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Structural Characterization of PFAS via Oxygen Attachment Dissociation: A Complementary Approach to CID

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are structurally diverse, making their analysis challenging with conventional CID-based MS/MS. Oxygen Attachment Dissociation (OAD) offers a complementary approach by inducing radical-driven fragmentation using neutral species such as atomic oxygen and atomic hydrogen. In this study, OAD was applied to various PFAS, including branched and unsaturated compounds. Unlike CID, OAD revealed distinct fragment ions and cleavage pathways, reflecting differences in structure. For example, unique fragments from branched PFOA and FDUEA were observed only under OAD. These results highlight the potential of OAD to enhance PFAS structural characterization, particularly for complex analogs.

2. Method

OAD (Oxygen Attachment Dissociation)-MS/MS for Lipids

OAD is a new fragmentation technology that enables identification of structural isomers not achievable with CID [1]. As shown in Fig. 1, charge-neutral atomic oxygen or atomic hydrogen are introduced into q2, inducing gas-phase dissociation. Applicable to ions of any charge state, including negative and multiply charged ions, OAD selectively dissociates C=C in lipids, allowing positional isomer differentiation.

$$[M+H]^{+} + O^{\bullet} \rightarrow [M + H + O]^{+\bullet} \rightarrow \textit{fragments}$$

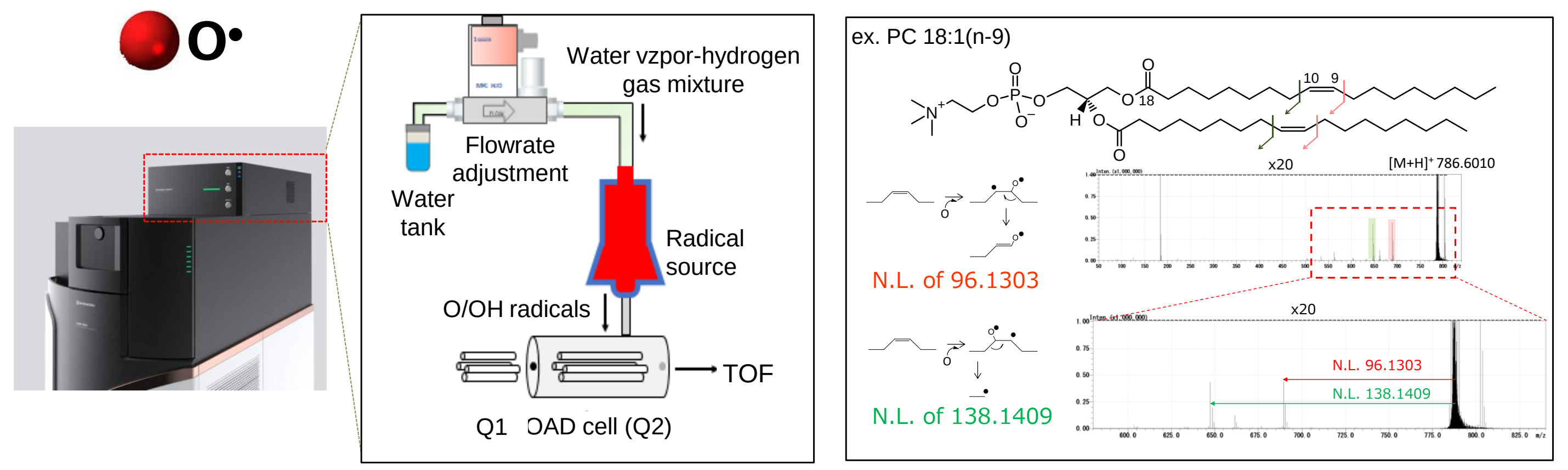
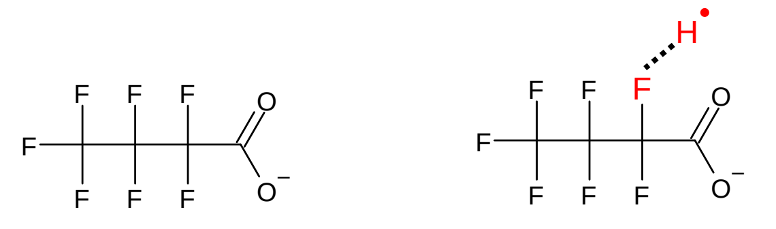


Fig. 1 Shimadzu LCMS-9050 (Q-TOF) with OAD unit.

