

**Characterizing the Occurrence and Fate of Micropollutants in Aqueous
and Environmental Samples: A Multidimensional Approach**

by

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Authors Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Statement of Contributions

Chapter 3 incorporates sections of a manuscript in preparation which is titled:

“Fragmentation Behaviour of Perfluorosulfonic Acids: Mechanisms and Pathways of SO_3^- and FSO_3^- Formation in the Gas Phase” with co-authors as listed:

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Abstract

Emerging contaminants such as pharmaceuticals and per-and polyfluoroalkyl substances (PFAS) present persistent challenges for environmental monitoring due to their chemical diversity, trace-level occurrence and limited removal in conventional wastewater treatment. This thesis presents a dual approach combining experimental two-dimensional separations with computational modeling to advance contaminant detection and mechanistic understanding. A targeted liquid chromatography-mass spectrometry (LC×DMS-MS/MS) method was developed and applied to complex pharmaceutical mixtures, revealing improved compound differentiation and class-based clustering across orthogonal retention time and compensation voltage dimensions. These trends demonstrate the potential of LC×DMS-MS/MS as a selective, high throughput workflow for micropollutant screening in aqueous matrices. Complementary to this, quantum chemical calculations were performed on PFAS molecules, specifically perfluorosulfonic acids (PFSAs), to elucidate degradation pathways and assess the influence of molecular conformation on fragmentation energetics. Calculations identified several concerted and non-concerted reaction pathways that contribute to product selectivity. Together these efforts establish a framework that integrates instrumentation and theory, to support more informed contaminant analysis and method development.

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Warm Regards,
Shannon Lemmens



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List of Abbreviations

CCS	Collision Cross Section
CEPA	Canadian Environmental Protection Act
CID	Collision Induced Dissociation
CI	Climbing Image
EIS	Extracted Internal Standard
ESI	Electrospray Ionization
FIF	Fragment Ion Flagging
GCB	Graphitized Carbon Black
HCB	Hexachlorobenzene
HRMS	High-Resolution Mass Spectrometry
IMS	Ion Mobility Spectrometry
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MCL	Maximum Contaminant Level
MD	Mass Defect
MS	Mass Spectrometry
NEB	Nudged Elastic Band
ng/L	Nanograms per liter
NIS	Non-extracted Internal Standard
OptTS	Transition State Optimization
PAH	Polyaromatic Hydrocarbon
PCB	Polychlorinated Biphenyl

PCP	Pentachlorophenol
PFAPA	Perfluoroalkylphosphinic Acids
PFAS	Per- and Polyfluoroalkyl Substances
PFCA	Short-Chain Carboxyl Compounds
PFHxS	Perfluorohexane Sulfonic Acid
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
PFOSA	Perfluoro Sulfonamides
PFPi	Perfluorophosphinic Acids
PFSA	Perfluorinated Sulfonic Compounds
Q	Quadrupole
QTOF	Quadrupole Time of Flight
RSD%	Relative Standard Deviation
RT	Retention Time
TS	Transition State
TWIMS	Traveling Wave Ion Mobility Spectrometry
U.S. EPA	United States Environmental Protection Agency
WAX	Weak Anion Exchange
WWTP	Wastewater Treatment Plant
XIC	Extracted Ion Chromatograms

Chapter 1. Introduction to Micropollutants: Their Impact on the Environmental and Human Health

1.1 Defining and Characterizing PFAS and Pharmaceutical Micropollutants

Micropollutants such as per- and polyfluoroalkyl substances (PFAS) and pharmaceuticals are emerging contaminants of increasing concern in Canadian water supplies.^{1,2} These chemicals, often detected at trace levels (ng/L to µg/L), originate from industrial, agricultural and domestic sources and are not fully removed by conventional wastewater treatment processes.³⁻⁵ PFAS in particular are a class of persistent organofluorine compounds, recognized by Health Canada as hazardous chemical pollutants.^{1,2} Characterized by their strong carbon-fluorine bonds, PFAS are highly resistant to environmental degradation and are commonly referred to as “forever chemicals”. Historically, produced as industrial byproducts, PFAS have been widely incorporated into consumer goods such as non-stick cookware, food packaging and personal care products.⁶ With over 4700 PFAS compounds identified to date, these substances vary significantly in regional distribution and environmental concentration.¹

Large-scale and repeated use of these products, pose significant health risks for our population and due to their long half-life, micropollutants are not only being introduced into our drinking water but they are known to bioaccumulate in our bodies.⁷ These risks include (but are not limited to) poor fetal development, behaviour and learning irregularities in children, infertility in women and hormonal imbalances in both males and females.⁷ Due to growing concern surrounding these pollutants, the Canadian Environmental Protection Act, 1999 (CEPA) and the U.S. Environmental Protection Agency (EPA) have enforced regulations to minimize our exposure to these pollutants where it possible.^{2,6} However, while these regulations are a step in the right direction, they do not address a fraction of the total pollutants

that may be present in our water supplies.^{8,9} Many drinking water thresholds focus primarily on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) with the occasional consideration of a few other PFAS.^{8,9} Even with these limited considerations, analysis ranges for these PFAS are between 20-30,000 parts per trillion (ng/L) which on the lower end is already five times the suggested maximum contaminant level of 4 ng/L proposed by the U.S. EPA with similar guidelines suggested in Canada.^{2,8}

Pharmaceuticals, likewise, are commonly detected in aquatic environments due to incomplete metabolism in humans and improper disposal practices.¹⁰⁻¹⁴ Common classes include analgesics, antibiotics, antidepressants and hormones, all of which have been detected in Canadian surface and drinking water.^{10,14,15} These contaminants can pose chronic effects on aquatic organisms and may pose long-term risks to human health through low-dose, continuous exposure.^{10,11,14,15} While they are less regulated than PFAS, pharmaceuticals do play a significant role of the broader micropollution issue when it comes to resisting conventional treatment and monitoring.^{10,11,14,15}

Together, PFAS and pharmaceuticals highlight similar challenges characteristic of many micropollutants: persistence and difficulty with detection, regulation and remediation. Continuing to study their occurrence, behaviour and removal is key to protecting public health and improving water quality in Canada.

1.2 Current Practices for Micropollutant Sample Preparation and Analysis

1.2.1 Liquid Chromatography Ion Mobility Mass Spectrometry (LC-IMS-MS)

Liquid chromatography coupled to ion mobility and mass spectrometry (LC-IMS-MS) has been used as a powerful analytical tool for the separation and characterization of

micropollutants, specifically PFAS and pharmaceuticals. Both compound classes pose analytical challenges due to their structural diversity, wide polarity range and frequent occurrence of isomeric or coeluting species. Instrumental approaches combining retention time, ion mobility separation and mass-to-charge ratio (m/z) offer increased selectivity and confidence in analyte identification.^{16–18} For example, some PFAS isomers, both linear and branched, have been identified which complicates quantification in conventional LC-MS workflows. Similarly, many pharmaceuticals such as beta-blockers, antibiotics or antidepressants may share structural characteristics or exhibit similar chromatographic behaviours, leading to peak overlap in complex environmental matrices.

Ion mobility spectrometry (IMS) adds, an orthogonal separation dimension based on the gas-phase mobility of ions, influenced by their size, shape and charge.^{18–22} This additional separation power is especially useful for distinguishing isomeric and isobaric compounds.^{18–22} Lanucara et al. demonstrated the utility of IMS-MS in pharmaceutical analysis by resolving structurally similar drug metabolites based on their collision cross sections (CCS), enabling separation of species such as isomers and protomers that would otherwise be indistinguishable by mass spectrometry alone.¹⁸ This highlights the important role IMS can play in improving selectivity and structural resolution for micropollutants in complex mixtures.¹⁸

Contemplating the instrumental approaches used by Boatman. A. K et al.²³ and Cserbik. D et al.²⁴, the fish fillet study used LC-IMS-MS to separate and identify PFAS within their samples using an untargeted approach and to acquire supporting mobility information such as drift times and collision cross sections (CCS). In contrast, the study of plasma samples uses

LC-MS/MS analysis to separate and quantify the targeted PFAS analytes within water samples.^{23,24}

While the sample preparation in both studies do mimic the same general practises for the preparation and storage of PFAS samples, the instrumental analysis varies between each method, although these variations are dependent on the targeted and untargeted approaches required. The use of LC-IMS-MS as a coupled instrumental analysis method is efficient for not only separation of analytes and isomers but the acquisition of their mobility behaviours.^{23,25} In some cases branched and linear isomers may be inseparable by traditional LC or IMS methods depending on the degree of structural variation.²⁶ In such cases, the addition of a multiple reaction monitoring (MRM) method can help identify these species if they produce slightly different product ion intensities or even different fragments entirely under collision induced dissociation (CID), due to the fragmentation kinetics or steric hindrances.²⁷ However, in the case that isomers are separable by LC or IMS, the use of a standard MRM approach is not necessary, because the features such as CCS and RT are sufficient enough to distinguish these species. Aderorho and Chouinard demonstrated the benefit of using ion mobility for isomer separation using fentanyl analogues, a group of synthetic opioids that are notoriously difficult to differentiate using traditional LC-MS due to their identical m/z values and similar fragmentation patterns.¹⁶ The study showed that isomeric fentanyl's (*e.g.* para-fluorofentanyl vs ortho-fluorofentanyl) could not be separated using LC or MS alone, but displayed distinct drift times in IMS.¹⁶ The formation of metal adducts further altered these structural conformations, leading to enhanced mobility separation due to the changes in ion shape and coordination behaviour.¹⁶ This instrumentation provides complementary features such as RT,

CCS and m/z that are otherwise collected separately and prolong the sample analysis process. Determining unique features can be critical in the untargeted identification of micropollutant analytes, for isomers, isobars or analytes that co-elute. In some cases the analytes could be separated based on their differential mobility characteristics.^{19,21,28–30} Additionally, differential ion mobility has useful applications when dealing with ultra trace detection limits, where known analytes are studied in a sample matrix.^{19,21,28–30} The DMS acts as a narrow pass filter that transmits pre-selected ions, removing any unwanted noise from the matrix and overall improves the signal-to-noise ratio (SNR). The works of this thesis compare retention time and differential ion mobility data using coupled and un-coupled LC-MS and DMS methods, to improve the separation and characterization of pharmaceuticals found in our water and environmental samples.²²

1.2.2 Analyzing Wastewater Water Treatment Influent, Effluent and Biosolids for Micropollutant Contamination and Effective Removal

Analyzing wastewater treatment influent, effluent and biosolids for micropollutant contamination is crucial for understanding the efficacy of current treatment processes and the environmental risks posed by residual micropollutants. A comprehensive review of pharmaceutical occurrences in freshwater systems from 2019 to 2024 highlights that wastewater treatment plants (WWTP) effluents are primary pathways for pharmaceutical contaminants entering aquatic environments.^{31–33} These compounds often persist through conventional treatment stages, leading to their detection in both effluent and biosolids.^{31–33} The variability in removal efficiency is influenced by the physicochemical properties of the pharmaceuticals, such as solubility, molecular structure, which affects their ability to degrade and their adsorption potential during the treatment process.^{31–33}

Furthermore, the management of pharmaceutical effluent presents significant challenges due to the complex composition of wastewater.^{31–33} The characteristics of pharmaceutical wastewater are influenced by various factors, including the diversity of compounds present and their concentrations.^{31–33} Microbial treatment processes, while beneficial, may not effectively degrade all pharmaceutical compounds, which emphasizes the need for integrating more advanced treatment technologies.^{31–33} The selection of appropriate treatment methods must consider the specific characteristics of the influent, the desired quality of the effluent and the potential environmental impacts of the biosolid produced.^{31–33}

In the context of biological treatment methods, recent studies have explored the efficacy of various bioreactor configurations in removing pharmaceutical contaminants.^{31–33} These biological processes leverage microbial communities to degrade organic pollutants, including pharmaceuticals.^{31–33} However, the effectiveness of these systems can be limited by factors such as the bioavailability of the compounds and the adaptability of the microbial species.^{31–33} Therefore, optimizing operational parameters and integrating additional treatment stages are essential strategies to enhance the removal of micropollutants and ensure the production of environmentally safe effluent and biosolids.^{31–33}

Link, G et al., reports key findings about the presence and characteristics of PFAS in wastewater treatment plant (WWTP) influent, effluent, and biosolids.³⁴ While the existence of PFAS in these contexts has been established for several years, considerable progress is still needed. The study highlights the importance of carefully considering and reporting analytical methods when conducting biosolids studies.³⁴ It also emphasizes the necessity of focusing on

fully processed biosolids for an accurate representation of PFAS concentrations prior to disposal.³⁴

Link, G et al., reveals that fluorinated long-chain sulfonic compounds (PFSA) dominate Michigan WWTP biosolids, but short-chain carboxyl compounds (PFCA) are also frequently detected.³⁴ Notably, PFOS remains a prominent PFAS identity in Michigan biosolids, despite manufacturing regulations.³⁴ The study raises concerns about sulfonate compounds, their role in PFAS content in biosolids, and the need for further research and regulation.³⁴ Lastly, the research highlights the variability of PFAS compounds in biosolids across the state and indicates trends in PFAS enrichment, which generally increase with compound hydrophobicity.³⁴ PFAS enrichment can be understood as the extent to which a PFAS compound exhibits a preference for the solid phase rather than the aqueous phase.³⁴ This behaviour dictates the extent to which various PFAS will accumulate in biosolids.³⁴

In conclusion, these studies suggest extensive future research efforts be directed towards understanding micropollutants in influent, effluent, biosolids and the environment.³¹⁻³⁴ As well as urging researchers to build upon these findings by including relevant precursors and performing regular sampling of treated solids.³¹⁻³⁴

1.2.3 Advancements in Micropollutant Analysis Techniques: Bridging the Gap between Targeted and Non-Targeted Methods

In the field of environmental analysis, the investigation of micropollutants such as per- and polyfluoroalkyl substances (PFAS) and pharmaceuticals is of growing importance due to their persistence, widespread occurrence and emerging health concerns. Traditional methods primarily use gas or liquid chromatography coupled to tandem mass spectrometry (MS/MS) to target a limited set of 20 to 40 specific PFAS compounds.¹⁷ However, as Raquel Gonzalez de

Vega et al. argue, these targeted approaches fall short of providing a complete picture due to the diversity of PFAS, encompassing a broad range of chain lengths, fragmentation patterns, functional groups and partially fluorinate species.¹⁷ Similarly, pharmaceutical pollutants span diverse therapeutic classes such as beta blockers, analgesics, and antidepressants, many of which are structurally similar and can occur as isomeric or coeluting species, complicating targeted analysis. As with PFAS, most pharmaceutical monitoring is limited to a targeted list, potentially overlooking structurally related compounds.

To overcome this limitation, more non-targeted methods are becoming popular. These non-targeted methods use high-resolution mass spectrometers (HRMS) to identify PFAS, taking advantage of exact mass determination and molecular formula prediction as a chemical identifier.¹⁷ Additionally, the study highlights the potential of ion mobility spectrometry (IMS), as a technology for environmental assays, which, when coupled with MS, allows for a more detailed structural analysis of PFAS by separating ions based on charge and collisional cross section (CCS).¹⁷ This approach mirrors efforts in pharmaceutical research, such as the work of Aderorho and Chouinard, who successfully used IMS-MS to distinguish fentanyl isomers by using conformational differences enhanced via metal adduct formation.¹⁶ In that study, isomeric fentanyl compounds that were indistinguishable by mass alone, were separated based on differences in CCS values, reinforcing the power of IMS for resolving structurally similar analogues.

