Possible matrix effect when using oxygen-18 labeled perfluorohexanesulfonate as a reference standard

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Abstract

The analysis of perfluoroalkylsulfonates in the environment has attracted a steadily increasing amount of interest. The introduction of mass-labeled perfluoroalkylsulfonate standards should be of great benefit in improving data reliability. Recently, oxygen-18 labeled perfluoroalkylsulfonates have become available as analytical standards. When $^{18}O_2$ mass-labeled perfluorohexanesulfonate is used as an internal standard for LC-electrospray ionization-MS (LC/ESI-MS) analysis, the actual and theoretical concentration ratios matched closely those for related native sulfonates as long as they did not co-elute. However, when they do co-elute, the peak intensities of the native species are enhanced by about 5%, while those of the labeled compound are suppressed by a similar amount. If this effect were not taken into account, the apparent concentration of the native would be inflated by 10%. This observation is possibly due to differences in the ability of ^{18}O versus ^{16}O to ion-pair and solvate in the droplets formed during the electrospray process.

1. Introduction

There continues to be an increase in the interest concerning the wide spread distribution of fluorinated chemicals, including perfluoroalkylsulfonate, in the environment and their accumulation in humans.[1-5] The production of perfluorooctanesulfonate (PFOS) derivatives from linear alkyl precursors using electrochemical fluorination is not a clean process.[6] Perfluorohexanesulfonate (PFHxS) is a known residual by-product found in PFOS-related products.[7] It has also been reported that PFHxS is produced and used as a building block for compounds incorporated into fire-fighting foams and specific post-market carpet treatment applications.[7] Many laboratories are now seeking to identify and quantitate PFHxS in a variety of samples.

The availability and use of mass-labeled perfluoroalkylsulfonate standards should be of great benefit in improving the reliability of the resulting data. $^{13}C_4$-PFOS, $^{18}O_2$-PFOS and $^{18}O_2$-PFHxS have recently been introduced as potential mass-labeled reference standards. When the mass-labeling is "hidden inside" the molecule, as is the case of carbon-13 in ^{13}C-labeled compounds, such compounds exhibit essentially identical physical characteristics as those of the native homologues. However, when the mass-labeled atoms in a compound are "exposed" to the physical environment, such as in deuterated compounds, it is known that the physical behavior of these compounds is affected. For example, shorter retention times are induced during gc analysis. In the case of ^{18}O-labeled sulfonates, the oxygen-18 atoms are exposed to their environment and this led us to question how this might impact their physical properties and whether this would have any consequences during LCMS analyses.

The objective of this work was to evaluate any potential for problems when sodium $^{18}O_2$-perfluoro-1-hexanesulfonate ($^{18}O_2$-PFHxSNa, see Figure 1) is used as a standard during LCMS analysis.

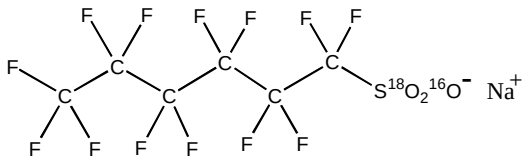

Figure 1. Structure of Sodium $^{18}O_2$-perfluoro-1-hexanesulfonate ($^{18}O_2$-PFHxSNa)

2. Materials and methods

2.1 Chemicals and standards

Sodium $^{18}O_2$-perfluoro-1-hexanesulfonate (>99% linear, $^{18}O_2$-PFHxSNa), sodium PFHxS (>99% linear, L-PFHxSNa), sodium PFOS (>99% linear, L-PFOSNa) and sodium PFDS (>99% linear, LPFDSNa) were synthesized at Wellington Laboratories using proprietary methods. HPLC grade methanol, acetonitrile and water were from Caledon.

2.2 LCMS experiments

LCMS experiments were conducted on a Waters Acquity Ultra Performance LC interfaced to a Micromass Quattro micro API mass spectrometer. Separations were performed on an Acquity UPLC BEH Shield RP_{18} column (1.7 um, 2.1 x 100 mm). A typical LC method started at 67% (80:20 MeOH: ACN) and 33% water (both with 10 mM NH_4OAc) at a flow rate of 350 µL/minute and held for 5.5 minutes. The program was then ramped to 90% (80:20 MeOH: ACN) over 0.5 minutes and held for 3 minutes before returning to initial conditions.

