# Analysis of perfluoroalkyl anion fragmentation pathways during LCMS: Evidence for fluorine migration prior to secondary and tertiary fragmentation.

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**Abstract**
It is known that under LC/ESI-MS/MS conditions, the $[R_FCO_2]^-$ anion first loses $CO_2$ to give a perfluoroalkyl anion $R_F^-$, $[(M-H)-CO_2]^-$, which can subsequently fragment to give (*inter alia*) lower mass carbanions. It has been suggested in a previous study that such secondary fragmentation involves cleavage of $C_nF_{2n}$ segments. The results of our study of the LC/ESI-MS/MS of a series of $^{13}C$-labeled perfluoroalkyl carboxylic acids (PFCAs) indicate that fragmentation of the $R_F^-$ anion does not involve simple "unzipping" of a primary perfluoroalkyl anion of the type $F_3C(CF_2)_xCF_2^-$. For example, we have discovered that the secondary transition for the mass-labeled PFOA, perfluoro-1,2,3,4-$^{13}C_4$-octanoic acid (MPFOA), gives two signals of equal intensity at m/z 169 and m/z 172. We propose a mechanism of fragmentation that involves rapid fluorine shifts, after the initial decarboxylation, which generate a series of new anions prior to secondary and tertiary fragmentation. The production of two product ions from a 'single' secondary transition of mass-labeled PFCAs may impact some work done in analytical labs since the response factors will be about half of those observed for the corresponding native PFCAs.

## 1.0 Introduction

LC-electrospray ionization-MS (LC/ESI-MS) and LC/ESI-MS/MS can be considered as the current standards for analysis of anionic perfluorinated compounds.[1] The majority of reports in the literature have employed LC/ESI-MS/MS as the analytical method. Multiple transitions of the molecular ion are used for qualitative and quantitative purposes. For perfluorocarboxylic acids (PFCAs), usually the dissociated acid (pseudo-molecular) ion $[M-H]^-$ is observed, and can be used for quantitative purposes in LCMS, or as the precursor ion in selected reaction monitoring (SRM) in LC/ESI-MS/MS.[2] However, to date, there has apparently been no published reports on systematic investigations of the fragmentation pathways of the PFCA-derived carboxylate anions ($R_FCO_2^-$) during LCMS.

It is known[1] that under LC/ESI-MS conditions, the $[R_FCO_2]^-$ anion first loses $CO_2$ to give a perfluoroalkyl anion $R_F^-$, $[(M-H)-CO_2]^-$, which subsequently fragments to give (*inter alia*) lower mass carbanions. It has been suggested in a previous study[3] that such secondary fragmentation involves cleavage of $C_nF_{2n}$ segments. However, as discussed below, the results of a study of the LC/ESI-MS/MS of a series of $^{13}C$-labelled PFCAs indicate that fragmentation of the $R_F^-$ anion does not entail simple "unzipping" of a primary perfluoroalkyl anion of the type $F_3C(CF_2)_xCF_2^-$.

The present work will hopefully shed some light on the fragmentation mechanism of perfluoroalkyl anions generated under LC/ESI-MS/MS conditions.

## 2.0 Experimental

### *2.1 Chemicals*

The mass-labeled perfluoroalkyl carboxylic acids ($R_f$-$CO_2H$) were synthesized at Wellington Laboratories using proprietary methods. HPLC grade methanol (MeOH) and water were purchased from Caledon.

### *2.2 LC/ESI-MS/MS*

The analyses were conducted on a Waters Acquity Ultra Performance LC interfaced to a Micromass Quattro *micro* atmospheric pressure ionization (API) mass spectrometer (MS). The compounds were dissolved in 80:20 MeOH:water and infused via syringe pump into the MS through a union tee that was also connected to the LC system. Both the aqueous and organic mobile LC mobile phases contained 10mM ammonium acetate and the LC inlet was held constant at 80% 80:20 MeOH:ACN and 20% water at a flow rate of 0.15 mL/minute. The Micromass

Quattro *micro* API MS was set up in the negative-ion electrospray mode and the tune parameters were optimized for the in-source fragmentation of each individual perfluoroalkyl carboxylic acid to produce $[R_FCO_2]^-$ (cone voltages: MPFHxA and MPFOA 30V; MPFNA, MPFDA, MPFUdA, and MPFDoA 35V). The product ion experiments involving the transition $[R_f–CO_2]^-$ to $[R_f]^-$ were optimized for each individual compound (argon collision gas ~ $3.5e^{-3}$ mbar, collision energies: MPFHxA 18V; MPFOA 20V; MPFNA, MPFUdA, and MPFDoA 25V; MPFDA 23V).