Gonzalez de Vega et al. presents a multi-dimensional PFAS characterization method using UHPLC-IMS-QTOF-MS, which combines retention time, CCS, mass, and fragmentation data. This was applied to both targeted and non-targeted workflows, including quantitative analysis

of 14 sulfonated PFAS compounds and the development of an automated data analysis workflow, incorporating mass defect (MD) and fragment ion flagging (FIF), for the simultaneous identification of non-targeted PFAS within the same chromatographic run.¹⁷ This study showcases the ever-evolving landscape of PFAS analysis, and efforts devoted to bridging the gap between targeted and non-targeted methods to offer a more comprehensive understanding of PFAS contamination.¹⁷

Gonzalez de Vega et al.'s study not only offered a comprehensive approach but also addressed specific challenges in identifying PFAS isomers. It employed a combination of CCS values, retention times, and drift times for distinguishing PFAS isomers, which are difficult to separate using HRMS alone as seen in Figure 1.1.¹⁷

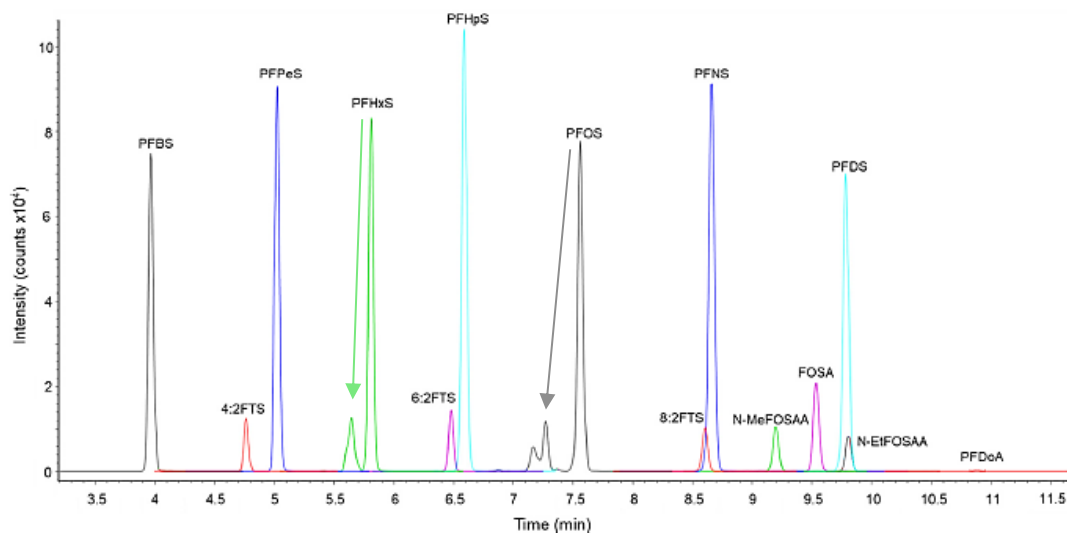


Figure 1-1. Separated extracted ion chromatograms (XIC) of 14 PFAS. A chromatogram displaying the intensity peaks of various perfluorinated compounds over time, with focus on the sulfonate-based subclass of PFAS ($C_nF_{2n} + SO_3H$) following the analysis of a 10 $\mu g/L$ standard mixture. Figure 1.1 is adapted from reference ¹⁷.

The study focused on PFHxS and PFOS as representative PFAS, proving the effectiveness of combining CCS and retention time analysis in reliable isomer identification.¹⁷ The CCS data

was collected and summarized in Table 1.1, for the isomers of both PFHxS and PFOS. Chromatograms as seen in Figure 1.2, identify isomers by their CCS values, enhancing the accuracy of PFAS isomer identification in environmental samples.¹⁷

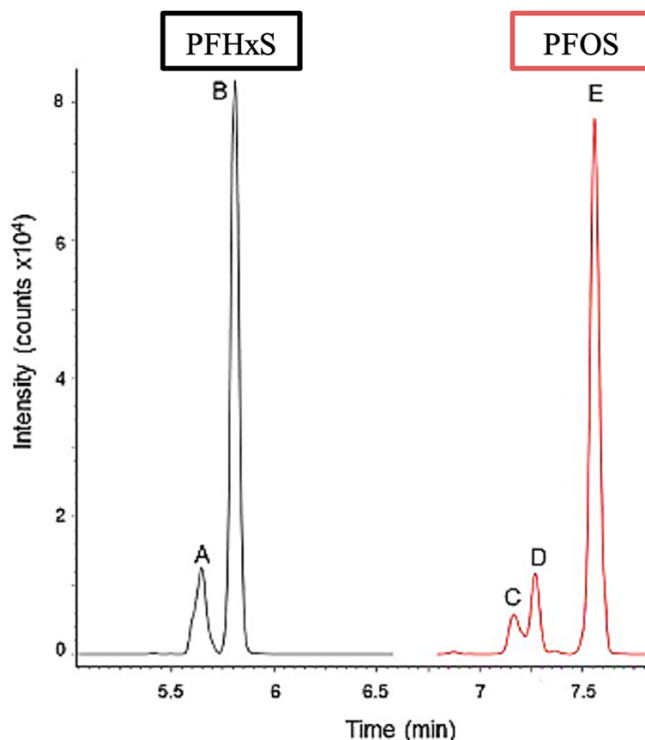


Figure 1-2. Chromatographic separation and detection of linear and branched PFHxS and PFOS isomers via LC-IMS-QTOF. A chromatogram that identifies the isomers of PFHxS and PFOS using their unique CCS values, collected via LC-IMS-QTOF analysis. Figure 1.2 is adapted from reference 17.

Table 1-1. Summary of observed m/z , retention times, CCS values, drift times and isomer assignment for major PFHxS and PFOS isomers. Table 1.1 is adapted from reference 17.

Compound	Peak	Observed m/z	Observed RT (min)	CCS ($\text{\AA}^2$)	Observed Drift (ms)	Structure
PFHxS	A	398.937	5.65	145.97	3.86	$\text{CF}_3\text{CFCF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$ CF_3
		398.937	5.81	147.15	3.91	$\text{CF}_3\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$
PFOS	C	498.930	7.17	161.53	4.47	$\text{CF}_3\text{CF}_2\text{CFCF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$ CF_3

D	498.930	7.27	163.87	4.56	$\begin{array}{c} \text{CF}_3\text{CFCF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^- \\ \\ \text{CF}_3 \end{array}$
E	498.930	7.56	164.75	4.59	$\text{CF}_3\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$

Their non-targeted analysis workflow could detect up to 700 potential PFAS candidates in each sample, ultimately narrowing these down to 107 potential PFAS candidates using mass defect filtering and analysis of common fragments and neutral losses.¹⁷ The combination of targeted and non-targeted analyses was able to identify a significantly larger number of PFAS compounds in non-targeted analysis (56-107) compared to targeted analysis (3-7).¹⁷ Notably, isolated structural-isomer standards for sulfonate PFAS are generally unavailable, therefore, commercial single-compound standards should be assumed to be mixtures of these isomers (e.g. linear and branched positional isomers) rather than a single, pure structure. Table 1.2 presents a summary of data for the 14-sulfonate based PFAS compounds studied by Raquel Gonzalez de Vega et al. Both LOD and LOQ values exhibit variability across the different compounds.¹⁷

Table 1-2. Summary of distinct PFAS compounds and their respective physiochemical identifiers. Table 1.2 is reproduced from reference 17.

PFAS Compound	Molecular Formula	Adduct	Observed <i>m/z</i>	R ²	LOD (µg L ⁻¹)	LOQ (µg L ⁻¹)	Observed CCS (Å ²)	CCS (%RSD)	Instrument Response (%RSD)
PFBS	C4HF9SO3	[M-H]	298.9441	0.9992	0.19	0.56	129.80	0.06	1.47
PFPeS	C5HF11SO3	[M-H]	348.9410	0.9972	0.41	1.25	138.10	0.12	1.30
PFHxS	C6HF13SO3	[M-H]	398.9374	0.9981	0.21	0.65	147.15	0.12	2.14
PFHpS	C7HF15SO3	[M-H]	448.9338	0.9963	0.36	1.08	155.64	0.09	1.02
PFOS	C8HF17SO3	[M-H]	498.9377	0.9973	0.24	0.74	164.75	0.09	1.46
PFNS	C9HF19SO3	[M-H]	548.9278	0.9980	0.43	1.28	173.33	0.06	2.50
PFDS	C10HF21SO3	[M-H]	598.9216	0.9992	0.27	0.81	182.25	0.18	2.56
PFDoA	C12HF23SO2	[M-H]	612.9518	0.9997	0.76	2.30	190.15	0.15	4.82
4:2 FTS	C6H4F9SO2	[M-H]	326.9750	0.9982	0.32	0.96	148.96	0.16	1.46
6:2 FTS	C8H4F13SO2	[M-H]	426.9683	0.9982	0.57	1.73	165.51	0.04	1.88

8:2 FTS	C10H4F17SO2	[M-H]	526.9612	0.9982	0.57	1.74	182.30	0.09	0.99
N-MeFOSAA	C9H6F17NO4S	[M-H]	569.9677	0.9994	0.34	1.02	189.82	0.08	2.08
N-EtFOSAA	C10H8F17NO4S	[M-H]	583.9844	0.9990	0.43	1.30	194.22	0.08	3.82
FOSA	C8H5F17NOS	[M-H]	497.9477	0.9984	0.39	1.19	165.91	0.03	2.16

^a All perfluoroalkylsulfonates are in the linear form except PFHxS and PFOS which both have linear and various known branched isomers. Here, the most abundant isomer corresponding to the linear form is listed.

A consistent challenge, however, in both PFAS and pharmaceutical monitoring remains quantification, as standards are not always available for many isomeric forms or fragmentation products. Gonzalez de Vega et al. note that elemental mass spectrometry and atomic spectroscopy may offer future solutions for more universal quantification strategies in regards to micropollutants.

1.2.4 Employing Machine Learning and Computational Methods to Predict Energy Barriers to PFAS Degradation Pathways

A study conducted by Raza. A et al., introduces the application of machine learning techniques to predict and rationalize carbon–fluorine (C–F) bond dissociation energies in per- and polyfluoroalkyl substances (PFAS), aiming to enhance their efficient treatment and removal.³⁵ Employing various machine learning algorithms, including Random Forest, Least Absolute Shrinkage and Selection Operator Regression, and Feed-forward Neural Networks, the research achieves highly accurate predictions for C–F bond dissociation energies, with deviations below 2.93 kJ/mol compared to analytically acquired PFAS reference data.^{35–38} The machine learning approach proves both accurate and efficient, requiring minimal training time and rapid predictions for new compounds.^{35–38}

Density functional theory (DFT) and coupled cluster singles, doubles, and perturbative triples (CCSD(T)) are powerful quantum mechanical methods employed to accurately calculate bond dissociation energies and reaction barriers for PFAS degradation.^{36–38} These

approaches provide deeper insights into the energetic requirements for breaking C–F bonds, considered the strongest single bonds in organic chemistry.^{36–38} DFT calculations, utilizing functionals such as B3LYP, ω B97X-D3, or M06-2X, are all effective methods for predicting relative energies of PFAS degradation barriers.^{36–38} Although DFT is more computationally efficient than CCSD(T), it can occasionally underestimate energetic barriers to reaction in highly fluorinated systems.^{36–38} This is a result of electron correlation effects which can be quite complex and not always well represented by a standard DFT functional.^{36–38} Regardless, DFT is widely used for screening a range of PFAS derivatives because of its balance between computational speed and accuracy.^{36–38} In contrast, CCSDT(T) calculations are considered the “gold standard” for quantum chemistry, as they provide highly accurate energy predictions while capturing the electron correlation effects that play a role in PFAS fragmentation.^{36–38} Although CCSD(T) calculations are of higher computational cost, the results are highly valuable for benchmarking and validating initial DFT predictions.^{36–38} The integration of machine learning with quantum mechanical calculations like DFT and CCSD(T) can optimize PFAS degradation research by combining speed with accuracy.^{36–38} While machine learning models, such as Random Forest or Feed-forward Neural Networks, excel at rapidly predicting trends in bond dissociation energies, quantum mechanical methods provide detailed insights into reaction mechanisms and specific molecular interactions.^{36–38}

Both computational and machine learning methods can elucidate reaction pathways for the defluorination of PFAS. By identifying intermediates, transition states, and energy barriers, these approaches help rationalize the conditions under which C–F bonds break.^{36–38} For example, DFT and CCSD(T) analyses can reveal how various factors influence the

fragmentation behaviours of PFAS compounds.^{36–38} Some of these factors include the effect of substituent groups on transition state stability, the influence of solvent environments in lowering energy for defluorination and the role of external catalysts or reactants in promoting C–F bond cleavage.^{36–38} Ultimately, these computational insights provide a foundation for bridging theoretical understanding with applicable solutions to mitigate PFAS contaminants in our water supplies.

1.3 Scope of this Thesis

Despite the persistent challenges in achieving widespread applicability, cost-effectiveness, and sustainability in current PFAS and pharmaceutical removal methods, it is evident that additional efforts are necessary to address key analytical and computational limitations. One major challenge is the difficulty in establishing optimal detection conditions that are consistent across many structurally diverse micropollutants. PFAS and pharmaceuticals can often vary significantly in polarity, chain length, ionization behaviour and functional groups, which overall complicates method standardization and can compromise sensitivity for some species under fixed conditions.

Additionally, separation techniques continue to be the bottleneck for accurate detection and quantification due to the presence of structurally similar species, isomers and isobars in complex sample matrices. As such, improved multidimensional separation techniques (*e.g.* LC×DMS, LC-IMS-MS) are gaining attention in the hopes of resolving these challenges by leveraging differences in gas-phase ion mobility and retention time.

Another significant issue is the identification of unknown or emerging compounds, particularly in non-targeted workflows. While high resolution MS enables exact mass

matching, it often lacks structural specificity, especially for newly detected compounds without commercial standards. Other techniques such as NMR, while highly useful for structural identification of unknown analytes, cannot be used at traces levels such as those seen in environmental samples. Although, trends among chemical subclasses, such as differences in drift times or CCS values between perfluoroalkyl carboxylic acids (PFCAs) and sulfonic acids (PFSAs), can guide feature annotation, along with characteristic fragment fingerprints such as loss of CO₂ (PFCA) or SO₃⁻ (PFSA).

Computational tools are also being developed to leverage structural and fragmentation trends across known PFAS and pharmaceutical analogues to support the classification of unknowns (*e.g.* Compound Discover, MS Dial, Patroon, etc.). Ultimately, integrating computational modelling, differential ion mobility separations and targeted validation with standards will be key to expanding our knowledge base, improving quantification and guiding remediation efforts in real-world environmental samples.

This thesis explores unknown patterns (*e.g.* production of *m/z* 99 fragment) in the gas-phase degradation of perfluorosulfonic acids, as well as the development of a 2D LC×DMS separation method for characterization of pharmaceuticals.

Chapter 2 covers the analytical and computational background for the experimental and theoretical work conducted throughout this thesis. I discuss techniques such as liquid chromatography, mass spectrometry, differential ion mobility and the fundamentals of a computational workflow.

Chapter 3 discusses the exploration of perfluorosulfonic acid degradation in the gas-phase using analytical and computational methods. This work applies density functional theory

(DFT) and coupled cluster with single, double and perturbative triple excitation theory (CCSD(T)) to search the potential energy surface for the most thermodynamically favourable PFSA degradation mechanisms.

Chapter 4 explores the development of a 2D LC×DMS-MS/MS separation method for characterizing pharmaceuticals of various drug classes. This technique uses both liquid chromatography, the gold standard for solution phase separation, and differential ion mobility, a technique that separates ions based on their gas-phase differential mobility, to identify and characterize pharmaceuticals of similar structure and function.

Chapter 2. Analytical and Computational Background

2.1 Analytical Background

2.1.1 Liquid Chromatography

Chromatography is an analytical technique used for the separation of various chemical compounds based on their relative physicochemical properties.³⁹⁻⁴¹ There are both gaseous and liquid-phase chromatography instruments that serve unique purposes for targeting samples of varying volatility.³⁹⁻⁴² Liquid chromatography is ideal for studying semi-volatile and non-volatile analytes whereas gas chromatography is used for volatile and semi-volatile analytes.³⁹⁻⁴¹ For our study of aqueous samples containing semi- and non-volatile PFAS analytes, liquid chromatography is the ideal instrumentation.^{39-41,43,44} The liquid chromatography (LC) system used for the experimental methods of these works is comprised of an autosampler, pump, injection valve and a high-pressure column (C18-extend and F5, reverse-phase) which collectively separate multiple analytes over the duration of a specified run time.^{39-41,43,44} Once the compounds have eluted from the column, they are then introduced into the mass spectrometer via the electrospray.^{39-41,43,44}

The LC system contains a column, that is packed internally with the stationary phase.^{39-41,43,44} The stationary phase typically consists of microporous silica particles that can be coated with different functional groups.^{39-41,43,44} Each column has specific chemical and physical operating conditions to maintain its optimal accuracy and precision of results.^{39-41,43,44} These operating conditions include pressure, pH, temperature, and solvent composition.^{39-41,43-45} The pH conditions for a typical C18 column are between 2-8 pH, solvents of higher basicity may dissolve the silica packing, similarly to solvents of low acidity, where such an environment will hydrolyze the functional group of the packing material.^{39,44,45} Solvated analyte fills the

pores of the non-polar silica particles (reverse-phase) and the length of this interaction is known as the retention time (RT) of the analyte.^{39–41,43–45}

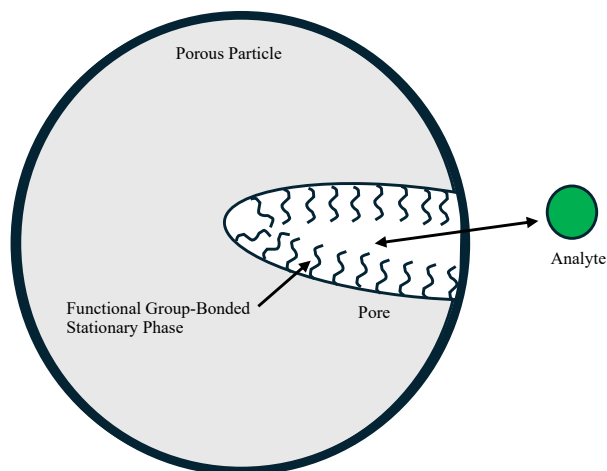


Figure 2-1. Stationary phase microporous particle.