OAD-MS/MS for PFOA

Since OAD is applicable to negative ions, it is also suitable for PFAS analysis, where negative ion mode is generally preferred. For example, in the case of $C_3F_7CO_2^-$, OAD of $C_3F_7CO_2^-$ yields unique product ions $C_3F_6^{-\bullet}$ (*m/z* 150) and $C_3F_5O^-$ (*m/z* 147) absent from CID. DFT calculations suggest an intricate pathway of H• attack onto a C-F bond, then loss of HF, decarboxylation, recombination with OH•, and finally or elimination of HF to give the products. This mechanism was elucidated by Richard O'Hair etc. al.[2]

e.g. $C_3F_7CO_2^- + H^{\bullet} \rightarrow C_3F_6CO_2^{-\bullet} + HF \rightarrow \textit{fragments}$



3. Results

Table 1 LCMS Analysis Parameters

Mass Spectrometer

System : LCMS-9050 with OAD unit
Polarity : Negative-ESI
MS/MS mode : CID/OAD
Fragment gas : Ar for CID, H_2O for OAD
Collision energy : 15-75 V for CID, 10V for OAD
Event time : 200 ms

LC

System : Shimadzu UPLC system
Analytical Column : Shim-pack GIST-HP C18-AQ

Samples

PFAS : All standards were purchased from Wellington Laboratories Inc.

Comparison of CID and OAD Fragmentation of PFOA

Fig. 3 shows a mirrored comparison of neutral loss spectra of PFOA obtained by CID and OAD. Both spectra were acquired from the carboxylate anion $C_7F_{15}CO_2^-$. CID primarily induces simple neutral losses such as $–C_3F_6$ and $–CO_2$, resulting in fragment ions. In contrast, OAD induces radical-driven fragmentation pathways, producing a broader range of ions, including $C_5F_9^-$, $C_6F_{11}^-$ and $C_7F_{13}^-$, which are absent in the CID spectrum.