**3.0 Results and Discussion**

**3.1 Perfluoro-1,2,3,4-$^{13}C_4$-octanoic acid (MPFOA) (1)**

In the analysis of native perfluorooctane carboxylic acid (PFOA) by LC/ESI-MS/MS, the primary fragmentation m/z 413 → 369 $[(M-H)-CO_2]^-$ and a secondary fragmentation m/z 413 → 169 $[C_3F_7]^-$are typically used[2] for identification and quantitation. Under similar conditions, the 1,2,3,4-$^{13}C_4$ analogue gives the expected $[M-H]^-$anion at m/z 417 and the primary fragmentation product at m/z 372 $[(M-H)-^{13}CO_2]^-$, but the corresponding secondary fragmentation now gives two product ions of similar intensities at m/z 169 and 172 (Fig. 1a) indicating equally facile formation of both $[^{12}C_3F_7]^-$ and $[^{13}C_3F_7]^-$ moieties. Additional secondary fragmentation products from MPFOA also appear in the mass spectrum (Fig. 1a) as pairs of signals of similar intensities at m/z 119, 121 ($[^{12}C_2F_5]^-$ and $[^{13}C_2F_5]^-$) and 219, 222 ($[^{12}C_4F_9]^-$ and $[^{12}C^{13}C_3F_9]^-$). These observations suggest that the precursor, m/z 372, to the secondary anionic fragments behaves as if it were a symmetrical species $[F_3CC_5F_9CF_3]^-$. Indeed, since it is well known[4] that the order of stabilities of perfluoro carbanions is $3^o>2^o>1^o$, it would not be surprising if the primary perfluoro carbanion formed upon loss of $CO_2$, $F_3C(CF_2)_3{}^{13}CF_2{}^{13}CF_2{}^{13}CF_2^-$, isomerizes rapidly *via* fluorine migrations to give secondary anions. We therefore propose the fragmentation mechanism for MPFOA outlined in Figure 2. After primary fragmentation by decarboxylation of MPFOA, the newly generated anion **1a** is converted into a series of secondary anions **1b**-**1f** *via* fluorine shifts. This fluorine (and resulting charge) migration has to be rapid relative to further fragmentation due to the fact that, in each pair, the product ions at m/z 119/121, 169/172, 219/222 are of similar intensities. Subsequent fragmentation of each of the possible secondary carbanions, by elimination of neutral $C_nF_{2n}$ moieties, then occurs as indicated in Figure 2. Note that signals were not detected at m/z 269 or 272, corresponding to fragmentation of the initially formed primary carbanion **1a** or the possible alternative primary carbanion **1g,** respectively. Further, signals attributable to the trifluoromethyl carbanion at m/z 69/70 were not observed consistent with the lower stability of this species in comparison with perfluoroethyl and higher perfluoro carbanions. Finally, the primary carbanions generated by fragmentation of isomeric precursors **1b**, **1c**, **1e** and **1f** would be expected to undergo isomerization to the corresponding secondary perfluoropropyl and perfluorobutyl carbanions.

**3.2 Perfluoro-1,2-$^{13}C_2$-hexanoic acid (MPFHxA) (2)**

If the initially formed carbanion from MPFHxA, $[(M-H)-^{13}CO_2]^-$ at m/z 270 undergoes rapid isomerization followed by fragmentation as suggested above for MPFOA, see Figure 3, then fragmentation pathways A, C, C′ and A′ would be deemed much less favorable than pathways B and B′. Indeed, product ions of strong, and equal intensities, are observed at m/z 119 and m/z 120 (see Fig. 1b) along with weak signals at m/z 69 and m/z 70.

**3.3 Perfluoro-1,2,3,4,5-$^{13}C_5$-nonanoic acid (MPFNA) (3)**

The product ion spectrum (see Fig. 1c) of the $[(M-H)-^{13}CO_2]^-$ion from MPFNA (m/z 423) is again consistent with the proposed mechanism outlined above. However, due to the longer chain length of MPFNA *vs* MPFOA, further fragmentation is possible. Thus the two carbanions (m/z 270 and 273) generated by secondary fragmentation should be susceptible to further fragmentation (tertiary fragmentation) similar to that observed for the $[(M-H)-^{13}CO_2]^-$ ion from MPFHxA (Fig. 3). In the case of MPFNA, the fragment with m/z 270 would be expected to produce fragments with m/z 119 and m/z 120 while the fragment with m/z 273 would be expected to produce fragments with m/z 120 and m/z 121. Indeed, signals are detected at m/z 119 and m/z 121 (arising from both secondary and tertiary fragmentation), along with a third signal at m/z 120 (arising from only tertiary fragmentation). This may be

contrasted with the spectrum of MPFOA (Fig. 1a) which, as expected, contains a pair of product ions at m/z 119/121 but shows no indication of a peak at m/z 120.

