CHALLENGES ASSOCIATED WITH THE ANALYSIS OF MONO-SUBSTITUTED POLYFLUORINATED PHOSPHATE ESTERS BY LCMS

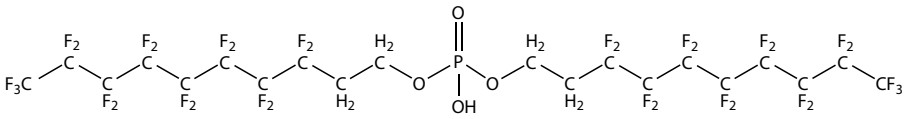
Riddell N[1], Stefanac T[1], McAlees A[1], McCrindle R[1,2], Chittim B[1]

[1]Wellington Laboratories Inc., 345 Southgate Drive, Guelph, Ontario, Canada, N1G 3M5; [2]University of Guelph, Chemistry Department, 50 Stone Road East, Guelph, Ontario, Canada, N1G 2W1

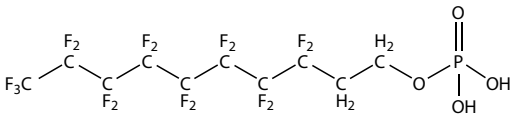
Introduction

The use of polyfluorinated phosphate esters (PAPs) in food-contact paper products, and the subsequent exposure of humans to these compounds, has resulted in an increased interest in their environmental fate and toxicological properties. Polyfluorinated phosphate esters can be mono-, di-, or tri-substituted (monoPAPs, diPAPs or triPAPs respectively) and the polyfluorinated alkyl chain can vary in length with the most commonly observed homologues being the 6:2 [$CF_3(CF_2)_5(CH_2)_2$] and 8:2 [$CF_3(CF_2)_7(CH_2)_2$] chains. It has been shown that perfluorinated compounds, such as PAPs, can leach out of food packaging and migrate into the food that we ingest.[1,2,3] The magnitude of this possible exposure route, and the fact that PAPs have been identified as potential sources of perfluoroalkylcarboxylic acids as a result of biotransformation studies in rats[4,5], has prompted researchers to screen for these compounds in various matrices[6]. Although the phosphate ester linkages for both mono- and di-PAPs have been shown to be stable to abiotic hydrolysis[5], cleavage can still occur within biological systems producing fluorinated alcohols which would likely be metabolized to perfluoroalkylcarboxylic acids[4]. Unfortunately the analysis of PAPs, specifically the monoPAPs, appears to present challenges unlike those previously observed for perfluorinated compounds analyzed by LCMS.

Although a variety of methods have been developed and validated for the analysis of poly- and per-fluorinated compounds, the applicability of these methods to mono- and di-substituted phosphate esters is limited. The unique chemical and physical properties associated with each sub-class of perfluorinated compounds can drastically affect their behaviour during extraction and analysis. The work presented here outlines some challenges that may be encountered during the analysis of the monoPAPs as well as possible solutions.



8:2 diPAP



8:2 monoPAP

Figure 1: Structural representation of the 8:2 diPAP and 8:2 monoPAP.

Materials and methods

The native 8:2monoPAP, native 8:2diPAP, mass-labelled [M+2]8:2monoPAP ($^{13}C_2$), and mass-labelled [M+4]8:2diPAP ($^{13}C_4$) were synthesized at Wellington Laboratories Inc. (Guelph, ON) using proprietary methods. The native 6:2monoPAP and native 6:2diPAP were received from Scott Mabury's research group (University of Toronto, Toronto, ON). Characterization of all of the polyfluorinated phosphate esters was accomplished using a combination of ^{1}H NMR, ^{19}F NMR, ^{31}P NMR and LC/MS/MS analysis. LC/MS

experiments were conducted on a Waters Acquity Ultra Performance LC interfaced to a Micromass Quattro micro API (triple quad mass spectrometer). Separations were performed on a Waters Acquity BEH C_8 column (1.7 um, 2.1 x 100 mm). MS data were collected in MRM or full scan mode (capillary voltage = 3.00 kV; cone voltage = 35.00 V). All LCMS samples were prepared using 75:25 methanol:water (pH 11) as the reconstitution solvent. NMR experiments were performed on an Avance-400 MHz Bruker instrument.