In chromatography, the mobile phase is the solvent that flows through the LC system to transport the sample from the injection site through to the mass spectrometer.^{39–41,43,44,46,47} In reverse-phase chromatography, when using gradient elution method, the initial mobile phase must be more polar than the stationary phase and may contain one or many solvents that aid in the elution of analyte from the column depending on their relative physiochemical properties.^{39–41,43,44,46,47} The solvent composition at varying times throughout the run plays a prominent role in the retention times of each analyte.^{39–41,43,44,46,47} During the separation process, solvents compete with the stationary phase for the analyte.^{39–41,43,44,46,47} The least polar solvent competes more intensely with the stationary phase for the analyte, thus, the less polar mobile phase is increased toward the end of the elution process to elute the more strongly interacting compounds from the column.^{39–41,43,44,46,47} This concept gives rise to the idea that as the polarity of the mobile phase decreases relative to its starting composition, the polarity of the compounds eluting from the column will also decrease, therefore typically the most polar

analytes will elute from the column first, although there may be some exceptions to this.^{39–41,43,44,46,47}

The technique of chromatography relies on the understanding that the process can be related by various equations. Notably column pressure effects and chromatographic efficiency play a large role in understanding liquid chromatography as a technique.^{39–41,43,44,46–49} Column pressure, P , is controlled by many different factors such as mobile phase viscosity, particle size and flow rate as shown below in Equation 2.1.^{48,49}

$$\Delta P = (\eta FL)/(K^0 \pi r^2 d_p^2) \quad \text{Eq. 2-1}$$

Where η is the viscosity of the mobile phase, F is the flow rate of the mobile phase typically in units of mL/min, L is the column length, K^0 is column permeability, r is column radius and d_p is the diameter of the stationary phase particles.^{39–41,43,44,46–49} The relationship between viscosity (η) and pressure (P) is linear in nature and can be described by Equation 2.2.^{48,49}

$$\eta = \eta_0 (1 + \alpha P) \quad \text{Eq. 2-2}$$

Where η_0 is viscosity of atmospheric pressure and α is a constant with values ranging from 5×10^{-3} to 1.2×10^{-2} per mPa for common organic solvents but around an order of magnitude smaller for water.^{48,49} The flow rate, column length and viscosity are all directly proportional to the change in column pressure.^{48,49} To describe the dependence of viscosity on pressure we can combine the Darcy equation (Equation 2.3) with Equation 2.2 to yield a comprehensive equation relating pressure and linear velocity, as shown in Equation 2.4.^{48,49}

$$u = K_0 d_p^2 \Delta P / \eta L \quad \text{Eq. 2-3}$$

$$u = K_0 d_p^2 \Delta P / L \eta_0 (1 + \alpha P) \quad \text{Eq. 2-4}$$

A less obvious inversely proportional relationship to column pressure is column temperature, not seen in the equation above.^{48,49} As the temperature of the column decreases, the viscosity of the mobile phase moving through the column increases which imparts a resistance to flow and overall increasing the pressure within the column.^{48,49} Other inversely proportional factors to column pressure are column permeability, column radius and particle diameter.^{48,49} From the relationship in Equation 2.1, the variability in pressure can be modified through instrumentation by altering solvent viscosity (temperature) and flowrate.⁴⁸ The remaining factors are heavily pre-determined by the choice of the column that is being used for the experimental methods, which reinforces that importance of selecting the proper candidate column for varying target analysis.⁴⁸

2.1.2 Mass Spectrometry

Mass spectrometry is an instrumental technique used to analyze the mass to charge ratio (m/z) of ions (cation and anionic).^{30,39,50–52} A mass spectrum is a visual interpretation of the number of ions detected at varying values of mass-to-charge ratios (m/z).^{30,39,50–52} To acquire a mass spectrum, the sample of interest must be ionized using one of many ionization techniques. The ionization technique used for the methods of this thesis is electrospray ionization (ESI) which is a soft chemical ionization technique.^{53,54}

ESI is carried out by applying a 3 kV to 5 kV potential to ionize the sample from its aqueous state at the tip of the capillary tube.^{53,54} Neutral compounds require chemical ionization via

protonation for positive ion analysis and deprotonation for negative ion analysis.^{53,54} The full ESI process as seen in Figure 2.2, starts by channeling a fine spray of charged droplets, that will in turn be desorbed from the solvent.^{53,54} This results in the ions ejection from the charged droplets which are carried from the ion source into the mass analyzer by the applied cone voltage.^{53,54}

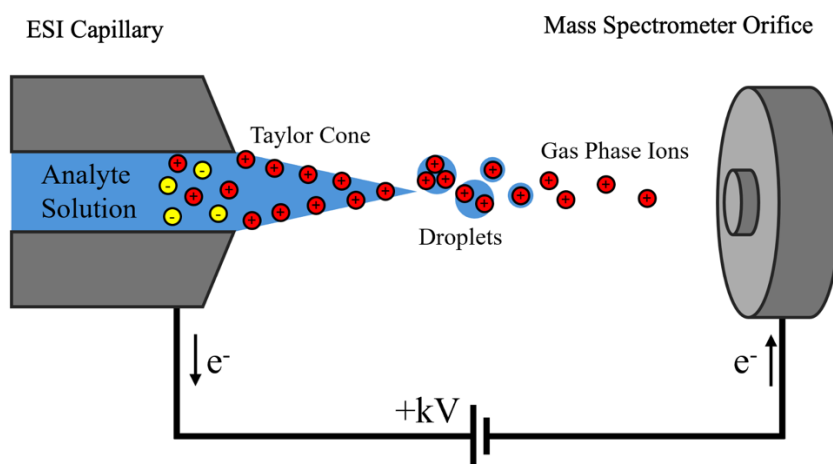


Figure 2-2. Mechanism of electrospray ionization.

Following the ionization process, the ions are transmitted into the mass spectrometer with the help of the cone voltage at the inlet.^{53,54} The mass analyzer used for these experimental methods is a tandem quadrupole system. This analyzer is commonly known as a “triple quad” due to the nature of its setup, having three independent quadrupoles aligned consecutively for a multi-step analysis. The analyte is selected by mass for transmission through the first quadrupole (Q1) where it will be passed into Q2 (the collision cell) to experience collision interactions with the collisional gas, most often N_2 or Ar.^{55,56} This process is followed by collision induced dissociation (CID) whereby ions absorb kinetic energy from these collisions which can induce further fragmentation of the analyte into smaller molecular fragments.^{55,56}

The fragments generated in Q2 act as a fingerprint to the analyte parent structure when they are analyzed further in Q3.⁵⁵

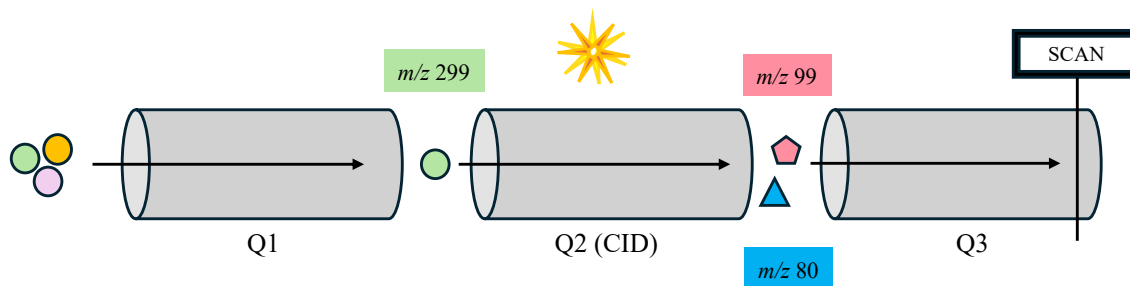


Figure 2-3. Mass analyzer-tandem quadrupole.

Before compounds can be analyzed they must be tuned in the mass spectrometer to determine their optimal conditions for transmission and detection. The four parameters that are optimized on the SCIEX 6500 Triple Quadrupole system are declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP).^{57,58} Declustering potential is an applied voltage at the orifice plate before Q0.^{57,58} Declustering potential is used to extract ions from the region of atmospheric pressure in the ion source while moving into the high vacuum region of the mass analyzer starting at Q0.^{57,58} Entrance potential is the potential difference between Q0, and ground and it is used to channel ions into Q0 for transmission.^{57,58} Collision energy is imparted during a collision between the ion and a neutral gas molecule.^{57,58} Since ions are separated based on their mass-to-charge ratio, collision induced dissociation (CID) is used to generate ion fragments that aid in structural determination.^{57,58} The collision cell exit potential is a parameter used to control the energy of generated fragments while they exit the collision cell.^{57,58}

2.1.3 Differential Ion Mobility Spectrometry

Ion mobility spectrometry (IMS) is a gas phase technique in which ions are separated based on their size, shape and charge as they move through a drift tube under the influence of an electric field. Among various ion mobility techniques such as drift tube IMS and travelling wave IMS, differential ion mobility spectrometry, also known as field asymmetric waveform IMS (FAIMS), explores nonlinear separations based on an ion's differential mobility between high and low electric field conditions.^{19,28,30}

Differential ion mobility is an ion separation technique where analyte separations occur based on their field-dependant mobility in a bath gas under the influence of an electric field.^{20–22,30,50,51} Although there are many ion mobility separation techniques used for a variety of analytes, I will be highlighting the use of differential ion mobility spectrometry (DMS), as seen in Figure 2.4, for the targeted separation of pharmaceutical and perfluorinated analytes.^{19,28,30}

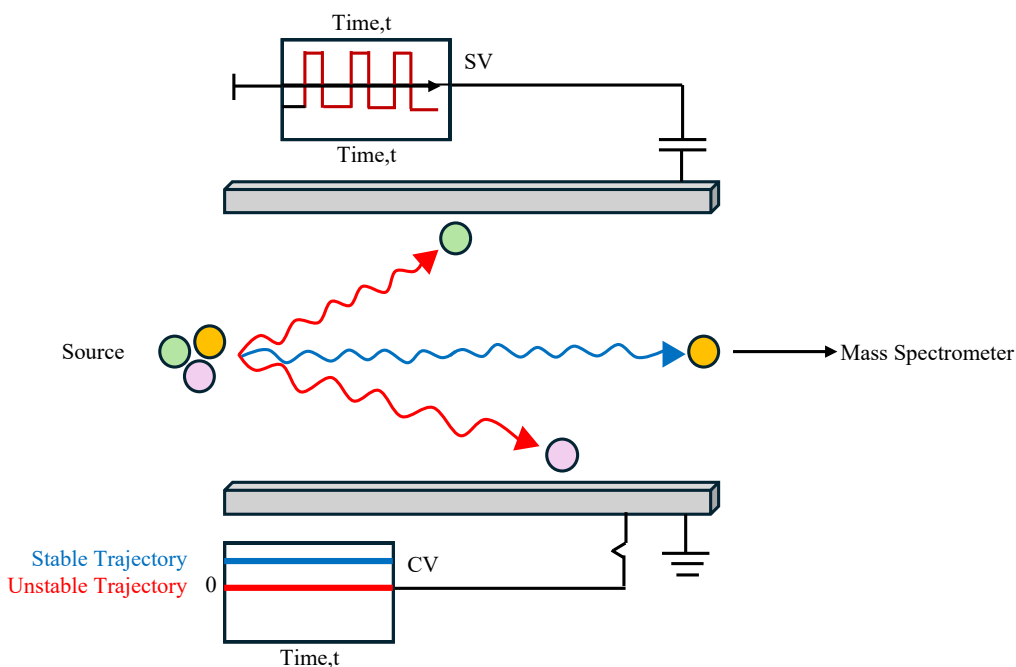


Figure 2-4. Visual representation of ion separation using DMS instrumentation.

The DMS process begins with ionization of the analyte and the resulting ions are guided into a DMS cell, as seen in Figure 2.4.^{19,28,30} This region is filled with a bath gas, in our case nitrogen.^{19,28,30} An asymmetric waveform, also known as a separation voltage (SV) is applied across parallel electrodes with alternating periods of high and low electric field strength.^{19,28,30} As ions move through the electric field their trajectories diverge towards the electrodes depending on how their mobility responds to the change in electric field.^{19,28,30} To selectively transmit an ion of interest, we can apply a direct current compensation voltage (CV) to correct for the divergence and realign the ion with its field free path to be transmitted into the mass spectrometer.^{19,28,30} Separation of two or more analytes occurs given a difference in their mobility under low and high electric field conditions.^{19,28,30} An ions low field mobility can be described by the following equation:

$$K = \frac{v}{E} \quad \text{Eq. 2-5}$$

where mobility (K) is directed related to the velocity (v) of the ion in a given electric field (E).^{19,28,30} This low field relationship assumes, that the electric field is too weak to effect the ions mobility (which is therefore constant), that the ion neutral gas collisions are low energy and elastic and that there is no clustering and declustering behaviour.^{19,28,30} This relationship, however, does not hold for high field conditions because the increased electric field strength plays a role in the ion's behaviour in the DMS cell.^{19,28,30} To best model the ions high field mobility, we use the following equation:

$$K(E) = K_0[1 + \alpha(E)] \quad \text{Eq. 2-6}$$

where K_0 is the field independent (low field) mobility and α is the field dependant correction factor.^{19,28,30}

The field dependant correction factor reveals interesting correlations regarding our ions mobility which can be classified into one of three categories: Type A, B or C. In the field of differential ion mobility, these “Type Classes” are often represented visually by what is known as a dispersion plot.^{19,28,30} These dispersion plots describe the ions differential mobility as a function of separation voltage as seen in Figure 2.5.

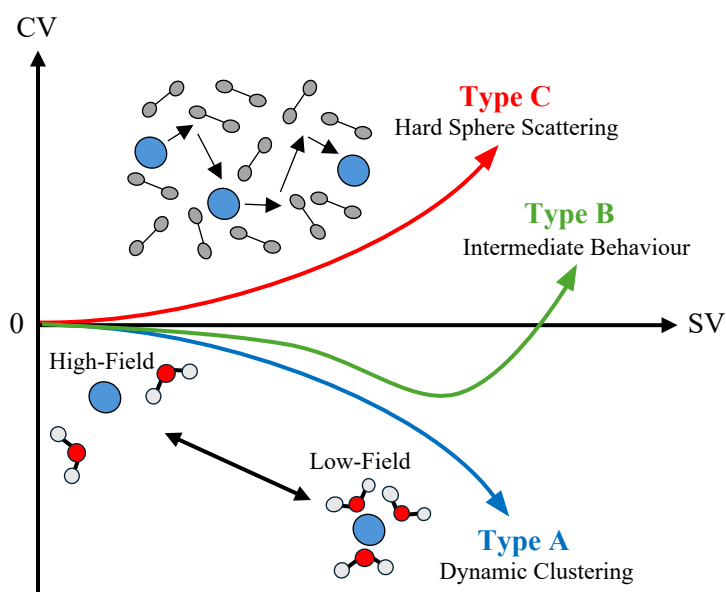


Figure 2-5. Field-dependant ion behaviour: CV-SV relationship for Type A, B and C.

