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Use of electron activated dissociation (EAD) to elucidate PFAS structures

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Introduction

Poly- and perfluoroalkyl substances (PFAS) are well-known environmental contaminants and are widely detected in humans and wildlife, water, soil and air.[1,2] PFAS are primarily used for their stain repellency properties as well as their surfactant characteristics, such as in aqueous film-forming foams (AFFF) to combat petroleum fires. Even though there are an estimated 5,000 unique PFAS manufactured, most monitoring efforts are focused on only 20-30 compounds. Non-targeted data acquisition using high resolution accurate mass spectrometry is beneficial for elucidating unknown compound structures, such as PFAS in complex samples. However, candidate structure assignment depends crucially on the collection of high-quality MS/MS spectral data. Traditional fragmentation methods using collision-induced dissociation (CID) can be too aggressive to form diagnostic MS/MS spectra (Figure 1). Alternatively, electron activated dissociation (EAD) has shown potential as a form of fragmentation to produce more robust spectra.[3] This study evaluated the use of EAD fragmentation qualitative PFAS structure elucidation and compared the results to those produced with MS/MS spectra achieved using traditional CID generated data.

Key features of the ZenoTOF 7600 system for structural elucidation

- The ZenoTOF 7600 system collects high resolution spectral data using multiple types of fragmentation
- Electron activated dissociation (EAD) is a "softer" fragmentation strategy compared to the commonly applied collision-induced dissociation (CID)
- The capacity to select different fragmentation types allows the user to collect the greatest number of unique MS/MS fragments, which can be used for structural elucidation
- User-adjustable parameters such as kinetic energy settings allow for further refinement and optimization of the acquisition method to achieve the best MS/MS data
- Utilizing the EAD fragmentation strategy was observed to be advantageous in analyzing AFFF for PFAS, as the softer fragmentation strategy produces a greater number of unique fragments from PFAS species
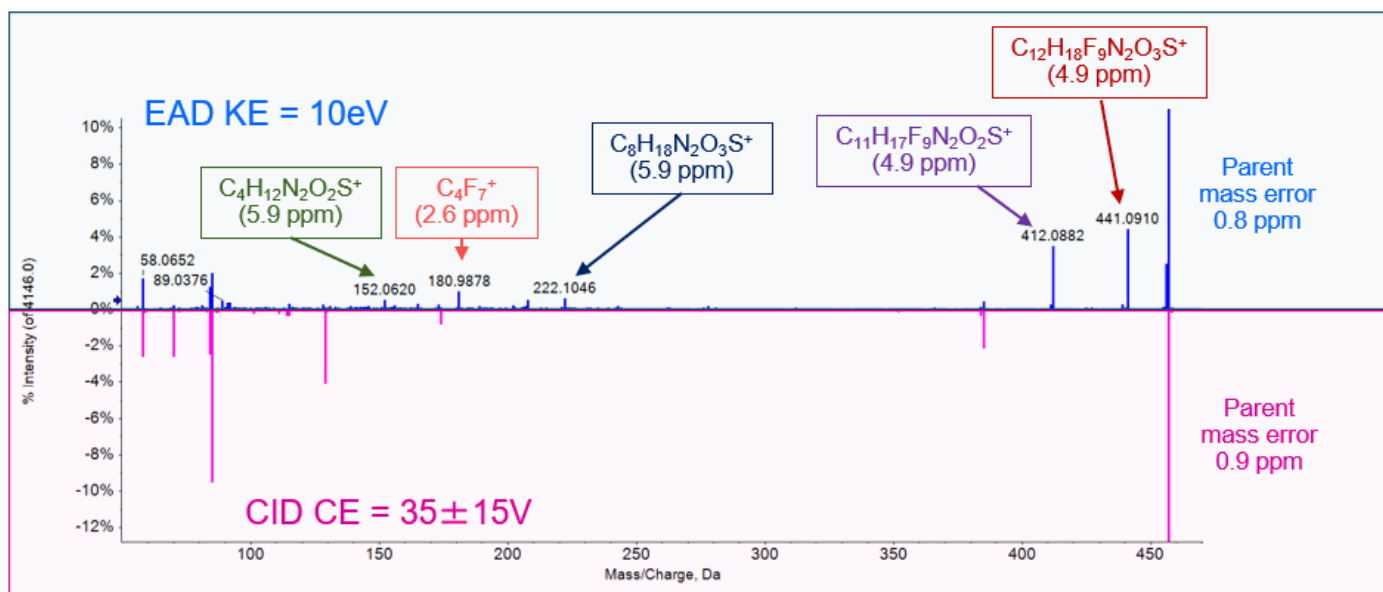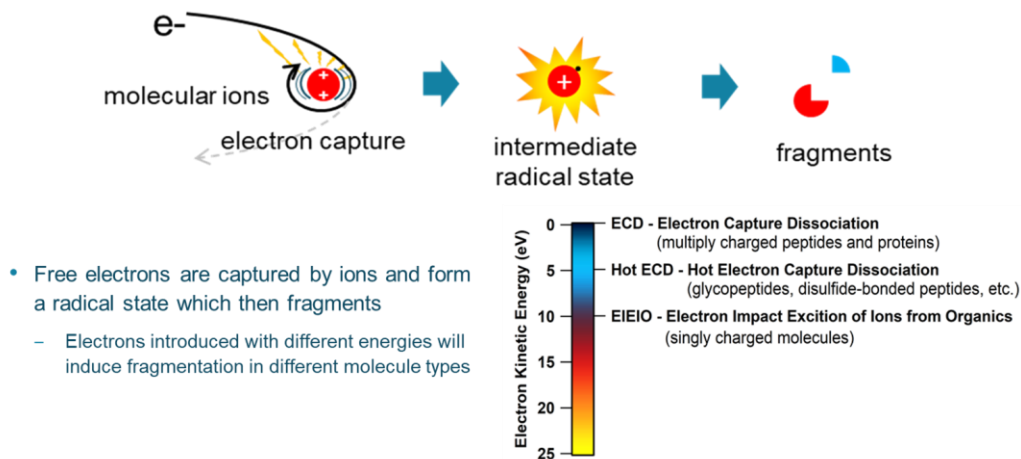

