also reported matrix interferences in food and blood for perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA),[10,11] suggesting uncharacterized analytical interferences do exist for other PFAS when using low resolution MS/MS instrumentation. In 2020, our laboratories undertook an investigation into PFAS in the placenta from high-risk pregnancies. The study utilized a HPLC method similar to many HPLC methods in the literature paired with low-resolution MS/MS instrumentation.[12] Numerous PFAAs were quantified and reported in that study; however, because of some uncertainty around PFBA in the initial study, values for PFBA were not published in the original report.[12] More specifically, examination of placental samples revealed a widespread ($n = 93/122$) chemical interferent in the quantitative ion channel for PFBA ($213 \rightarrow 169$), and as a result of the interferent, PFBA concentrations were influenced up to ∼3 ng/g. Unfortunately, the small size of PFBA results in limited fragmentation of the molecule, yielding one MS/MS transition for quantification and none for verification, increasing the likelihood of interference. A second parameter often utilized for identification is matched retention time (RT) with an isotope labeled PFBA standard. However, because of the short RT for PFBA and the RT allowance window for many programs that automatically integrate peaks during high throughput analysis, misidentification is still possible. There-

fore, the current study was undertaken to identify the PFBA interferent present in the placental samples through HRMS and putatively assign a structure.

■ MATERIALS AND METHODS

Initial, targeted HPLC-MS/MS analysis of placental samples was completed as detailed in Bangma et al. 2020.[12] More details on the Bangma et al. MS/MS parameters can be found in the Supporting Information.

Following identification of an interference in low-resolution MS/MS, additional HRMS analysis of the samples was conducted on a separate system that employed an Agilent 1200 UHPLC coupled to an Agilent 6546 quadrupole time-of-flight mass spectrometer with electrospray ionization in negative mode. Chromatographic separation on the second system utilized a Waters BEH C18 column (50 mm × 30 mm, 1.7 μm). As before, 5 μL of extract was injected for each sample. Similar to the previous method, each sample run involved a ramping LC solvent gradient with methanol and deionized water both containing 2.5 mmol/L ammonium acetate. In brief, the LC gradient began at 25% organic and was held steady for the first minute, then ramped to achieve 55% organic at 2 min. A second ramp from 2 to 6 min was undertaken to achieve 65% organic. From there, the gradient was ramped to 100% organic at 8 min and held for 2 min to flush the column. System chromatographic pressure maintained around 700 bar (max system pressure 1300 bar). Under the new conditions, the unknown interferent was not observed to coelute with $^{13}C_4$ PFBA internal standard. Therefore, to identify the interfering compound, several placenta samples from the previous study were selected, divided into groups with the highest levels of interferent (∼3 ng/g based on previous PFBA quantitation methods), midlevel of interferent

(>1 ng/g to <3 ng/g), and no observable interferent (less than 0.5 ng/g). All samples contained a mass labeled PFBA internal standard ($^{13}C_4$PFBA), and samples were run alongside a method blank (previously processed for the original study) and solvent blanks on the QTOF. Method reporting limits (MRLs) for the HRMS were set based on the lowest point calibration standard with replicate precision <20% RSD and resulted in an MRL of 0.3 ng/g. Data was collected in 10 GHz high dynamic range mode at resolution ~20 000 and scan rate of 2 Hz. Additional parameters for the HRMS can be found in Supporting Information, Table S1.

■ RESULTS AND DISCUSSION

The presence of PFBA in placental samples previously analyzed using low-resolution MS was examined utilizing additional chromatographic and HRMS instrumentation. On the HRMS, PFBA presence or absence was investigated by extracting the deprotonated monoisotopic masses of PFBA ($[M - H]^-$, 212.9792 amu) and $^{13}C_4$−PFBA internal standard ($[M - H]^-$, 216.9926 amu) with a 10 ppm window (Figure 1B) and compared to previous data collected from the low-resolution instrument (Figure 1A). Samples previously observed to contain chemical interferent in the PFBA ion channel (213 → 169) were found to contain no PFBA based on the absence of a detectable ion peak for the PFBA exact precursor mass across the entire chromatographic window.