The dispersion behaviour of an ion can tell us information about its size, shape, charge distribution and solvent/clustering interactions.^{19,28,30} This trend arises because ions tend to form clusters with neutral molecules in low-field conditions, but these clusters dissociate under high-field conditions, revealing mobility differences driven by underlying molecular properties. Type A behaviour is an increase in the ions mobility with electric field strength, which results in a negative CV shift as SV increases.^{19,28,30} Type A behaviour is commonly

seen for smaller and more rigid molecules because their compact structure allows for more efficient acceleration under high field conditions, which therefore increases its mobility.^{19,28,30} Type C behaviour is characterized by a decrease in an ions mobility with increasing field strength, which results in a positive CV shift as SV increases.^{19,28,30} Finally, Type B ions show minimal change in mobility across different electric field strength, resulting in a moderately sloped dispersion curve.^{19,28,30} Unlike Types A and C, Type B ions lack strong field dependence, therefore we characterize them by their own group.^{19,28,30}

The presence or absence of solvent clustering may also influence an ions classification. In the low field region, ions often retain loosely bound neutral solvent vapours such as water, isopropanol (IPA), methanol (MeOH) and acetonitrile (ACN), forming ion-neutral clusters.^{19,28,30} In the high field region, cluster tend to dissociate due to an increase in collisional and internal energy, this process is known as declustering.^{19,28,30} When an ion either clusters or declusters, it can change its effective mass and shape depending on the extent of micro-solvation, a shift in differential mobility will likely be observed.^{19,28,30} Understanding an ions type classification can aid in identifying the optimal compensation voltage for maximum transmission and sensitivity.^{19,28,30} When coupled with mass spectrometry, DMS can serve as a narrow pass filter removing background and enhancing ion selectivity in complex sample matrices where isobaric or isomeric species may be present, such as surface water for example.^{19,28,30}

2.2 Computational Background

To develop proper remediation strategies for PFAS, it is important that we understand how they degrade. Experimentally this can be challenging because there are significant limitations

to observing multi-step processes with many intermediates and products. These limitations are exacerbated due to the low reactivity and stability of PFAS in an environmental setting. Using computational chemistry as a complementary approach, we can gain insight into the reaction pathways and energy barriers of these forever chemicals. In my work, quantum calculations are performed to model the behaviour of parent PFAS molecules and their predicted degradation products. This allows us to better understand the energetics of each possible pathway and uncover the most thermodynamically favourable products.

The calculations conducted in this thesis use density functional theory (DFT), which balances computational efficiency with chemical accuracy, and coupled cluster theory (CCSD(T)), which accounts for deviations in energy based on electron correlation effects.^{36–38,59–64} The B3LYP D3BJ hybrid functional is used for geometry optimizations, of all minima and transition states, as well as intrinsic reaction coordinate (IRC) calculations because of its proven ability to accurately predict molecular geometries and vibrational frequencies.^{36–38,59–64} Geometry optimizations are performed by minimizing the total electronic energy with respect to nuclear positioning and calculating the gradient slope at each point along the optimization trajectory.^{36–38,59–64} To reduce the computational cost and increase efficiency, the R2Scan-3c functional was used for the nudged elastic band (NEB) calculations in order to capture intermediate geometries along the potential energy surface between the global minima.⁶⁵ NEB calculations work by generating a set number of “system images” from reactant to product.^{66–68} There are two forces that these system images are optimized with, the first being the true force from the PES projected onto the path, and secondly, an artificial spring force to maintain even spacing between the interpolated images.^{66–68} The NEB-CI method

identifies the highest energy image and maximizes its energy until it reaches the first order saddle point and thus a true transition state geometry.⁶⁶⁻⁶⁸ The accuracy of the electronic energies were improved using DLPNO-CCSD(T) single point energy calculations.⁶⁹ This is a post-SCF energy calculation that uses the domain-based local pair natural orbital (DLPNO) approximation to CCSD(T), which reduces the computational cost while maintaining a high level of accuracy.⁶⁹

Quantum calculations were conducted using the orca suite (version 6.0.1) and molecular structures were either built using Gaussview 16 software or interpolated between minima.⁷⁰⁻⁷³ For the NEB calculations, the NEB-CI keyword was used to locate the first order saddle point on the PES via the climbing image NEB (NEB-CI) method.⁶⁶⁻⁶⁸ The initial TS search interpolates 16 images from reactant to product using tight convergence (VeryTightSCF) criteria to generate a mechanistic coordinate.⁶⁶⁻⁶⁸ Using 16 images provides a narrow grid approximation of the minimum energy path which allows for the identification of shallow intermediates or multi step reactions which is crucial when searching a PES.⁶⁶⁻⁶⁸ The addition of the VeryTightSCF criteria reduces oscillations when evaluating forces as a result of electronic interactions, leading to a smoother trajectory.⁶⁶⁻⁶⁸

The transition state energy was further refined using the OptTS function to confirm the existence of a single imaginary frequency.^{72,73} The function of this calculation is to adjust the TS structure to locate a stationary point on the PES with only one negative eigenvalue in the Hessian matrix.^{72,73} By verifying there is only one negative eigenvalue we can ensure that it correspond with a true first order saddle point.^{72,73}

Intrinsic reaction coordinate (IRC) paths were computed to confirm that the optimized transition states belongs to the initially identified reactant and product minima.^{74,75} The IRC calculations work by numerically integrating the descent pathway with the steepest slope on either side of the TS. If the TS corresponds to the desired minima they will be generated as the result of the descent trajectories.^{74,75}

Energies of the optimized minima and TS were extracted from the orca output files using the PodPals GUI.⁷⁶ Relative Gibbs energies were calculated from the difference between the reactant energy and the calculated transition states to acquire quantitative data for the degradation mechanisms being studied. These energies represent the energy required to overcome the barrier between reactant and product and are crucial to understand the kinetics of a mechanism. Full reaction coordinates were visualized using Avogadro software to animate system images and better understand the dynamics of the MEP.⁷⁷

Chapter 3. Fragmentation Behaviour of Perfluorosulfonic Acids: Mechanisms and Pathways of SO_3^- and FSO_3^- Formation in the Gas Phase

3.1 Introduction

Per- and polyfluoroalkyl substances (PFAS) are recognized by Health Canada, United States Environmental Protection Agency and European Union as hazardous chemical pollutants of growing concern in our water supplies.^{1,7,78–84} These organic compounds, known as “forever chemicals,” are characterized by strong carbon-fluorine bonds and significant resistance to degradation in the environment.¹ PFAS pollutants are a common industrial byproduct.^{85–87} Health Canada has reported the existence of approximately 4,700 human-made PFAS compounds in the environment, varying by regional distribution and concentration.¹ Concerningly, PFAS are found in many common household and personal items, including non-stick pans, food packaging, and personal care products.^{88–90} Due to their long half-lives, PFAS rapidly bioaccumulate in humans and exposure to these species pose significant health risks.^{7,82} These risks include poor fetal development, behaviour and learning irregularities in children, infertility, hormonal imbalances across all genders and several forms of cancer.^{7,82,91,92}

Long-chain PFAS have been scrutinized for their toxic health impacts on multiple groups.^{81,82} In particular, focus has been directed towards physicochemical characteristics such as their strong carbon-fluorine bonds, high thermal and chemical stability and behaviour such as environmental mobility, bioaccumulation, and toxicities of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS).^{93–96} However, as negative attention surrounding long-chain PFAS has risen in recent years, industries have shifted production to short-chain perfluorinated compounds as a possible replacement for the long-chain species.^{97,98} Despite this shift, several long-chain PFAS continue to linger in the environment.⁹⁷ Additionally, the

degradation of long-chain perfluorinated compounds often results in the formation of poorly characterized short-chain species, emphasizing the need for further research on these compounds.⁹⁷

Given the high sensitivity and specificity of mass spectrometry, it is widely regarded as the method of choice for the detection and quantification of per- and polyfluoroalkyl substances (PFAS).^{99–101} However, despite its efficacy, a comprehensive understanding of the gas-phase ion chemistry of short-chain per- and polyfluoroalkyl sulfonic acids (PFSAs) remains limited. This gap in our knowledge poses challenges in our analytical workflows. As an example, using ion-mobility mass spectrometry (IMS-MS), collision cross sections (CCS) are often used to support the identification of an ion. Although, some short chain perfluorinated carboxylic acids (PFCAs) do not produce stable molecular ions under normal electrospray ionization (ESI) conditions. Instead, these compounds often undergo in-source fragmentations, specifically decarboxylation, which leads to the formation of fragment ions such as fluorocarboxylate anions (FCO_2^-).¹⁰² As a result, the experimentally derived CCS values often correspond to that of the decarboxylated parent rather than the intact parent structure. This can complicate the assignment of CCS data and potentially lead to ambiguous or misleading structural interpretations.

While the fragmentation behaviour of some short chain PFSAs is an experimental inconvenience, it also reflects the gas-phase reactivity of these species that is not yet well understood. Recent work by Lee et al. investigated this issue and found that for several short chain PFCAs, the FCO_2^- fragment was not just a minor product but rather a dominant ion being produced under specific ionization and dissociation conditions.¹⁰² This means that the

decarboxylation pathway is energetically favourable and likely represents a major degradation route during the ionization process. Importantly, Lee et al. explored PFCA fragmentation behaviour under two dissociation techniques, collision induced dissociation (CID) and infrared multiphoton dissociation (IRMPD), finding the relative product branching ratios varied largely between the two. CID promotes dissociation through collisional activation and energy transfer from neutral gas molecules (usually nitrogen), whereas IRMPD uses vibrational excitation via multi-photon absorption.¹⁰² The difference in branching ratios suggests that the dissociation pathways are energy-dependant and potentially involve distinct transition states or gas-phase rearrangements.¹⁰²

The findings from this paper highlight the presence of complex, technique selective gas-phase ion chemistry in PFAS anions. This behaviour can complicate mass spectral interpretation, limit the reliability of CCS-based identification strategies, and hinder efforts to develop a robust and transferrable quantification method. Therefore, elucidating these mechanisms could enhance the reliability of qualitative analyses and improve our ability to detect these compounds in complex environmental matrices, ultimately contributing to more accurate risk assessments and regulatory measures.

Commonly, detection and quantification of PFSA anions involves monitoring the loss of SO_3^- (m/z 80) from the deprotonated parent molecule via mass spectrometric multiple-reaction monitoring (MRM).^{103,104} Figure 3.1 provides an example of this fragmentation pathway for deprotonated perfluoropentanesulfonate. This fragmentation pathway is well established for sulfonate anions, however, in perfluorinated species, an additional product channel is accessed resulting in the formation of FSO_3^- (m/z 99).^{103,105} While this fragment is commonly used as a

qualitative reference for PFSA identification, the exact mechanism of FSO_3^- formation remains unclear.^{103–105}

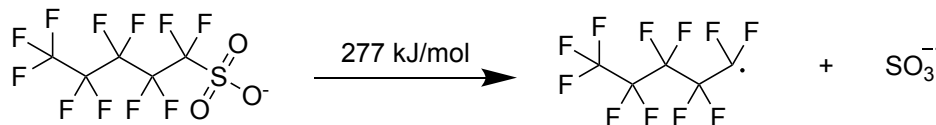


Figure 3-1. Possible CID fragmentation to produce SO_3^- radical anion from perfluoropentanesulfonate, PFPeS.

Here, we examined the fragmentation mechanisms of deprotonated PFSAs ranging from perfluoropropane sulfonate to perfluoroheptane sulfonate, which all exhibit SO_3^- and FSO_3^- fragmentation products following collision-induced dissociation (CID).^{20,106,107} Although the fragmentation pathway for production of SO_3^- is somewhat intuitive (*i.e.*, homolytic bond cleavage), the mechanism for production of FSO_3^- is less clear; the geometries of the FSO_3^- nor the C_nF_{2n} products are unknown, as are the energetics of the unimolecular decomposition reactions. In Figure 3.2, we propose many possible closed and open-shell reaction pathways for dissociation of deprotonated PFPeS (as a case study) to form FSO_3^- ; these can be categorized into concerted and non-concerted mechanisms. As per the closed-shell pathways, we identify two possible concerted pathways. The first is the reaction from **A** to **B**, which involves the direct elimination of a fluorosulfonate group through fluorine abstraction at the β -carbon, yielding a perfluorinated terminal alkene. The second is the reaction from **E** to **F**, which follows a similar β -abstraction mechanism but produces a different conformation of the terminal alkene product. Although, **A** to **B** and **E** to **F** lead to the same net product (via fluorine abstraction and rearrangement) they differ in their initial confirmation, which dictates which fluorine atom is abstracted. In isomer **A**, the geometry position favours one specific fluorine

atom for abstraction, whereas in isomer **E** (a rotated conformer), the spatial orientation changes, leading to abstraction of a different fluorine atom. In contrast, the non-concerted pathways are more complex and begin with sulfonate isomerization from the α -carbon to the β -carbon. For example, reaction **A-C-B** and reaction **E-G-F** involved isomerization prior to fluorine abstraction at the α -carbon to produce FSO_3^- and the perfluorinated terminal alkene product. Alternatively, reaction **A-C-D** and reaction **E-G-H** involve fluorine abstraction at the γ -carbon after isomerization, leading to the formation of FSO_3^- and perfluorinated internal alkene products. Note that the conformational changes that can occur (*i.e.*, **A** to **E** or **C** to **G**) ultimately lead to *cis* or *trans* internal alkene products. These isomerizations occur via rotation around single bonds within the perfluorinated carbon chain. These are purely conformational changes that alter the spatial arrangement of the atoms without breaking any bonds.

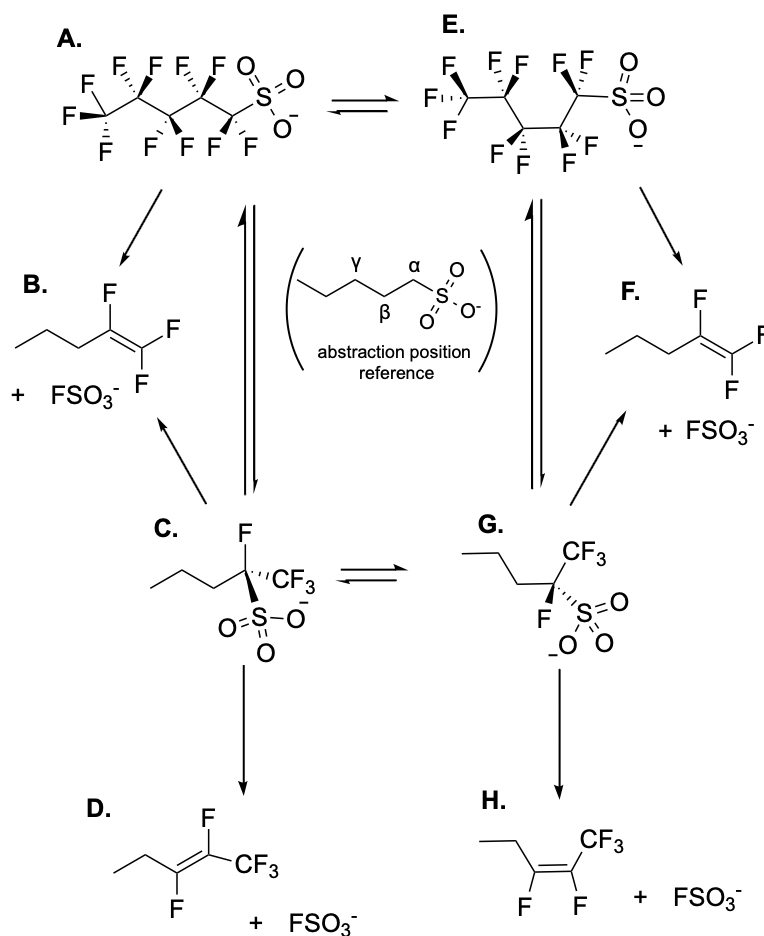


Figure 3-2. Summary of possible FSO_3^- formation mechanism in the gas-phase. (*For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from the diagrams; however, all species are fully perfluorinated*)

Whether sulfonate isomerization plays a role in the unimolecular decomposition of deprotonated PFSA's or if FSO_3^- formation occurs via β -carbon abstraction and direct elimination is an open question. To investigate these gas phase ion reactions, we employ mass spectrometry, differential ion mobility and high-level electronic structures calculations.

3.2 Methods

3.2.1 Experimental Methods

Mass spectrometric experiments were conducted using a SCIEX QTRAP 5500 MS/MS system at the University of Waterloo.^{108,109} Perfluoropropanesulfonic acid (PFPeS) was sourced from the Internal Organization for Standardization (ISO) 2 $\mu\text{g mL}^{-1}$ Native PFAS standard, purchased from Accustandard (Connecticut, USA). This solution of standards is employed for Environmental Protection Agency (EPA) method 1633A and, in addition to PFPeS, this solution includes a wide variety of other PFAS commonly found in ground, tap and wastewater.¹¹⁰ HPLC-grade acetonitrile (ACN), methanol (MeOH) and isopropanol (IPA) were purchased from Sigma-Aldrich (Missouri, USA). Ultrapure water was obtained from a Milli-Q system (Millipore, 18.2 $\text{M}\Omega\text{ cm}^{-1}$). Standard was diluted in MeOH to working a concentration of 100 ng mL^{-1} . Analyte was ionized via negative mode electrospray ionization (ESI⁻) and mass-selected prior to characterization.^{111–113} Enhanced product ion scans were performed while monitoring CID for collision energies of -180 to -10 eV with a step size of 1 eV to generate a breakdown curve.^{47,114,115} To ensure the absence of gas-phase isomeric interferences, differential ion mobility spectrometry (DMS) was employed prior to mass selection. By using DMS, we allow for gas-phase separation of ions based on their differential mobility under high- and low-field conditions, enabling isolation of isomeric species if separation is possible.