### 3.4 Perfluoro-1,2-$^{13}C_2$-decanoic acid (MPFDA) (4)

The intervention of tertiary fragmentation processes is also seen in the product ion spectrum for MPFDA (Fig. 1d). Clearly, the intensities of the signals for m/z 119/120 and m/z 169/170 are significantly skewed towards the non-labeled fragments, m/z 119 and m/z 169. This observation is readily explained by the fact that the product ions m/z 269 and m/z 319 can further fragment to give only the non-labeled ions at m/z 119 and m/z 169, therefore increasing the intensity of these signals. Similar observations and conclusions were reached with compounds **5** and **6**.

### 3.5 Possible issues to analytical labs

The production of two product ions from a 'single' secondary transition of mass-labeled PFCAs may impact some work done in analytical labs since the response factors will be reduced to half relative to those of the native compounds when analyzed by LCMS/MS in SRM mode. Laboratories must take this into account when using mass-labeled PFCAs as internal or recovery standards.

## 4. Conclusions

The fragmentation mechanism for perfluoroalkyl anions generated from perfluoroalkyl carboxylic acids involves rapid fluorine shifts to generate new anions prior to secondary and tertiary fragmentation. This can result in a reduction (of *ca* 50%) in the response factors for the mass-labeled PFCAs relative to their native counterparts.

## References


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3. Lyon PA, Tomer KB, Gross ML. *Anal Chem.* 1985; **57**: 2984.
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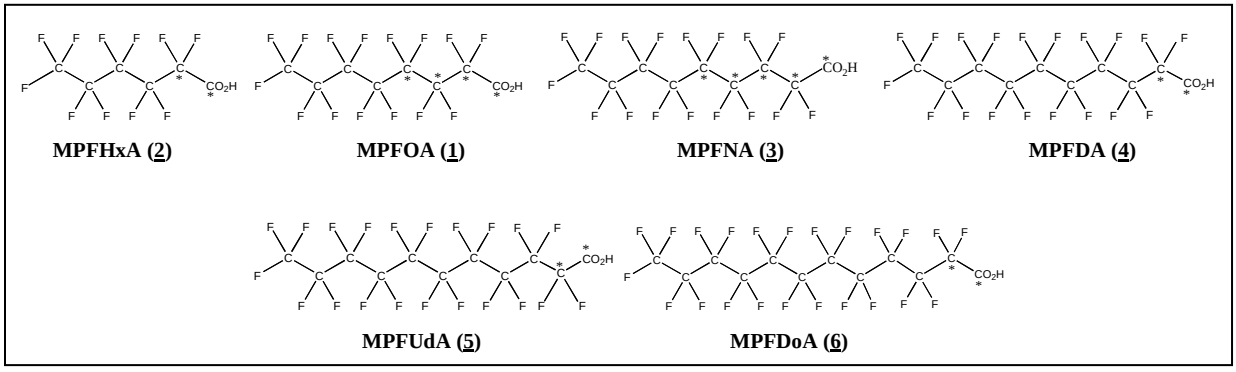