Because of altered chromatographic conditions (column and system pressure capabilities) on the HRMS system, the interfering ion no longer eluted in time with $^{13}C_4$−PFBA internal standard. Therefore, to identify the previously coeluting ion, we recreated the low-resolution scan data by extracting ion chromatograms of PFBA with a ±0.5 m/z window, allowing us to replicate the chromatographic traces observed in the low-resolution instrument. We were then able to pinpoint the interfering ion through the observation of the increasing peak size of an unknown ion corresponding to the previously estimated quantity of PFBA interferent in the various placental samples (P1−P5, Figure 1C). Investigation of the mass spectrum of the identified interferent peak at retention time (RT) = 4.7 min identified an ion with monoisotopic mass of 213.1496 m/z (Figure 1D), capable of causing interference in the ~1 Da precursor isolation window of a typical quadrupole. We hypothesize the identified ion undergoes an equivalent neutral loss of 44 amu (i.e., decarboxylation) to interfere with the MS/MS transition (213 → 169) in low resolution instrumentation.

The precursor mass spectrum of the interferent was processed using Agilent MassHunter 10 to predict an empirical formula for the compound (elements and limits allowed included C,3−50; H, 0−120; C, l0−10; S, 0−3; F, 0−100; O, 0−10). The most plausible predicted formula was assigned as $C_{12}H_{22}O_3$ with a score of 99.91 (composite scoring of monoisotopic mass, isotope cluster abundance, and isotope cluster spacing). The assigned formula of $C_{12}H_{22}O_3$ was searched against the Environmental Protection Agency CompTox Chemicals Dashboard to yield 149 plausible chemical structures (Supporting Information). Results were rank-ordered by the number of associated data sources which have been used in the past to identify probable compound identity and examined based on exogenous compounds that mothers might have a potential for exposure. Two exogenous compounds (ethyl 2-acetyloctanoate, CASRN 29214-60-6 https://comptox.epa.gov/dashboard/dsstoxdb/results?search=

DTXSID3051969; and tetrahydro-3-pentyl-2H-pyran-4-yl acetate, CASRN 18871-142, https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID7044556) listed as cosmetic and/or food additives were selected, standards purchased, and determined to not match the spectra of the interferent in the placentas. We then investigated the possibility that the interferent was an endogenous compound and searched the Human Metabolome Database (HMDB)[13,14] to identify endogenous molecules with plausible ionization in negative electrospray ionization; product ion species were predicted using CFM-ID 3.0.[15] We assigned the most likely chemical class to be a saturated oxo-fatty acid (SOFA), which comprised seven of the 149 chemical structures identified in the CompTox Chemicals Dashboard search. SOFAs are involved in fatty acid biosynthesis pathways in all eukaryotes, contain a carboxylic acid group for negative mode ionization, and have been documented in bacteria,[16] yeast,[17] human breath,[18] human plasma,[19] rabbit plasma,[20] and cows and goats milk.[21]

Of the several potential dodecanoic SOFAs with the assigned formula $C_{12}H_{22}O_3$, we hypothesize that 3-oxo-dodecanoic acid (Figure 2) is the major chemical interferent
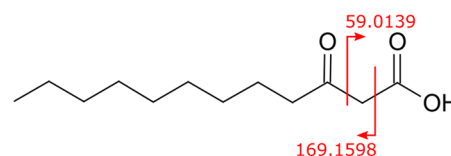
Figure 2. Structure of 3-oxo-dodecanoic acid with major (59.0139) and minor (169.1598) ion fragmentation annotated.

observed in the placenta samples, with the interfering 213 → 169 transition occurring as a minor, secondary fragment (Figure 2). CFM-ID predictions yielded a primary fragment at 169.1598 m/z, which matches the observed interference in the 213 → 169 ion channel in the low-resolution instrument, but empirical measurements of the small concentrations of interfering precursor mass on the HRMS observed 59.0134 amu as being the major ion fragment (Supporting Information, Figure S1). Fragmentation of 213.1496 amu at HCD 10, 20, and 40 all yielded the same resulting spectra, with the 59.0134 fragment being the major detected fragment on the HRMS. This is consistent with previous reports of this lipid in exhaled human breath,[18] with a primary 59 fragment and the observed 169 fragment appearing as a minor transition (~1% relative intensity). We predict the 3-oxo fatty acid as the main SOFA present in placental samples due to the preferential generation of the 59-fragment resulting from the relative positioning of the ketone at the 3 position (Figure 2).

In addition to the main interfering SOFA, several additional SOFA isomers were observed in the placenta. These peaks are discernible as minor peaks eluting prior to 4.7 min in the HRMS spectra at 213.1496 m/z (Figure 1D). Other isomer forms of dodecanoic SOFAs with the ketone in a different position could yield similar interferents in a MS/MS spectrum, although the relative yield of 213 → 169 transitions (and potential for interference) will vary. With this information combined, we are confident in a level 3 identification on the Schymanski scale with a tentative candidate of 3-oxo-dodecanoic acid as the major SOFA present in the placenta samples in this study.[22] Identifying the exact isomeric structure of the major interfering SOFA with a chemical standard would

be ideal and improve the Schymanski identification confidence level; however, several SOFAs appear to be present in the placenta in varying abundances and verifying with single a SOFA chemical standard not to mention several SOFA chemical standards is cost and time prohibitive.