3.2.2 Computational Methods

Calculations employed the ORCA computational suite.⁷² Geometry optimizations and thermochemical corrections were carried out using the B3LYP D3BJ/def2-TZVP functional.^{116–119} The B3LYP D3BJ functional was chosen for its capability to correct for spin

contamination when investigating the spin state of our homolytic desulfonation, as well as for its accuracy and efficiency in optimizing structures.¹²⁰ For NEB-CI calculations, the r2scan-3c functional was used because it provides an approximation to the transition state (TS) without requiring extensive computational time.¹²¹ To further improve the accuracy of electronic energies, single point energies were computed for the geometry optimized species at the DLPNO-CCSD(T)/def2-TZVP level of theory.^{69,122}

Having identified the parent and product molecules for each pathway, the nudged elastic band climbing image approach (NEB-CI) method was used to identify the transition states (TS) corresponding to formation of FSO_3^- via the proposed degradation mechanisms.^{66–68} Sixteen discrete points were selected along the potential energy surface to map the reaction pathway, with particular emphasis on geometries proximal to the predicted transition states. This method ensures a precise representation of the energy profile and structural changes occurring at key stages of the reaction. The TSs identified by the NEB method were subsequently optimized at the B3LYP D3BJ/def2-TZVP level of theory to ensure that they were local maxima and to compute accurate energies and thermochemical corrections.¹²³ Optimized atomic XYZ coordinates, and the results from intrinsic reaction coordinate calculations (IRC; to verify NEB results) are provided in the supplementary information along with frequencies for each TS.¹²⁴

Conformational ensembles for each of the five PFSA studied were initially generated using the CREST (conformer-rotamer ensemble sampling tool) program developed by the Grimme group.¹²⁵ CREST uses the GFN-xTB semiempirical tight-binding method to perform a comprehensive search of the conformational space. It leverages a combination of metadynamic simulations, genetic z-matrix recombination and clustering algorithms to

generate and refine a diverse conformational ensemble.¹²⁵ Following this, redundant and high-energy structures are parsed through using Featherstone Suite, which filters structures based on relative energies. Selected low-energy conformers were then geometry optimized using DFT and further refined using DLPNO-CCSD(T) single point energy calculations. These optimized geometries served as inputs for MobCal-MPI to calculate the theoretical Boltzmann weighted collision cross sections (CCSs) of each conformer. These CCS values are computed by applying a thermal population distribution at 298 K based on the relative Gibbs free energies of the conformers.

Additionally predicted ultraviolet photodissociation (UVPD) and infrared (IR) spectra were calculated for each optimized conformer using time-dependent DFT (TD-DFT) and harmonic frequency analysis respectively.¹²⁶ TD-DFT is a widely used method for simulating electronic excitation spectra, providing insights into the electronic transitions corresponding to absorption.¹²⁶

3.3 Results & Discussion

The deprotonated PFSAAs that we studied yield two product ions, m/z 80 and m/z 99, which correspond to SO_3^- and FSO_3^- , respectively. Although SO_3^- formation is expected to occur via a simple impulsive dissociation mechanism, the mechanism for FSO_3^- production is not as obvious. To confirm that SO_3^- was not a secondary product derived from FSO_3^- , we first conducted MS^3 experiments. An MS^3 scan in mass spectrometry (MS) is a form of tandem mass spectrometry (MS/MS/MS) where the ion of interest is first isolated (MS^1), then fragmented (MS^2) to produce product ions, and then one of the product ions is further isolated and fragmented again (MS^3). The addition of an extra fragmentation steps allows us to

better understand and characterize the origins of a specific fragment, in our case SO_3^- . To confirm that the SO_3^- peak is not a direct product of the FSO_3^- peak, an MS^3 experiment would first isolate the deprotonated PFPeS molecule (MS^1) and then fragment to form FSO_3^- and SO_3^- (MS^2) as seen in the MS/MS spectra for deprotonated PFPeS. Following this, the FSO_3^- is then isolated, fragmented and the resulting mass spectra is collected. If SO_3^- is not observed as a fragment of FSO_3^- in the MS/MS/MS spectra under collision induced dissociation (CID) conditions, it suggests that the SO_3^- ion detected in the MS/MS spectra arises independently, rather than through fragmentation of the FSO_3^- species. That said, the MS^3 experiments designed to test whether SO_3^- originated from FSO_3^- fragmentation were inconclusive, as no characteristic fragment ions of FSO_3^- were observed. The differentiation of these pathways is crucial for mapping out accurate fragmentation mechanisms and understanding the gas-phase chemistry of the analyte. Travelling wave ion mobility spectrometry (TWIMS) measurements of collision cross sections (CCSs) were conducted to support the assignment of both parent and product ions. The experimentally determined CCS values showed good agreement with those reported in the literature and those predicted using MobCal-MPI 2.0 software. For the FSO_3^- ion, no experimental CCS reference value was found in the literature. Computational analysis suggests that the isomer with the fluorine directly bound to the sulfur atom is energetically favoured by 415 kJ/mol over the alternative isomer with the fluorine bound to an oxygen. The calculated CCS for this lower-energy FSO_3^- structure $^-$ ($\Omega_{\text{FSO}_3^-} = 109.4 \pm 1.1 \text{ \AA}^2$) aligns more closely with the experimental measurement ($\Omega_{\text{FSO}_3^-} = 103.7 \pm 1.5 \text{ \AA}^2$) than the calculated CCS for the SO_3F^- isomer ($\Omega_{\text{SO}_3\text{F}^-} = 113.7 \pm 1.2 \text{ \AA}^2$). Similarly, the experimental CCS of PFPeS $[\text{M-H}]^-$ ($\Omega_{[\text{PFPeS-H}]^-} = 139.99 \pm 0.21 \text{ \AA}^2$) closely aligns with both the literature

value ($\Omega_{[\text{PFPeS-H}]^-} = 142.20 \text{ \AA}^2$) and the calculated CCS for its linear structure ($\Omega_{[\text{PFPeS-H}]^-} = 141.09 \pm 1.26 \text{ \AA}^2$), further supporting its assigned geometry.¹²⁷

We will focus our discussion on deprotonated PFPeS, since this analyte exhibited experimental responses that were typical of the other PFSA's studied, and because it was the focus of our computational work. The top panel of Figure 3.3 displays the mass spectrum resulting from the collision induced dissociation (CID) of the deprotonated PFPeS parent ion, $[\text{M-H}]^-$ (m/z 349), averaged across the entire collision range. Prominent fragment ions include SO_3^- (m/z 80) and FSO_3^- (m/z 99), indicating distinct dissociation pathways. The bottom panel of Figure 3.3 plots the breakdown curve for deprotonated PFPeS; the breakdown curves for other PFSA's are available in Supplementary Information. Both SO_3^- and FSO_3^- appear at $\text{CE} \approx 35 \text{ V}$, and SO_3^- remains the dominant product ion throughout the scanned voltage range. Similar behaviour was observed for the other PFSA's, with slight shifts in the experimental CE required for fragmentation onset (see Figure A-1 in Appendix A). In these cases, fragmentation onsets rise with chain length not because the S–C cleavage barrier changes significantly, but because heavier ions are partitioned lower center-of-mass collision energies (E_{cm}) than lighter ions at a given acceleration voltage.¹²⁸

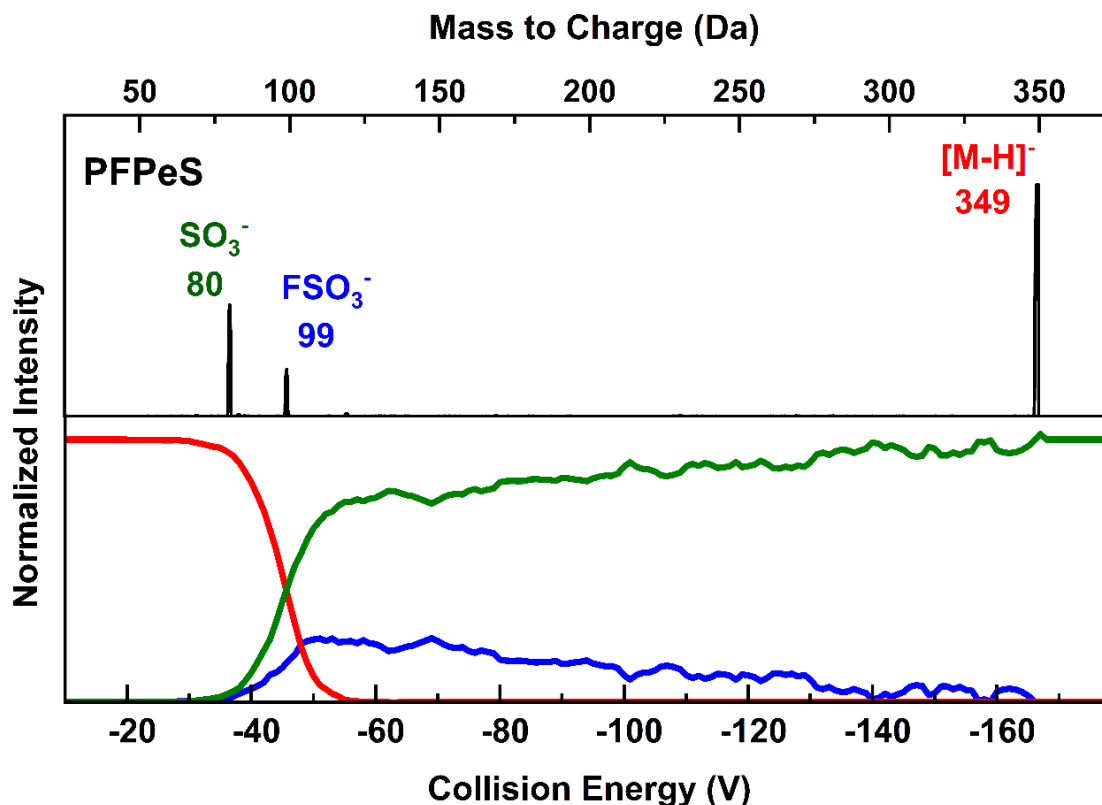


Figure 3-3. Mass spectrum and fragmentation breakdown curves for PFPeS. **(Top)** Mass spectrum displaying the deprotonated parent ion $[M-H]^-$ at m/z 349, and fragment ions SO_3^- at m/z 80 and FSO_3^- at m/z 99. **(Bottom)** Breakdown curves showing normalized intensity of each ion as collision energy increases: $[M-H]^-$ (red), SO_3^- (green), and FSO_3^- (blue).

To understand the gas-phase fragmentation of deprotonated perfluoropentanesulfonic acid, we investigated the closed-shell pathways through computational analysis, and derived reaction energy profiles along with mechanistic analysis. The calculated reaction energy diagram highlights six principal fragmentation routes: Pathway AB, Pathway ACB, Pathway ACD, Pathways EF, Pathway EGF and Pathway EGH. These pathways are divided into non-concerted and concerted mechanisms based on the synchronicity of bond cleavage and fluorine abstraction.

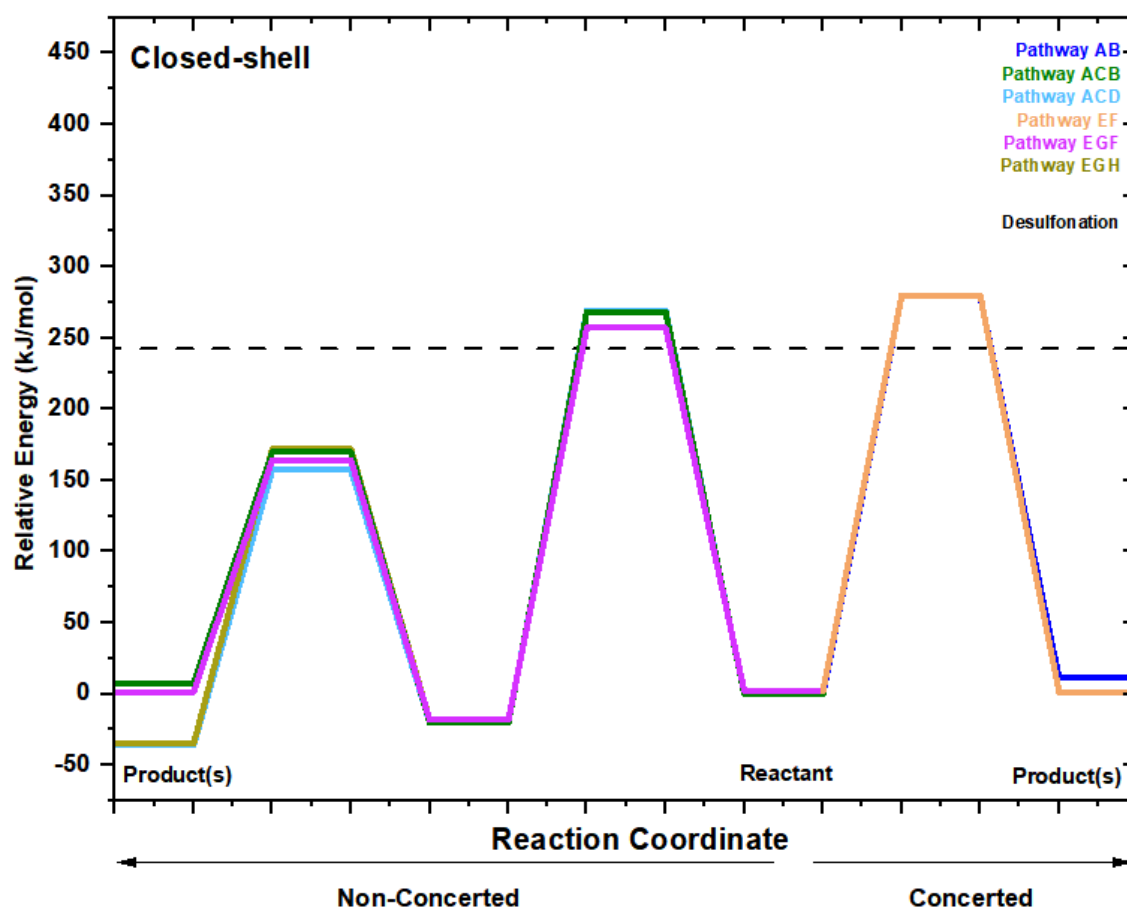


Figure 3-4. Reaction energy profile for closed-shell fragmentation pathways of deprotonated PFPeS. Pathways AB and EF represents concerted pathways, while ACB, ACD, EGF and EGH follow non-concerted processes. The plot illustrates distinct barrier heights and minima for each pathway, with the dashed line indicating the desulfonation threshold.

As depicted in the reaction energy profile (Figure 3.4) the non-concerted pathways, which include ACB, ACD, EGF and EGH, follow a stepwise dissociation process with similar energy barriers. Pathways AB, ACB and ACD all initiate from the same precursor conformation, whereas EF, EGF and EGH have a slightly different (kinked backbone) conformation as seen in Figure 3.5.