Figure 1. Fragmentation spectra generated using EAD fragmentation in an AFFF mixture. EAD spectral data (top spectrum) showed the generation of several unique fragments compared to CID fragmentation (bottom spectrum).

Figure 2. Mechanism of EAD fragmentation. Note that different kinetic energy settings produce fragmentation for different types of molecules.
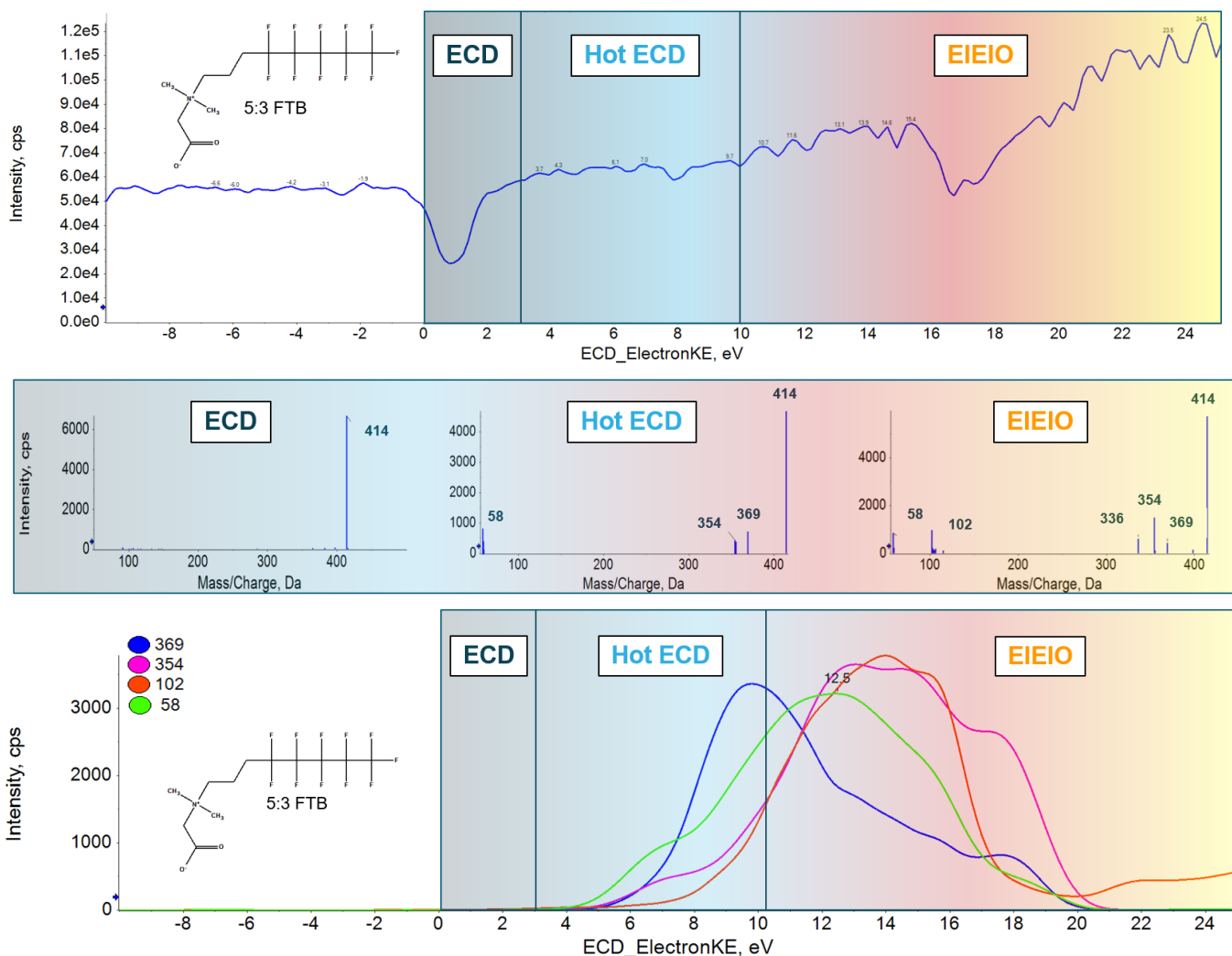
Figure 3.   Kinetic energy ramping from -10 to 25 V using EAD fragmentation mode for the 5:3 FTB. Increased fragments were generated in the "hot ECD" (3 to 10 V) and "EIEIO" (10-25 V) regions.

Methods

Standard solutions of 50 PFAS compounds including 5:3 fluorotelomer betaine (5:3 FTB), 5:1:2 FTB, AmPr-FHxSA, TAmPR-FHxSA and 6:2 FTSA-PrB were purchased from Wellington Laboratories (Guelph, ON). The standards were infused on the ZenoTOF 7600 system using both CID and EAD fragmentation modes. Figure 2 shows a brief visualization of the mechanism behind EAD fragmentation, including designation of relevant kinetic energy zones.[3] In separate EAD experiments, the kinetic energy (KE) was ramped from -10 to 25 V and the electron beam current ramped from 0 to 8000 V. Further, 10, 35, and 100 ms reaction times were tested. Finally, an AFFF mixture was injected on a reverse-phase LC column and subject to gradient conditions to compare EAD and CID fragmentation in a real-world PFAS AFFF sample. Data processing and evaluation were performed in the SCIEX OS software.

Results

Kinetic energy (KE) ramping

Initial EAD KE ramping experiments were performed using the 5:3 FTB. Results showed that low KE values (< 3 V) were insufficient to cause precursor compound fragmentation (Figure 3). However, fragmentation was observed as the KE increased into the "hot ECD" and "EIEIO" regions (Figures 2,3). Specifically, fragments m/z 369, m/z 354, m/z 102 and m/z 58 were detected as the KE values increased to greater than 5 V.

All fragments showed maximum intensity in the EIEIO region, except for the m/z 369 fragment.