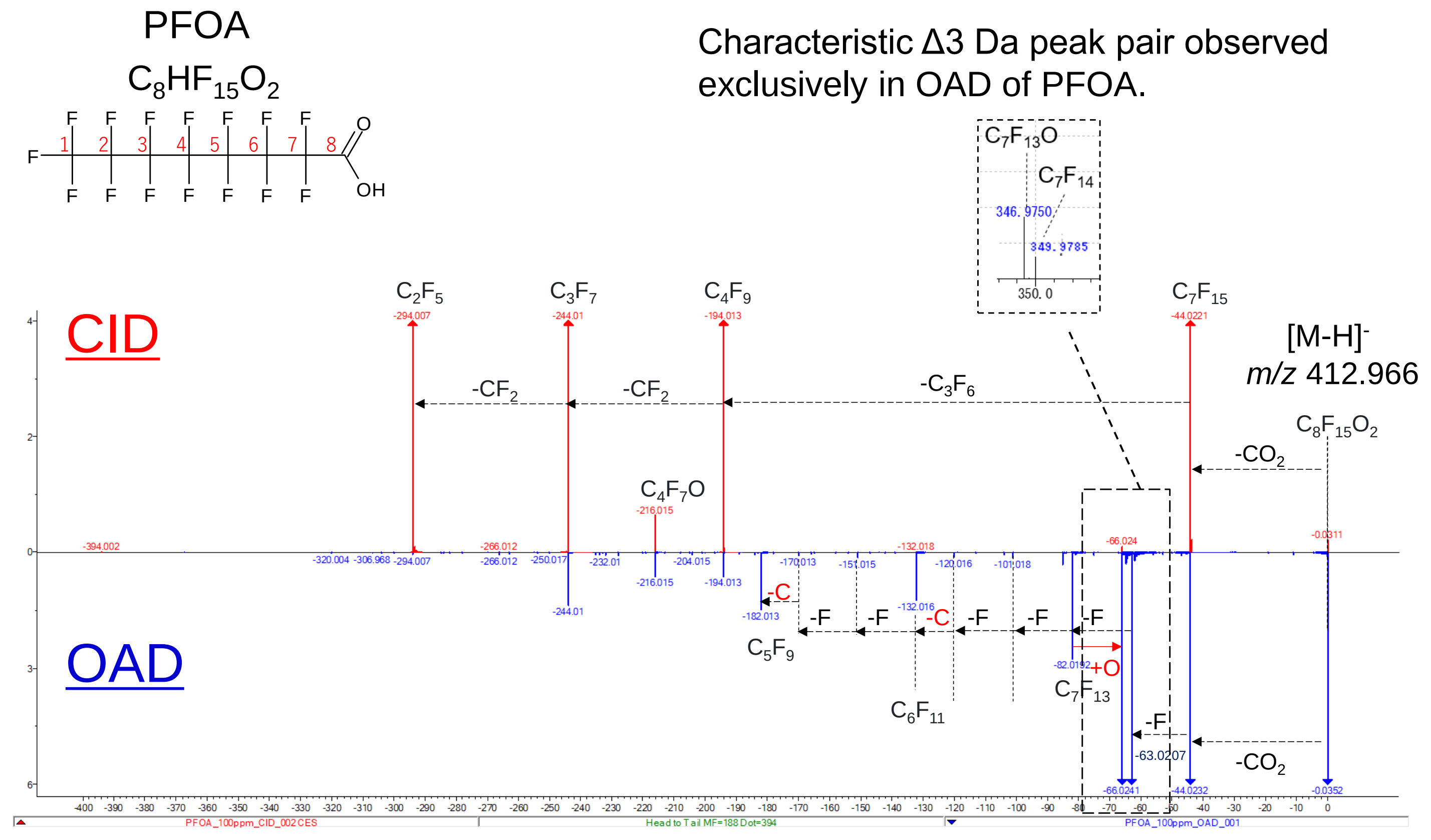


Fig. 3 Neutral loss spectra of PFOA by CID and OAD showing distinct fragmentation patterns.

Terminal-Specific Fragmentation Behavior in CID vs. OAD

To investigate the fragmentation pathway in FDUEA by CID and OAD, we compared neutral loss MS/MS ions using both unlabeled and ^{13}C-labeled standards. The ^{13}C labels were introduced near the carboxylic terminus, allowing us to track which end of the molecule was retained in each fragment ion. In CID (Fig. 4A), fragments derived from both terminal ends were observed, indicating symmetric cleavage behavior. In contrast, OAD (Fig. 4B) predominantly provided fragment ions retaining the ^{13}C-labeled moiety, suggesting that cleavage occurs mainly from the non-carboxyl end. These results reveal mechanistic differences between CID and OAD, with OAD favoring unidirectional fragmentation pathways.

Unlike the typical OAD reactions observed in unsaturated fatty acids, where oxygen adducts (e.g., $[M–H+O]^-$) are often detected at C=C positions, no such oxygen adducts were observed in FDUEA. This absence indicates that the molecule lacks reactive π-bond sites and suggests that the C=C moiety does not contribute to OAD fragmentation in this case.