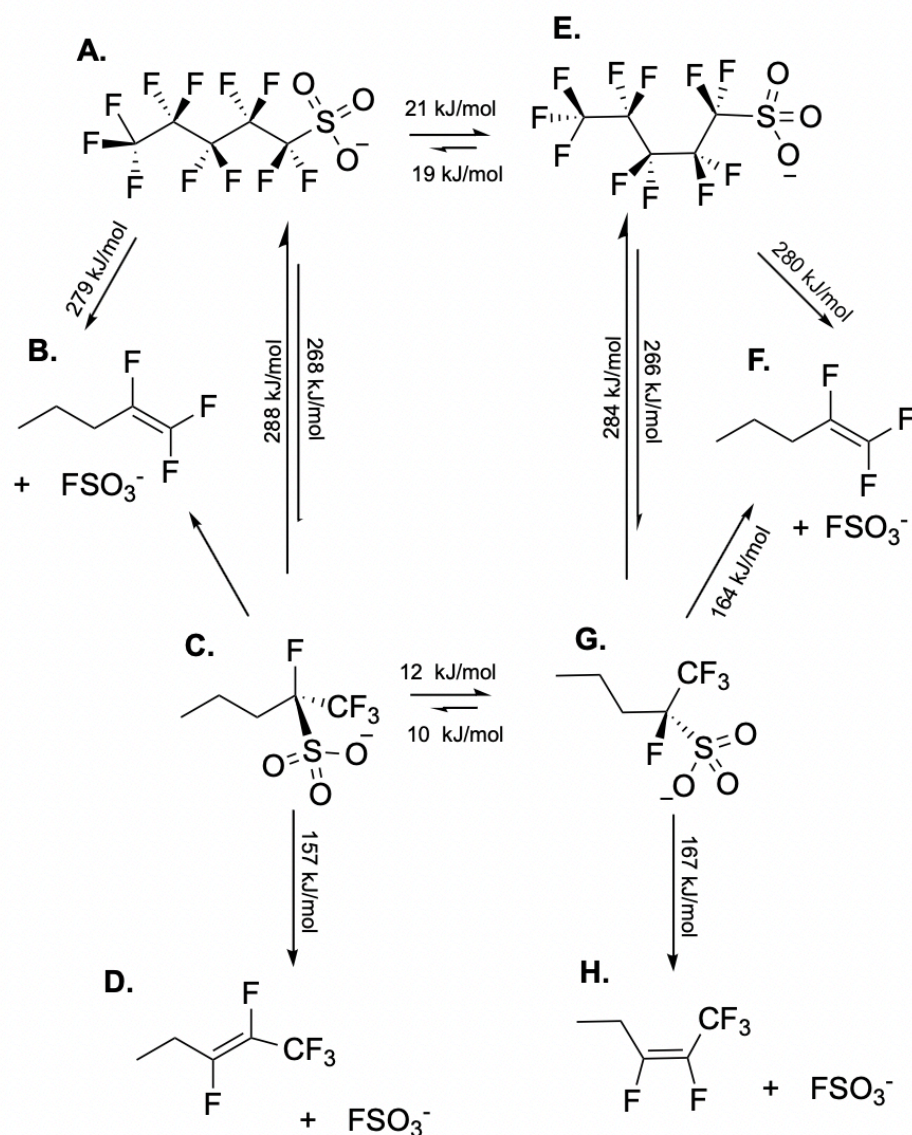


Figure 3-5. Calculated gas-phase fragmentation pathways of perfluoropentanesulfonic acid. Pathways proceed through isomerization or bond cleavages, resulting in the formation of FSO_3^- , and a perfluorinated alkyl fragment, with energy barriers indicated. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from the diagrams; however, all species are fully perfluorinated).

The concerted pathways AB and EF, involve synchronous bond cleavage and fluorine abstraction. In pathways AB, fragmentation proceeds directly from the linear precursor A, where the sulfonate group is eliminated simultaneously with β -fluorine abstraction, yielding

FSO_3^- and a linear alkyl fragment B. This concerted elimination requires an energy input of 279 kJ/mol as illustrated in Figure 3.5, which reflects the high stability of the sulfonate-carbon bond. Similarly, pathway EF follows a concerted mechanism, but initiates from the kinked precursor E. This pathway leads to product F and FSO_3^- with an associated energy barrier of 280 kJ/mol, nearly identical to the linear mechanism. The kinked geometry likely raises steric strain during the synchronous bond cleavage and F-abstraction, accounting for the slightly higher energy barrier.

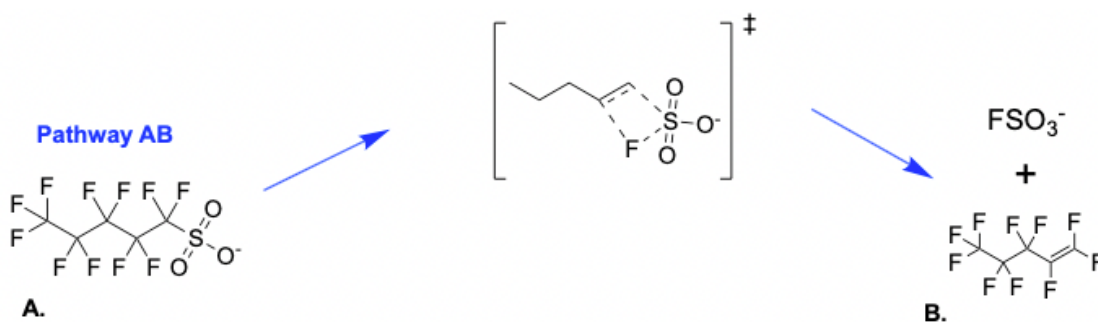


Figure 3-6. Proposed concerted fragmentation mechanism for Pathway AB, involving simultaneous cleavage of the C–S and C–F bonds to yield FSO_3^- and a neutral alkene fragment. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from one or more structures in the diagram; however, all species are fully perfluorinated).

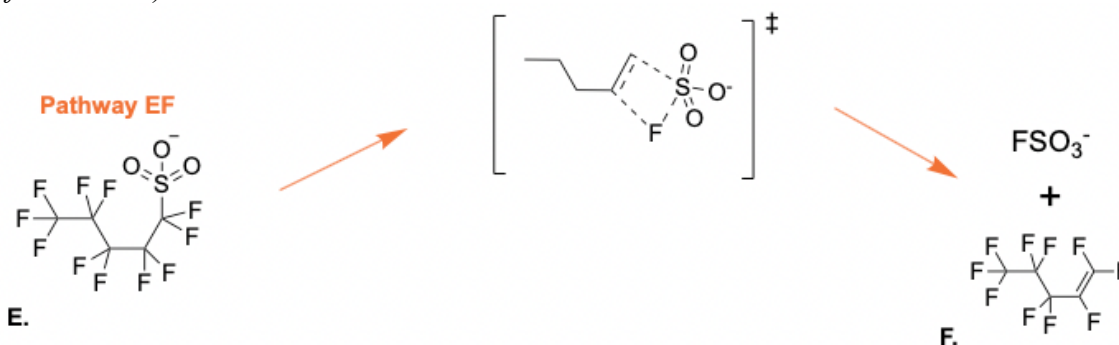


Figure 3-7. Concerted fragmentation mechanism proposed for Pathway EF, featuring simultaneous cleavage of the C–S and C–F bonds to yield FSO_3^- and a neutral alkene fragment. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from one or more structures in the diagram; however, all species are fully perfluorinated).

In contrast, the non-concerted pathways, ACB, ACD, EGF and EGH, proceed through stepwise dissociation pathways that involve isomerization of the deprotonated parent structure that lowers the energy barrier to FSO_3^- formation. For pathways originating from the linear precursor A, fragmentation proceeds via ACB or ACD. In Pathways ACB and ACD, the deprotonated parent, first isomerizes from A to C, with an energy barrier of 268 kJ/mol. This rearrangement positions the sulfonate and β -fluorine in an anti-coplanar diamond like transition state, optimizing orbital overlap for a subsequent atom shift. This pathway then proceeds via CB or CD with energy barriers of 170 and 157 kJ/mol, respectively. The transition from structure C to B, involves the abstraction of the α -fluorine co-planar to the sulfonate group, to form a terminal alkene and FSO_3^- . Similarly, the transition from C to D, involves the abstraction of the γ -fluorine co-planar to the sulfonate, resulting in the formation of a trans-internal alkene and FSO_3^- .

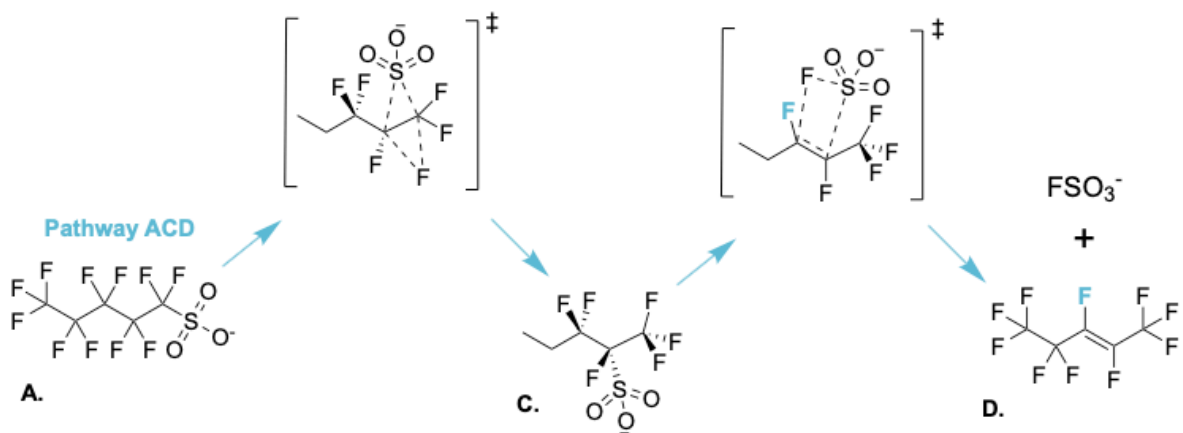


Figure 3-8. Stepwise fragmentation mechanism proposed for Pathway ACD, proceeding through sequential C–S and C–F bond cleavages to yield FSO_3^- and a rearranged fragment. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from one or more structures in the diagram; however, all species are fully perfluorinated).

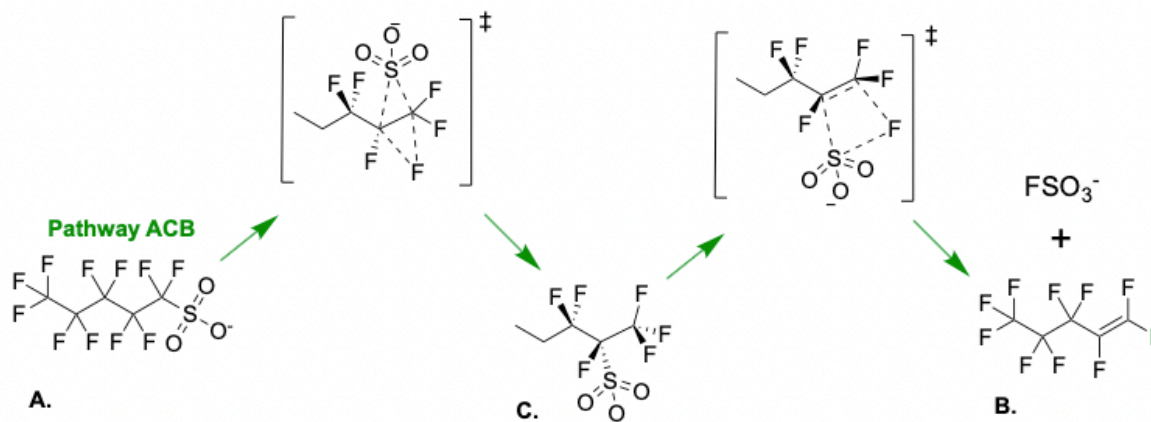


Figure 3-9. Proposed non-concerted fragmentation mechanism for Pathway ACB, initiated by C–S bond cleavage followed by a stepwise carbon–fluorine bond cleavage, resulting in the formation of FSO_3^- and a terminal alkene fragment. (*For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from one or more structures in the diagram; however, all species are fully perfluorinated*).

The non-concerted pathways originating from the kinked precursor E, include EGF and EGH, both of which utilize the bent backbone conformation which alters the possible alkyl product formation. Both the EGF and EGH pathways begin with the same diamond transition state isomerization as the ACB and ACD pathways, just slightly altered conformation with the bent backbone. From structure G, the pathways can proceed to form product F, a terminal alkene, or H, a cis-internal alkene, with barriers of 164 kJ/mol and 167 kJ/mol, respectively.

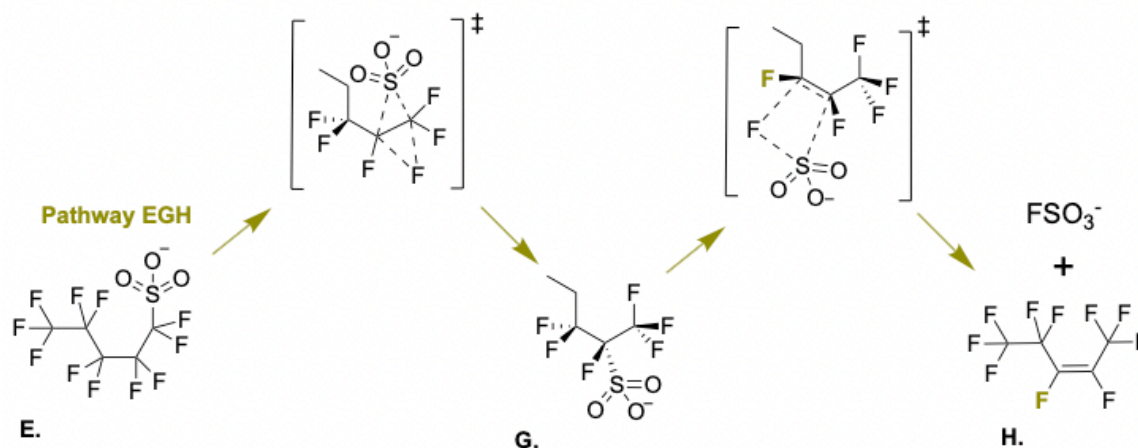


Figure 3-10. Non-concerted fragmentation mechanism proposed for Pathway EGH, originating from the kinked PFPeS precursor conformation. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from one or more structures in the diagram; however, all species are fully perfluorinated).

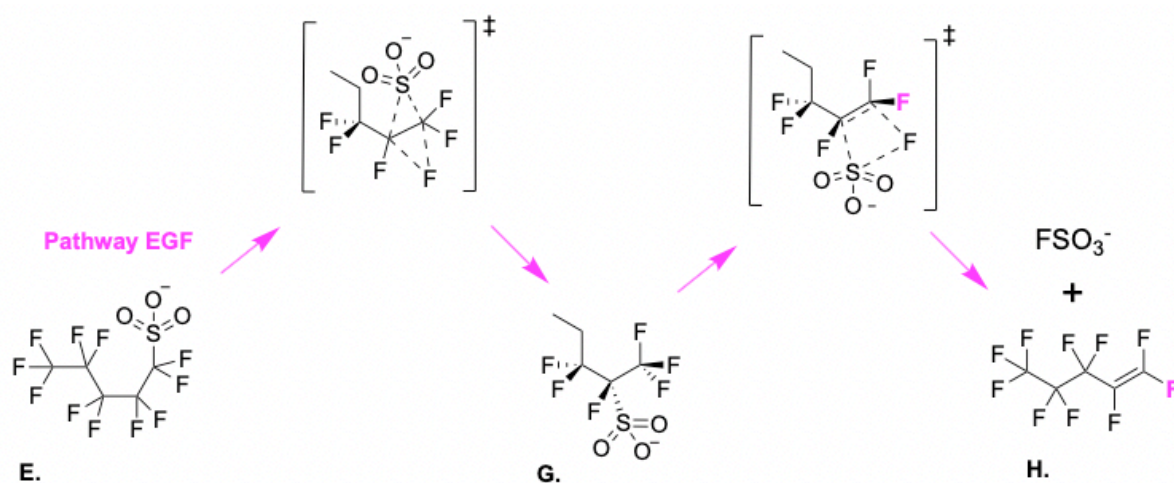


Figure 3-11. Stepwise fragmentation mechanism proposed for Pathway ABD, proceeding through sequential C–S and C–F bond cleavages to yield FSO_3^- and a terminal alkene fragment. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from one or more structures in the diagram; however, all species are fully perfluorinated).

The energy diagram (Figure 3.4) showcases the differences between the concerted and non-concerted fragmentation. Concerted mechanism requires higher initial energy inputs due to the

simultaneous nature of desulfonation and fluorine abstraction, while non-concerted pathways benefit from an intermediate isomerization that lowers the energy of the deprotonated parent structure and repositions the functional group for efficient removal of both α and γ -fluorines. As well, the linear (A) versus kinked (E) precursor conformation is showcased by the distinct energy barriers required for isomerization and FSO_3^- formation. The dashed line represents the desulfonation threshold, which confirms that all six pathways surpass the energy required for sulfonate cleavage, which aligns with our experimental findings as seen in Figure 3.4. Further insight into the isomerization process that facilitates FSO_3^- formation is provided in the calculated dispersion interaction density (DID) plot below (Figure 3.12).