Results and discussion:

Although the general physiochemical properties of poly- and per-fluorinated compounds (PFCs) are well known, the variability associated with sub-classes of perfluorinated compounds can result in complex analytical challenges. For instance, although perfluorinated sulfonic acids are amenable to liquid chromatography tandem mass spectrometry (LC-MS-MS), their extremely low pKa's have necessitated that they be run in buffered systems if traditional C18 columns are utilized for separation. Providing the analytes of interest with counter-ions not only improves retention behaviour on C18 columns, but also improves separation of structural isomers and homologues. Unfortunately, the use of buffer in the mobile phases presents the problem of adduct formation for the fluorotelomer alcohols. This limits the number of analytes that can be included in a comprehensive PFC analysis if the molecular ion is being targeted. Similarly, the tendency of perfluoroalkylphosphonates to form mono-layers on the surfaces of solid substrates such as metals and glass has resulted in their utilization as soil resistant coatings, anti-reflective glass coatings, and release coatings[7]. In our experience, the affinity of these compounds to metal surfaces has also resulted in peak tailing during liquid chromatography. This effect seems to be instrument and column dependent, but it is possible to improve peak shape and form the mono-anion by utilizing a relatively high pH in the reconstitution solvent and at the beginning of the gradient and then slowly buffering the pH over the course of the gradient.

Initially, LCMS analysis of the polyfluorinated phosphate esters was attempted using conditions similar to those previously developed for perfluoroalkylphosphonic acids (PFAPAs) (Table A – Gradient A). Peaks were observed for the 6:2 mono- and di-PAPs as well as the 8:2diPAP, but the 8:2monoPAP eluted from the column as an extremely broad lump instead of a sharp peak. A series of mobile phase combinations were evaluated and it was determined that the response of the monoPAPs could be optimized and peak tailing minimized by utilizing a methanol:water gradient (with the pH of the water adjusted to 11 with ammonium hydroxide, Table 1 – Gradient B and Figure 2). The higher pH required to minimize peak tailing and also observe the mono-anion of these compounds indicates that their pKa's are substantially higher than those previously determined for the PFAPAs.

Table A: A summary of initial (Gradient A) and final (Gradient B) LC conditions utilized for the LCMS analysis of polyfluorinated phosphate esters.

	Gradient A	Gradient B
Mobile Phase A	Water (pH 9 adjusted with ammonium hydroxide)	Water (pH 11 adjusted with ammonium hydroxide)
Mobile Phase B	80:20 MeOH:ACN with 10 mM NH_4OAc	Methanol
Flow	0.300 ml/min	0.300 ml/min
Initial (%B)	0 min (40% B)	0 min (60% B)
Time 1 (%B)	6 min (90% B)	5 min (90% B)
Time 2 (%B)	8.5 min (90% B)	8.5 min (90% B)
Time 3 (%B)	9 min (40%B)	9 min (60%B)

Sodium adducts of the 6:2- and 8:2-monoPAPs were also observed during infusion experiments. The addition of sodium hydroxide completely suppressed the signal, but the addition of ammonium hydroxide resulted in an increase in signal strength. The fact that the ion-pair was surviving the electrospray ionization process was initially quite surprising. This property could also have an effect on the extraction efficiency of these compounds from certain matrices.

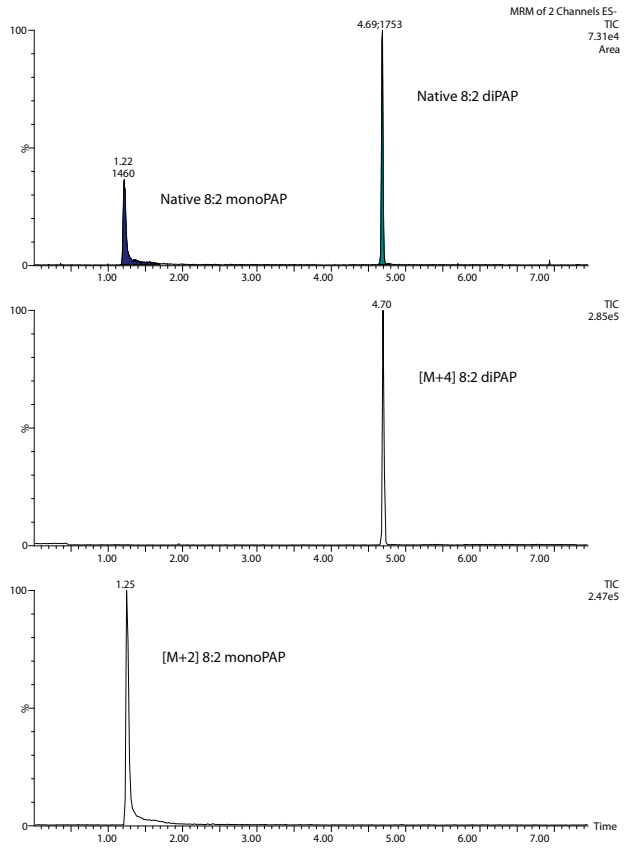


Figure 2: Chromatogram illustrating the response and peak shape achieved for the 8:2 mono- and di-PAP using gradient B.

Although much of the unique behaviour associated with perfluorinated compounds can be attributed to their poly- or per-fluorinated alkyl chains, it is evident that the functional groups associated with each sub-class can have a large impact on the ease of their analysis.

Acknowledgements:
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