Comparing CID vs EAD fragmentation

To further explore the potential benefits of EAD fragmentation, the 5:1:2 fluorotelomer betaine was infused using both CID and EAD fragmentation. The MS/MS spectra generated from CID fragmentation showed only formation of the m/z 58.0651 Da fragment ($C_3H_8N^+$) under the 3 voltage ranges of collision energy (CE) tested; 10-20 V, 30-40 V and 50-60 V (Figure 4). In contrast, the MS/MS spectra generated from EAD fragmentation showed many more fragments produced in the 3 KE ranges, particularly at KE=16 V (Figure 5). EAD showed the generation of several unique fragments as compared to CID fragmentation.

Using EAD fragmentation to identify PFAS in an AFFF mixture

The AFFF mixture that was separated using liquid chromatography with gradient conditions showed the presence of the perfluorobutane sulfonamido propyl dimethyl quaternary amine propanoate when using both EAD and CID fragmentation. However, the EAD fragmentation spectrum showed additional, numerous unique fragments (Figure 5) that were not observed during CID fragmentation (Figure 1). Therefore, EAD fragmentation may act as an additional, orthogonal source of confirmation for the identification of unknown PFAS compounds.
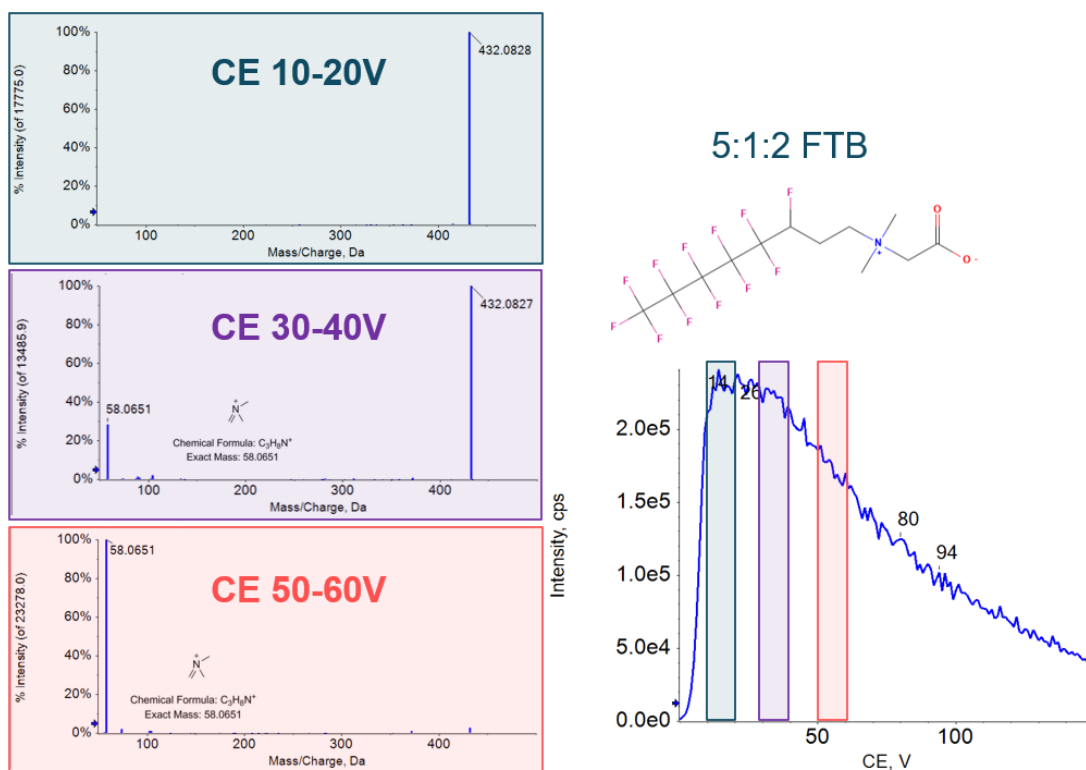

Figure 4. Left panels show fragmentation spectra generated using CID fragmentation of the 5:1:2 fluorotelomer betaine. Spectra are separated by the three CE ramps from 10-20V (top), 30-40V (center) and 50-60V (bottom). The only fragment observed during CID was the $C_3H_8N^+$ fragment at m/z 58.0651.