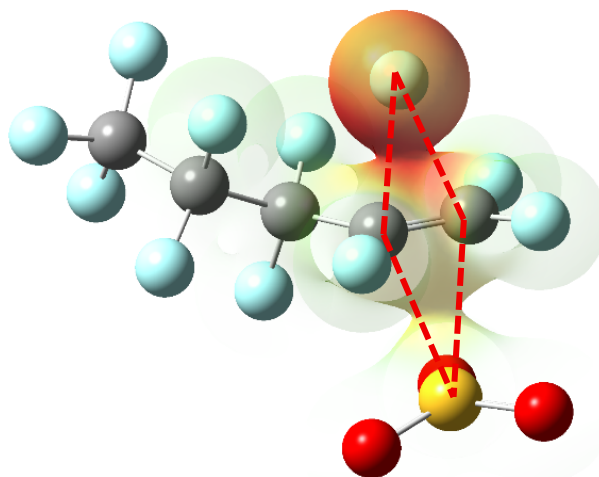


Figure 3-12. Dispersion interaction density (DID) plot for Reaction AC transition state. This diagram highlights the anti-coplanar diamond geometry required for this isomerization mechanism, along with the dispersion of electron density (red represents substantial electron density.)

This visualization depicts the geometric diamond configuration transition state geometry that occurs during the isomerization of the deprotonated PFPeS precursors, unique to the non-concerted pathways. The DID plot highlights the areas of electron density, which feeds us

information about electron flow throughout this transition. In the displayed structure above, the diamond-like geometry is characterized by the anticonplanar alignment of the sulfonate group and the β -fluorine atom. This spatial arrangement maximizes orbital overlap and enhances hyperconjugative interactions between the fluorinated carbon chain and the sulfonate group. Notably, the DID plot reveals a region of electron delocalization (green/yellow contour) that stabilizes the opposing charge density during bond rearrangement. The red density near the fluorine signifies an area of electron localization, which effectively forms a bridged charge distribution across the transition state and lowers the barrier to FSO_3^- formation (atomic x,y,z coordinates for each TS are provided in the supplementary material).

Building on the closed-shell fragmentation mechanisms, we also explored an additional six open-shell reaction pathways to further understand the dissociation behaviour of deprotonated PFPeS and other PFSA. These pathways introduced radical intermediates and unique bond cleavage patterns to contrast with the concerted and non-concerted mechanisms observed in the closed-shell processes, providing deeper insights into the molecule's gas-phase reactivity. The explored mechanism are shown below, along with the associated energy profile, Figure 3.13.

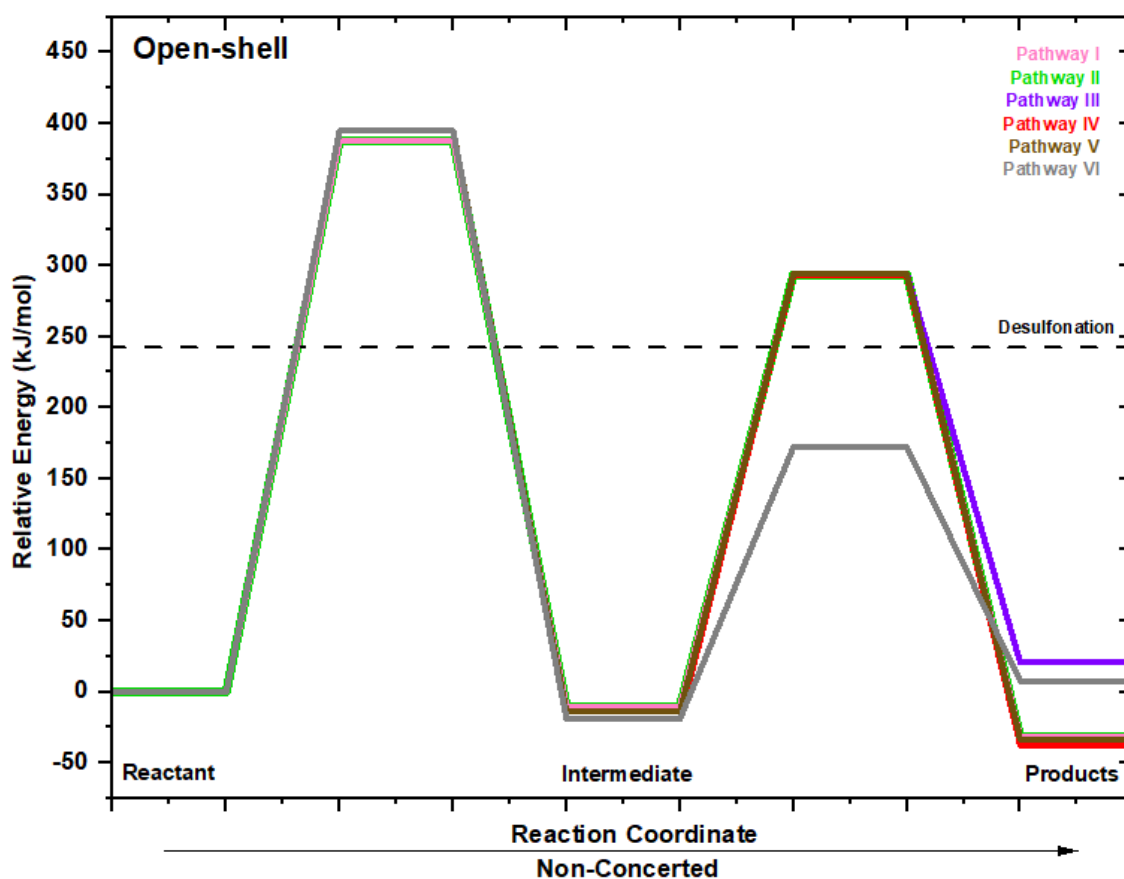


Figure 3-13. Reaction energy profile for open-shell fragmentation pathways of deprotonated PFPeS. The diagram displays six distinct pathways (I-VI), highlighting variations in energy barriers and stabilization. The dashed line marks the desulfonation threshold, indicating the minimum energy required for sulfonate loss.

Table 3-1. Summary of calculated Gibbs free energies for transition states across pathways I–VI.

Pathway	TS1 Gibbs Energy (kJ/mol)	TS2 Gibbs Energy (kJ/mol)
I	388	294
II	388	294
III	395	294
IV	395	293
V	395	294
VI	395	172

Pathway I and pathway II both initiate through a shared mechanistic route that involves the formation of a radical intermediate. This initial step proceeds via homolytic cleavage of the sulfonate group, generating a reactive sulfonate radical and a perfluorinated carbon chain radical. The critical difference between the two pathways emerges during the fluorine abstraction step. In pathway I, the abstraction occurs from the fluorine that is coplanar with the sulfonate group, which results in forming FSO_3^- and a cis-internal alkene. In the same fashion, pathway II abstracts a fluorine from the same carbon however, it is antio-planar with the sulfonate group, therefore forming a trans-internal alkene. The variation in fluorine abstraction influences not only the geometric rearrangement of the intermediate but also the fragmentation product that is formed. Despite the shared initial mechanism, the differentiation in fluorine abstraction showcases the sensitivity of the gas-phase reaction and subtle geometric or electronic changes to the mechanistic framework.

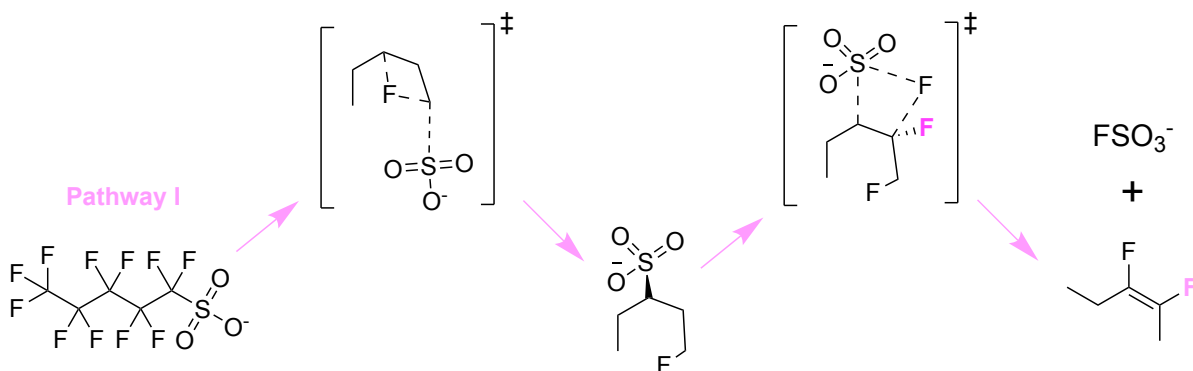


Figure 3-14. Proposed multi-step open-shell fragmentation mechanism for Pathway I, initiated by homolytic cleavage of the C–S bond, followed by sequential C–F bond dissociation. (*For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from the diagrams; however, all species are fully perfluorinated*).

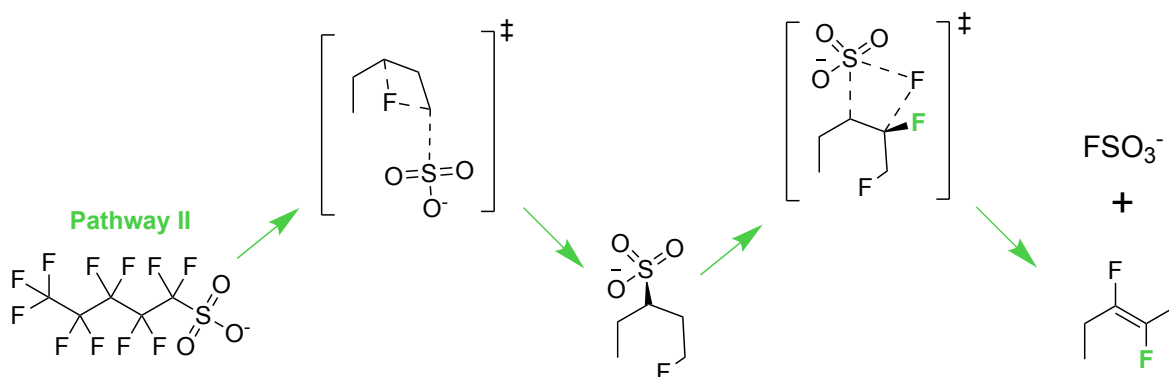


Figure 3-15. Stepwise open-shell fragmentation mechanism proposed for Pathway II, beginning with homolytic C–S bond cleavage and followed by sequential C–F bond dissociation. (*For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from the diagrams; however, all species are fully perfluorinated*).

Pathway III, IV, V and VI share a common mechanistic foundation, where they each proceed through the same initial rearrangement to form a stabilized radical intermediate. This intermediate is generated via homolytic cleavage of a γ -fluorine that transfers through a bridging mechanism at the terminal carbon and is subsequently replaced with the highly reactive sulfonate radical. From this shared intermediate, the mechanisms diverge based on the site of fluorine abstraction. In pathway III, fluorine abstraction occurs at the terminal carbon, enabling the closure of a 4-membered ring. This cyclization is driven by the reactivity of the radical species and the alignment of the α -fluorine, resulting in a compact cyclic product. Contrastingly, pathway IV abstracts fluorine from the γ -carbon that is antio-planar to the sulfonate group, initiating the formation of a trans-internal alkene. Similarly, in pathway V the sulfonate group abstracts a co-planar fluorine from the γ -carbon to form a cis-internal alkene product, via then same elimination mechanism. Finally, Pathway VI begins with the same radical intermediate but follows a slightly different fluorine abstraction compared to the IV

and V pathways previously mentioned. This pathway is characterized by the formation of slightly higher energy conjugated product, a terminal alkene.

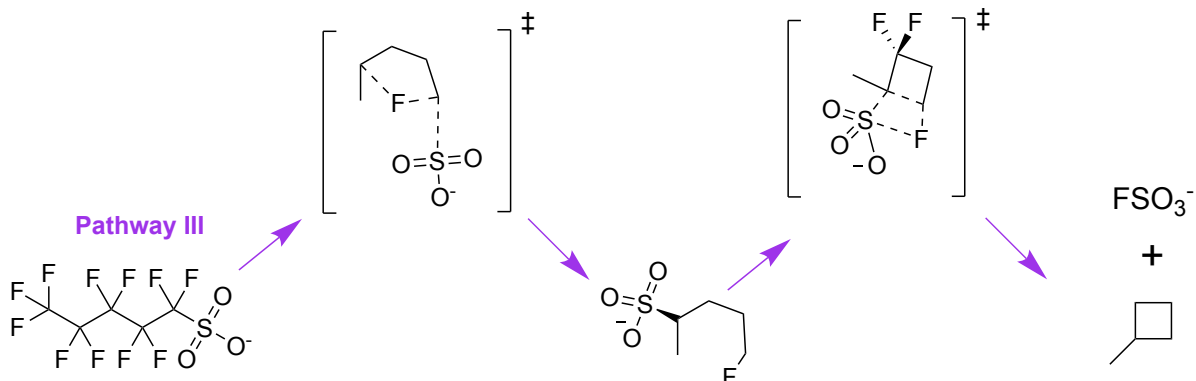


Figure 3-16. Pathway III proposed a linear PFPeS precursor undergoing radical-driven C–S and C–F bond cleavage forming FSO_3^- and a perfluorinated closed-shell ring. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from the diagrams; however, all species are fully perfluorinated).

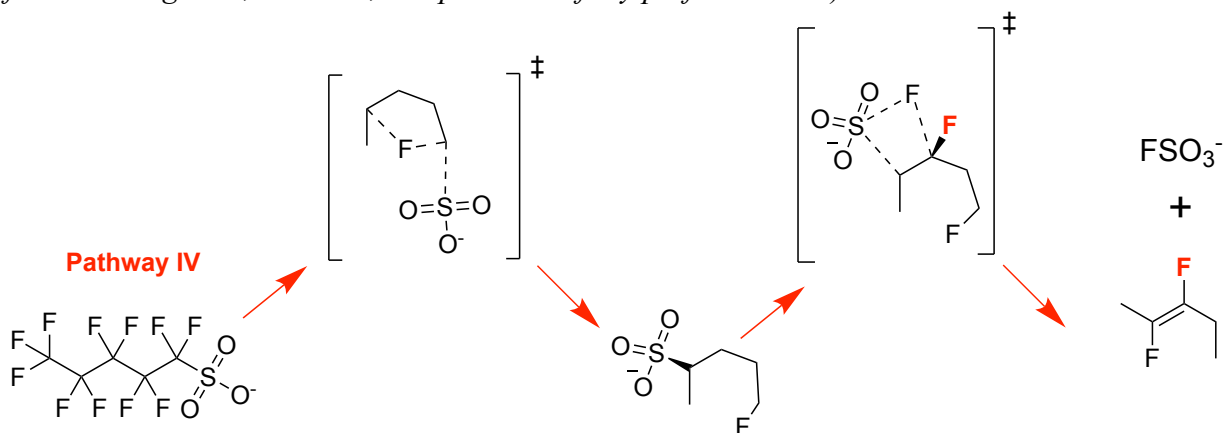


Figure 3-17. Pathway IV involves radical-induced fragmentation from the linear PFPeS precursor, with homolytic C–S and C–F bond cleavages yielding FSO_3^- and a fluorinated internal alkene. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from the diagrams; however, all species are fully perfluorinated).

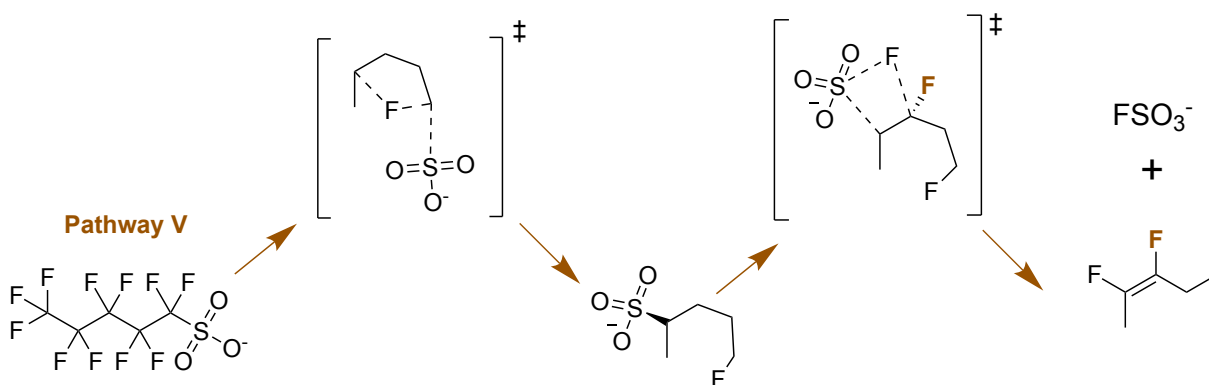


Figure 3-18. Proposed multi-step open-shell fragmentation mechanism for Pathway V, initiated by homolytic cleavage of the C–S bond, followed by sequential C–F bond dissociation. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from the diagrams; however, all species are fully perfluorinated).