For quantitative determination, the Micromass Quattro *micro* atmospheric pressure ionization (API) mass spectrometer was set up in the negative-ion electrospray mode with the following conditions: Capillary Voltage (kV) = 2.60; Cone voltage (V) = 60.00; Cone Gas Flow (L/Hr) = 60; Desolvation Gas Flow (L/Hr) = 650; Desolvation Gas Temperature (°C) = 325; Collision Gas (mbar) ~ 3.50e-3; Collision Voltage (V) = 40. The transition $[M - K]^-$ (m/z = 499) to $[FSO3]^-$ (m/z = 99) was optimized and used for quantification. A dwell time of 0.2 s was used to monitor each transition.

2.3 Preparation of solutions

All solutions were prepared from in-house 50 µg/mL standards of sodium perfluoro-1-hexanesulfonate (PFHxSNa), sodium $^{18}O_2$-perfluoro-1-hexanesulfonate ($^{18}O_2$-PFHxSNa) and PFOSNa by serial dilution and were brought to volume with 80:20 methanol:water. For a semi-quantitative determination of suppression and/or enhancement of the native and mass-labeled PFHxS, a solution of PFHxSNa (20 µg/mL), PFOSNa (20 µg/mL), and $^{18}O_2$-PFHxSNa (250 ng/mL) was compared to a solution containing only PFHxSNa (20 µg/mL) and PFOSNa (20 µg/mL). Similarly, a solution of $^{18}O_2$-PFHxSNa (20 µg/mL), PFOSNa (20 µg/mL), and PFHxSNa (250 ng/mL) was compared to a solution containing only $^{18}O_2$-PFHxSNa (20 µg/mL) and PFOSNa (20 µg/mL). Using the PFOSNa as an internal standard in both cases, it was possible to determine the effect of a small amount of native PFHxS on the mass-labeled species and *vice versa*. All data were acquired on the LCMS system in SIM mode.

3. Results and Discussions

In experiments involving $^{18}O_2$-PFHxSNa, an interesting matrix effect was observed when a small amount of $^{18}O_2$-PFHxSNa (250 ng/mL) was added to a 1:1 mix of PFHxSNa and PFOSNa (20 µg/mL each). The peak intensity of the native species, using PFOSNa as an internal standard, appeared to be enhanced by approximately 5% when compared to the ratio obtained for a mixture containing only PFHxSNa and PFOSNa (20 µg/mL each). An opposite effect was observed upon addition of a small amount of PFHxSNa (250 ng/mL) to a 1:1 mix of $^{18}O_2$-PFHxSNa and PFOSNa (20 µg/mL each). In this case, the labeled compound appeared to be suppressed by approximately 5%. Importantly, if this labeled compound were used as an internal standard in an analysis for PFHxS, the reported concentration of the native could be inflated by as much as 10%. The cause of this matrix effect has not been elucidated but co-elution of such isotopomers seems to be a prerequisite. A possible explanation is that, in the electrospray ionization process[8-10], there is an asymmetrical distribution of the isotopomers in the droplets with a larger proportion of the native species positioned on the negatively charged surface. Presumably inside a

droplet, anions are stabilized by both ion-pairing and solvation, while the latter plays a larger role at the negatively charged surface. If this is the case, the ^{18}O-labeled anions may prefer to be inside the drops, since ^{18}O species form weaker hydrogen bonds than their lighter isotope analogues.[11]

4. Conclusions

When $^{18}O_2$ mass-labeled perfluorohexanesulfonate was used as an internal standard for LC/ESI-MS analyses, the actual and theoretical concentration ratios matched closely those for related native sulfonates as long as they did not co-elute. However, when they did co-elute, the peak intensities of the native species were 10% greater than those for the $^{18}O_2$ mass-labeled perfluorohexanesulfonate. This effect would inflate the apparent concentration of the native species by 10%.

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