Our study investigating PFAS in the placenta revealed that SOFAs are the suspected chemical interferents in the PFBA ion channel. Additionally, our study also revealed that depending on the LC conditions related to the column and system pressure capabilities, the major interfering SOFA can be separated chromatographically from PFBA, as observed in the differences between the original LC method (Figure 1A) and the follow-up UHPLC method utilized in tandem with HRMS (Figure 1B,D). In the future, we suggest caution when observing widespread PFBA measurements by low-resolution MS/MS in biological matrices. On the basis of the current literature, we hypothesize that the suspected interfering SOFA is likely only to be an interferent in biological matrices and not present in high enough quantities in environmental samples such as tap water and/or groundwater, although this cannot be ruled out. In addition, as a short chained PFAS, PFBA is rapidly eliminated from the body via urine in humans[23] and animals[24,25] and therefore is unlikely to be observed at a high frequency in biological samples unless there is a chronic point source exposure to the humans or wildlife in question. In fact, a recent study from Germany has questioned previous reports of PFBA in human tissues[26] after finding little to no PFBA in human lung and kidney biopsies from a separate cohort study that employed HRMS instrumentation. Our followup analysis in placenta is similar to Abraham and colleague's study in that it seeks to verify or refute the presence of PFBA in biological samples previously thought to contain PFBA. It is with this in mind that we have built upon Abraham's and colleagues' findings to tentatively identify the PFBA interfering ion in the placenta as 3-oxo-dodecanoic acid. Additional research is warranted to verify 3-oxo-dodecanoic acid is in fact the SOFA of concern in the placenta.[27]

Armed with this knowledge, researchers can make more informed decisions as to whether it is necessary to confirm the presence or absence of PFBA in biological samples via secondary methods. Confirmation analysis can be undertaken with altered chromatographic conditions and or HRMS, analyzing only a handful of the samples (a few with suspected high PFBA and a few samples with no PFBA) to confirm the presence or absence of interfering SOFAs. Another avenue that may prove useful for investigators with limited access to additional chromatographic or HRMS instrumentation would be to scan for the $213 \rightarrow 59$ transition to indicate if an interfering SOFA elutes at the same retention time as PFBA. Authors reason that most if not all SOFA isomers with the molecular formula $C_{12}H_{22}O_3$ should theoretically produce the 59 fragment ($-COOH$ loss). However, authors are hesitant at this time to declare the $213 \rightarrow 59$ transition as an absolute guaranteed way to identify interfering SOFAs in a sample. While it is a valid method for the placental samples analyzed in this study, we reason that different isomeric SOFAs or changes in ionization conditions may lead to different major fragments besides the 59 fragment, and thus, depending on instrument settings and sensitivity, may lead to false negatives. More research on SOFA isomers is warranted to verify the $213 \rightarrow 59$ transition. Alternatively, sample preparation methods to eliminate interfering lipids[28] and/or enhanced chromatographic separations to eliminate coelution may reduce the

potential for false-positives, but such sample preparation methods have not been investigated as a possibility for removing SOFAs from extracted tissues. Last, it is imperative that researcher closely monitor the retention time for PFBA and isotope labeled PFBA from investigations to confirm exact matching elution time as there is no confirmatory ion for ion ratio flagging.

In spite of this apparent interferent, we acknowledge that PFBA is likely to be present in various collected environmental samples[29] and may pose a health risk to highly exposed individuals.[30] For example, recent analysis of groundwater in China using HRMS has observed PFBA as one of the dominantly detected PFAS.[6] Another study utilizing MS/MS and HRMS observed high frequency of PFBA in seabird livers.[7] We hope this information will help to guide PFAS researchers in how to best verify PFBA quantification in biological matrices in the future to reduce potential over reporting of PFBA in the literature.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.1c00901.

> Additional materials and methods, average fragmentation spectrum for $213.1496 \rightarrow [52.0000-223.0000]$, and ESI source parameters for MS1 data collection (PDF)

> Plausible chemical structures for the assigned formula of $C_{12}H_{22}O_3$ from the Environmental Protection Agency CompTox Chemicals Dashboard, (XLSX)

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