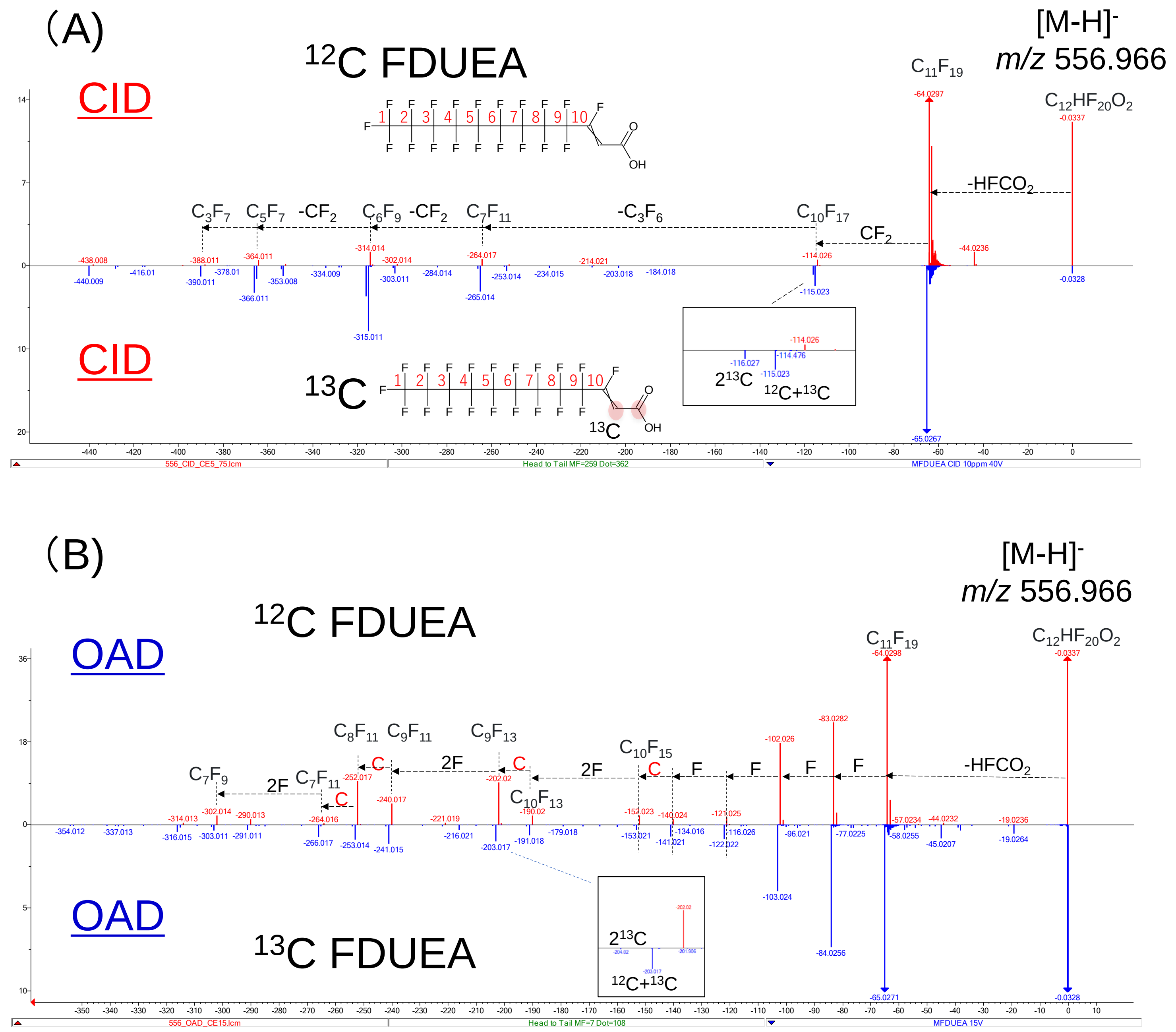


Fig. 4 Neutral loss spectra of FDUEA obtained by CID (A) and OAD (B). CID produces symmetric fragmentation, whereas OAD shows a unidirectional pattern with preferential retention of the ^{13}C label.

OAD Fragmentation of Linear and Branched PFOA

OAD-MS/MS analysis of linear and branched PFOA revealed differences in fragment ion distributions. Linear PFOA produced a characteristic Δ3 Da peak pair ($C_7F_{13}O^-$ and $C_7F_{14}^-$), also observed in Fig. 3, suggesting radical-driven cleavage followed by oxygen attachment. In branched PFOA, the corresponding peaks appeared at lower *m/z* values ($C_4F_7O^-$), reflecting the effect of molecular branching on the fragmentation pathway. These findings support the utility of OAD for probing subtle structural variations in PFOA.