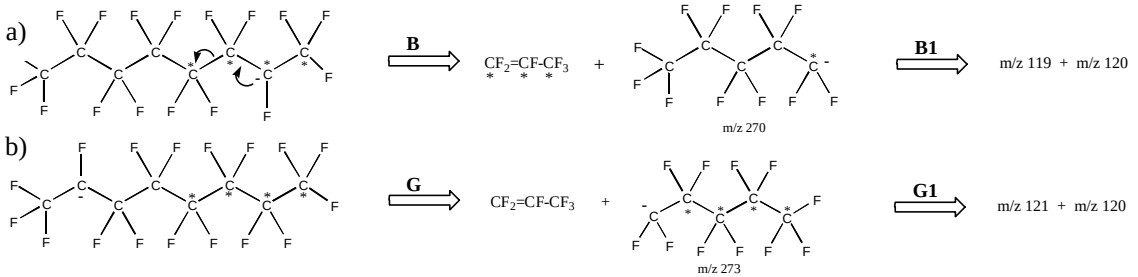


**Figure 4. Generation of m/z 119/120/121 from 3**

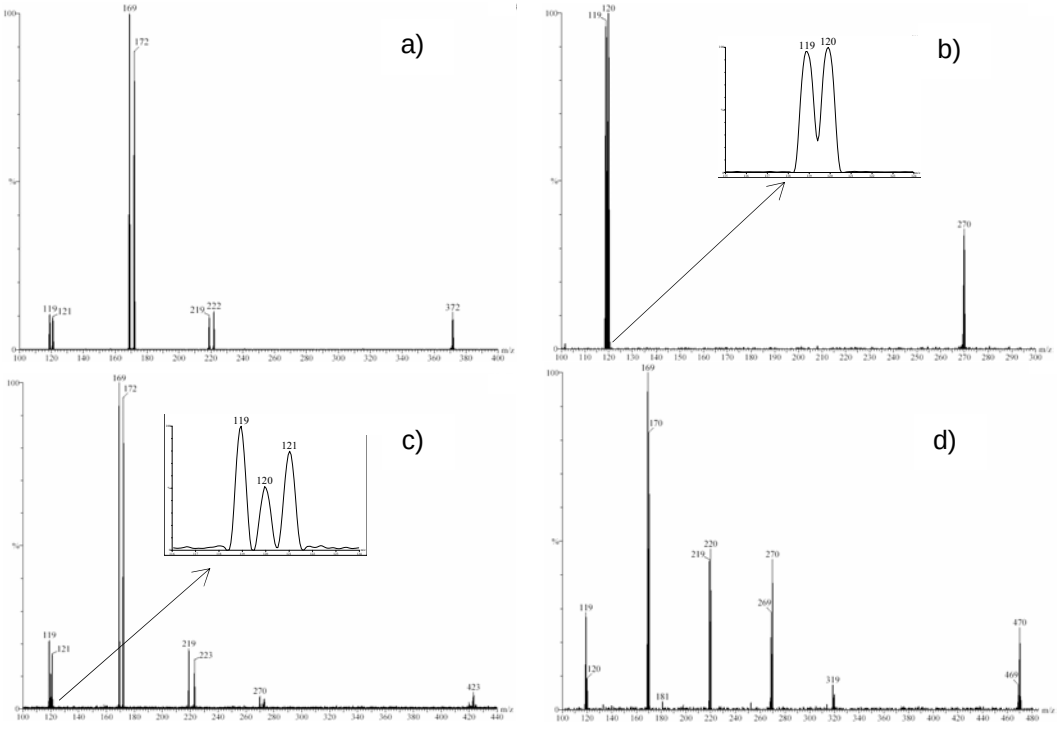


**Figure 1. Mass Spectra for compounds a) MPFHxA (2), b) MPFOA (1), c) MPFNA (3) and d) MPFDA (4)**

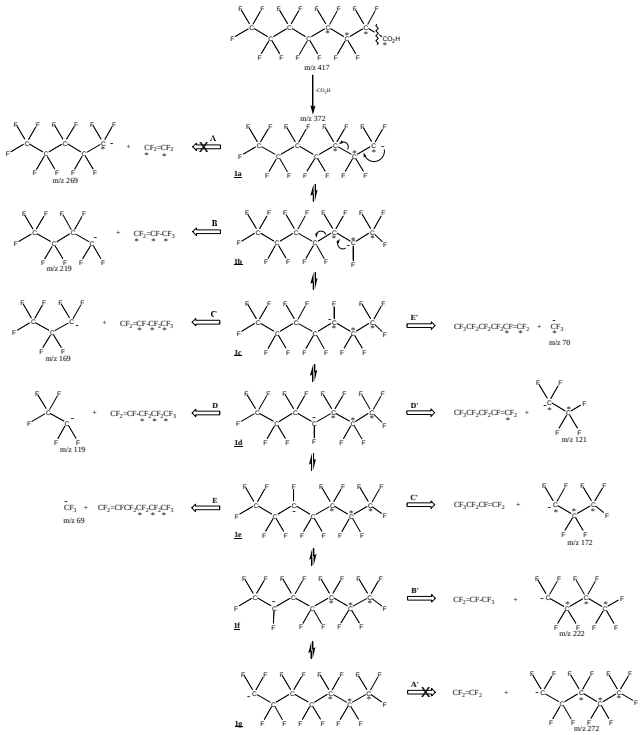

**Figure 2. Possible fragmentation pathway for 1**

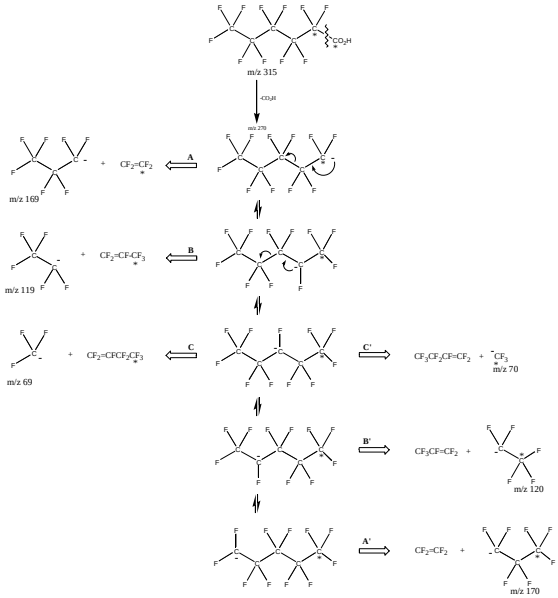

**Figure 3. Possible fragmentation pathway for 2**