Conclusions

In general, the MS/MS spectra collected using EAD generated more fragments which could be beneficial for confirming compound identity during nontarget analysis. During KE ramping, it was observed that different energy ranges produced different fragmentation patterns. Ramping the KE to "hot ECD" and EIEIO values showed unique fragments as compared to lower KE values. This study ultimately showed that the EAD fragmentation may provide additional or orthogonal spectral information for the identification of nontargeted PFAS structures.

References

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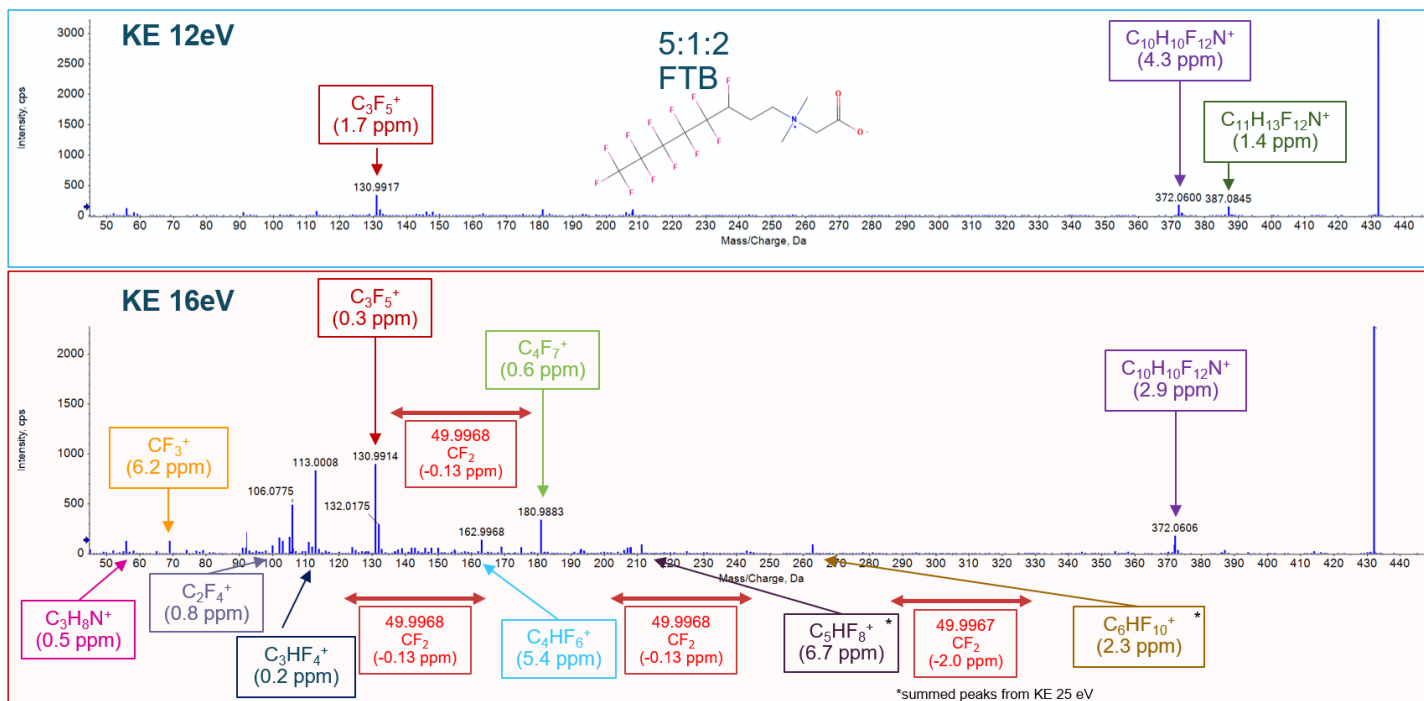
Figure 5. Fragmentation spectra generated using EAD fragmentation of the 5:1:2 fluorotelomer betaine at KE values of 12 eV (top) and 16 eV (bottom). The KE 12 eV spectrum showed the formation of 3 fragments while the KE 16 eV spectrum showed the formation of 16 unique fragments.