OAD-specific "Δ3 Da peak pair" for PFOA, as seen in Fig. 3

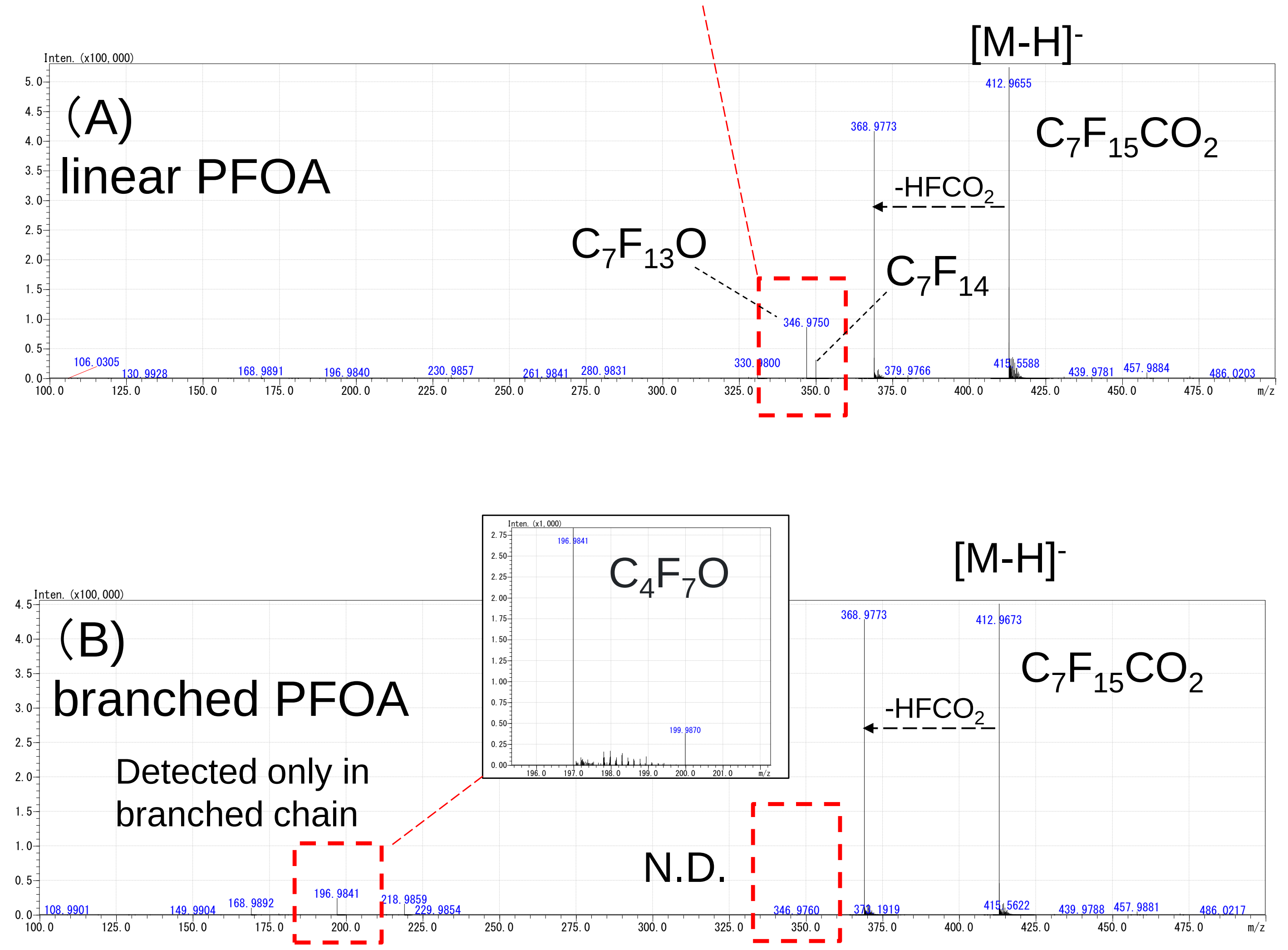


Fig. 5 OAD-MS/MS spectra of linear (A) and branched (B) PFOA showing "Δ3 Da peak pair" shift due to branching.

4. Conclusion

- OAD gave distinct PFOA fragmentation patterns from CID.
- ^{13}C labeling confirmed unidirectional cleavage in OAD.
- In branched PFOA, the characteristic Δ3 Da peak pair, typically observed in OAD, appeared at a different position from the linear isomer.
- OAD complements CID for PFAS structural analysis.

[1] Takahashi et al., Anal. Chem. 2018, 90(12), 7230–7238.

[2] Li et al., Rapid Commun. Mass Spectrom. 2025, 39(3), e9953.