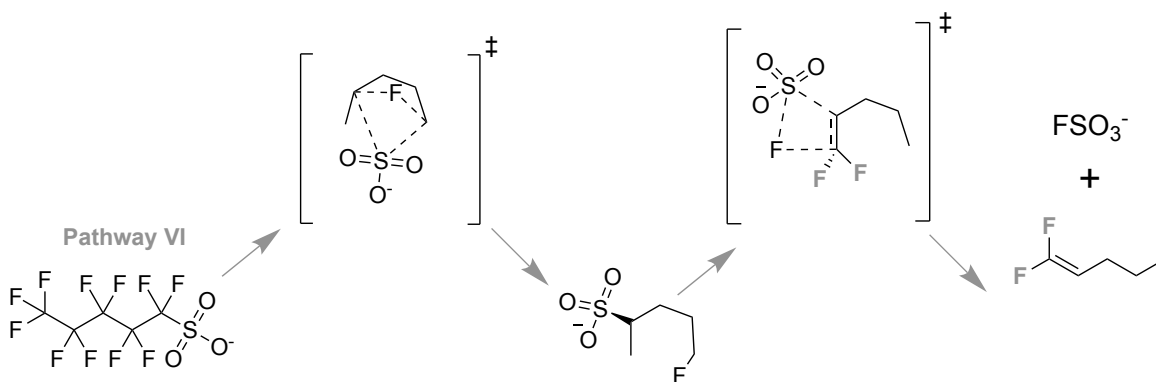


Figure 3-19. Stepwise open-shell fragmentation mechanism proposed for Pathway VI, beginning with homolytic C–S bond cleavage and followed by sequential C–F bond dissociation to form FSO_3^- and a fluorinated terminal alkene. (For clarity, peripheral fluorine atoms not directly involved in the fragmentation mechanism were omitted from the diagrams; however, all species are fully perfluorinated).

To explore the impact of chain length on the energetic profile of a proposed mechanism, I performed a sample calculation of pathway ACD across five different PFSA chain lengths. This analysis was aimed at understanding how extending or shortening the perfluoroalkyl chain influences the activation barriers and overall reaction feasibility. Given the various conformations that can be present at longer chain lengths, it is expected that experimental

barrier would differ from the computed values, however, this study was conducted on a simple linear conformation, and the results are shown below in Figure 3.20.

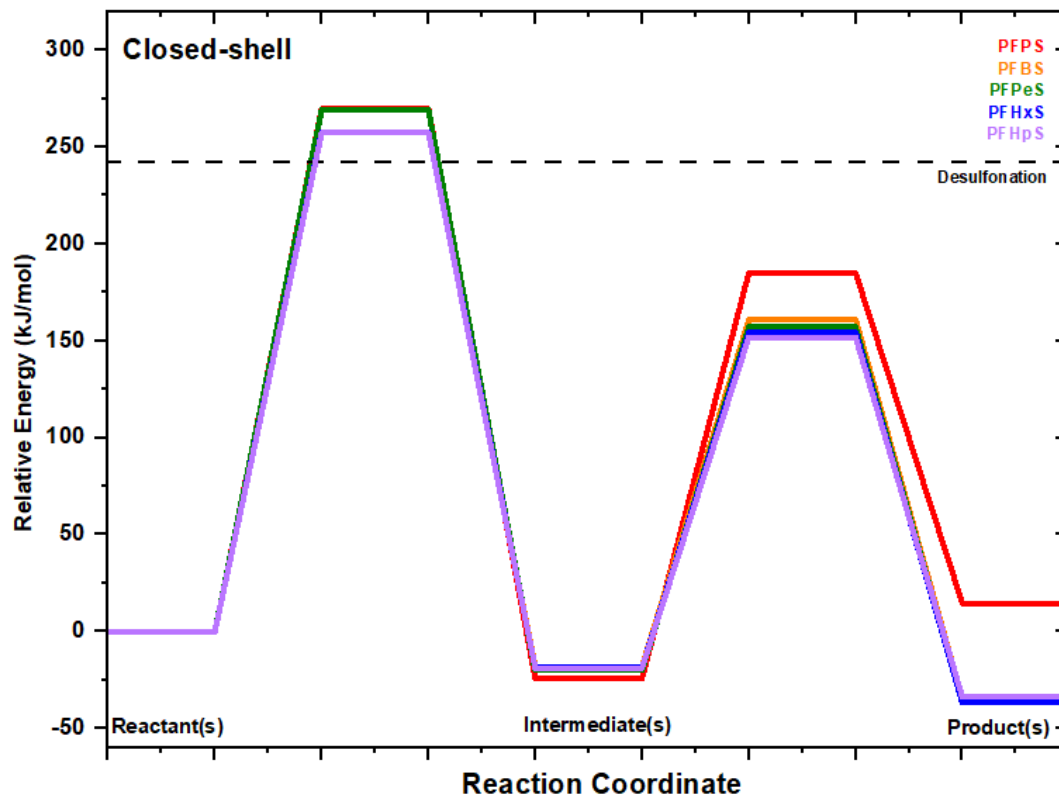


Figure 3-20. Reaction energy profile for closed-shell fragmentation pathways of five deprotonated PFSA. The diagram displays the non-concerted pathway ACD, highlighting variations in energy barriers as a result of perfluoroalkyl chain length. The dashed line marks the average desulfonation threshold, indicating the minimum energy required for sulfonate loss.

Table 3-2. Summary of calculated Gibbs free energies for transition states across pathway ACD for PFPS-PFHpS, where n is the number of carbons in the parent backbone.

Parent	TS1 Gibbs Energy (kJ/mol)	TS2 Gibbs Energy (kJ/mol)
PFPS ($n=3$)	270	185
PFBS ($n=4$)	257	161
PFPeS ($n=5$)	269	157
PFHxS ($n=6$)	256	154
PFHpS ($n=7$)	257	152

The reaction coordinate diagram (Figure 3.20) compares the relative energies of the closed-shell fragmentation pathway ACD, for several short-chain perfluoroalkyl sulfonates (PFSA), including PFPS, PFBS, PFPeS, PFHxS and PFHpS. All compounds exhibit a two-step mechanism with a distinct intermediate, and the overall relative fragmentation energetics are generally similar despite the increasing chain lengths. Variation may be attributed to differences in CF₂ backbone orientation or local conformation strain that affect the geometry of the transition state. However, further analysis is needed to confirm whether these fluctuations reflect true structural effects or are the results of minor computational artifacts within the convergence thresholds of the electronic structure calculations. Regardless, all species remain close to the desulfonation threshold, indicating a shared low-energy fragmentation channel that competes with direct SO₃⁻ dissociation.

To compliment the computational analysis of the five chain lengths of PFSA, I also calculated the IR and UPVD spectra for the perfluorinated terminal alkene for each species. These spectra provide valuable insight into the vibrational modes and electronic transition characteristics of each PFSA structure, which serves as a potential fingerprint for experimental validation. Unfortunately, due to current limitations of the available instrumentation in our lab, conducting these spectroscopic experiments is not feasible at this time. However, integrating experimental IR and UVPD analysis remains a priority for future work, as it would allow for direct comparison with the computational predictions and further validate the mechanistic pathways proposed.

3.4 Conclusions

The formation of FSO_3^- and C_nF_{2n} counter products via Reaction ACB is thermodynamically favoured when compared to all the proposed pathways. This is in part due to the isomerization of the reactant prior to fluorine abstraction. In a perfluoropentane sulfonate molecule, placing the sulfonate group on the β -carbon rather than the α -position enhances the abstraction of a fluorine atom from the C-F backbone. The sulfonate group is an electron-withdrawing group (EWG) that decreases the electron density on the carbon chain, making the carbon more electrophilic and thereby more susceptible to nucleophilic attack or bond cleavage. When the sulfonate group is positioned on the β -carbon, the electron-withdrawing effect is amplified inductively through the sigma bonds, further increasing the electrophilicity of the adjacent carbons, where the C-F bond is located. As the C-S bond elongates, the electron density is imparted on the carbon which then helps to stabilize the highly electrophilic carbon of the elongated C-F bond, which ultimately results in the formation of FSO_3^- and a π -bond. This isomerization effectively provides a more stable geometry for all the fluorine abstraction mechanisms by increasing the electron withdrawing groups acting upon the carbon being extracted from. Although Reaction ACB has the lowest activation barrier, this does not mean the other pathways are not possible. In fact, many of the other pathways have barrier within a few kilojoules per mole of Reaction ACB meaning that we may be forming a mixture of these products during gas-phase experimentation.

In conclusion, this study provides significant insights into the gas-phase fragmentation mechanisms of PFPeS, particularly focusing on the formation of FSO_3^- ions. Our findings reveal that multiple pathways may contribute to the production of these fragments, with the assistance of a parent isomerization prior to fluorine abstraction. The results of our

computational analysis, particularly those obtained via the nudged elastic band (NEB) method, underscore the impact of perfluorination on the transition states that lead to FSO_3^- formation. This knowledge deepens our understanding of PFPeS fragmentation, which is crucial for enhancing PFPeS detection and environmental management strategies. Future studies should further investigate how perfluorosulfonate chain length influences fragmentation behaviour, with particular attention to the formation and environmental persistence of perfluorinated alkene products. Additionally, structural characterization of the neutral fragments generated during dissociation could be enhanced through infrared (IR) spectroscopy, which may offer valuable insight into the geometry and bonding of product species. Ultimately, this research lays the groundwork for more targeted efforts to mitigate the environmental and health risks posed by micropollutants in our water systems.

Chapter 4. Application of a Two-Dimensional LC×DMS-MS/MS Separation Technique for Characterizing Pharmaceuticals in Aqueous Samples

4.1 Introduction

The detection and characterization of pharmaceutical compounds in aqueous environments has sparked attention in the science community due to their potential ecological and human health impacts.^{12,13,129} There are many ways in which pharmaceuticals can spread through our water systems, such as wastewater discharge, improper disposal (flushing), and agricultural runoff.^{12,13,129} Even in very low concentrations these pollutants can pose significant effects on our aquatic ecosystems and even cause antibacterial resistance.^{12,13,129} As a result, accurately detecting, separating and characterizing pharmaceuticals in aqueous matrices is a critical challenge to overcome for the betterment of the environment and our own health.

Traditional methods such as liquid chromatography and gas chromatography paired with mass spectrometry have been used as powerful tools in the identification and characterization of pharmaceuticals.^{130–132} However, there are limitations to these techniques when analysis involves structurally similar compounds, isomers and isobars. Although techniques such as LC and DMS have been independently and successfully used to separate analytes in simple environmental matrices, combining these techniques provides a complementary approach to address limiting challenges presented by structurally and chemically similar species. Using a two-dimensional approach takes advantage of stationary phase interaction-based separations with orthogonal analysis using DMS as a gas-phase filter dependant on differential ion mobility in an oscillating electric field. Rather than improving speed or simplifying the analysis process, 2D LC×DMS-MS/MS is used for the specific purpose of enhanced separation and characterization from either dimension, which increases confidence in compound

identification. This method can be especially useful for complex sample matrices where traditional LC or DMS methods may fall short.

One of the earliest applications of 2D LC×DMS-MS/MS was in a quantitative steroid hormone analysis. A study by Woroniecki & Moshin (2014) demonstrated the integration of the SelexION® DMS device with a QTRAP 5500 LC-MS/MS platform to analyze steroids in oral fluids.¹³³ By filtering out problematic isobaric interferences, the DMS-enhanced workflow improved signal to noise ratios and quantifier/qualifier agreement which enhanced specificity without compromising sensitivity.¹³³ Another important application is found in lipidomics, where DMS has been used to deconvolute complex isomeric and isobaric lipid species. In a study by Xu et al. (2019), LC×DMS-MS/MS was used to analyze glycosphingolipids in human plasma and cerebrospinal fluid.¹³⁴ Their approach enabled the concurrent quantification of multiple GlcCer and GalCer species, which they indicate as difficult species to distinguish via LC-MS/MS alone.¹³⁴ The addition of DMS greatly improved specificity, allowing for more confident identifications and quantitation within a single analytical run.¹³⁴ This shows the value of 2D LC×DMS-MS/MS workflows for resolving closely related analytes particularly in complex biological samples.

In this study I present a 2D LC×DMS-MS/MS technique, designed to address the challenges associated with pharmaceutical detection mainly focused on improving the separation of structurally similar species. This method allows for enhanced confidence in the identification of a small group of pharmaceuticals in aqueous sample matrices. Particularly, it is a beneficial tool for orthogonal separation of small molecules where a traditional one-dimensional approach is not sufficient.

4.2 Experimental Methods

4.2.1 Chemicals and Reagents

Twenty-nine individual pharmaceutical standards (See Appendix B, Table B-1) were first prepared as stock solutions at approximately 1000 mg L⁻¹ (ppm) in HPLC-grade methanol (Sigma-Aldrich, Missouri, USA). The compounds were selected from various classes of pharmaceuticals such as beta-blockers, PDE5 inhibitors, benzodiazepines, among others. From these stocks, three mixtures of common pharmaceuticals were created by combining selected standards to achieve randomized batches, each with a final summed total concentration of approximately 200 ng mL⁻¹ (ppb). These mixtures include representative compounds from various pharmaceutical classes frequently detected by researchers studying environmental waters.^{10,13,129} A full list of compounds included in each batch is provided in Appendix B (Table B-1).

All solvents, including HPLC-grade methanol, acetonitrile (ACN), and isopropanol (IPA), were obtained from Sigma-Aldrich (Missouri, USA). Ultrapure water (18.2 MΩ cm⁻¹) was produced using a Milli-Q system (Millipore, Massachusetts, USA). All solution were prepared in methanol without the use of internal standards, filtration, spiking, or additional sample processing was used prior to analysis.

4.2.2 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Chromatographic separations were performed using an Agilent 1100 series liquid chromatography system equipped with a quaternary pump and autosampler. An Ascentis Express F5 column (50 x 2.1 mm, 2.7 μm particle size, Supelco, USA) was used for separation and set at 30°C throughout the analysis. The autosampler temperature was set to 4°C, and an injection volume of 5 μL was used for all runs. The mobile phase consisted of ultrapure water

with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), set at a constant flow rate of 550 $\mu\text{L min}^{-1}$. The mobile phase gradient is shown in Table 4.1.

Mass spectrometric detection was carried out using SCIEX QTRAP 5500 hybrid triple quadrupole/linear ion trap mass spectrometer equipped with a turbo electrospray ionization (ESI) source operating in positive mode. The ion source temperature was maintained at 300°C, and nitrogen gas was used as the nebulizing, auxiliary and collision gas. Collision-induced dissociation (CID) was performed under high-pressure conditions (CAD gas, 7 mTorr, as defined in Analyst software). Data acquisition was conducted in multiple reaction monitoring (MRM) mode, with a dwell time of 25 ms per transition. Compound specific transitions and related parameters are provided in Appendix B (Table B-2 – B-4). LC-MS/MS and DMS-MS/MS analyses were conducted independently to assess the degree of separation achieved by each 1-dimensional (1D) technique, prior to implementing the combined LC×DMS-MS/MS method. Blanks (MeOH) were run between standards in order to reduce analyte contamination in future runs.

Table 4-1. LC Mobile phase gradient for pharmaceutical analysis. Mobile phase A is ultrapure water with 0.1% formic acid and mobile phase B is acetonitrile with 0.1% formic acid.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Flow Rate ($\mu\text{L min}^{-1}$)
0.0	95	5	550
0.5	95	5	550
12.0	0	100	550
17.0	0	100	550
17.1	95	5	550
24.0	95	5	550

4.2.3 Differential Ion Mobility Spectrometry (DMS/MS)

Differential mobility separations were performed using the SelexION DMS interface (SCIEX) mounted between the turbo ESI source and the MS inlet. The DMS cell had a 1 mm gap height

and was operated in unit resolution mode at a separation voltage of 3200 V. The cell temperature was maintained at 150°C. A chemical modifier of 1.5 mol% isopropanol (IPA) was T-infused into the carrier gas to enhance ion separations. Nebulizing gas and auxiliary gas (GS1 and GS2) were set to 40 psi and 30 psi, respectively. Compensation voltage (CV) scanning was performed to determine optimal ion transmission for each target analyte. These CV values, along with their corresponding MRM transitions, are summarized in Table B-2 – B-4 in Appendix B. All data acquisition and instrument controls were performed using Analyst software (SCIEX).

4.2.4 2D LC×DMS-MS/MS

Two dimensional LC×DMS-MS/MS experiments were conducted by integrating chromatographic separations using an Ascentis Express F5 column with gas-phase ion separation using the SelexION® differential mobility spectrometry (DMS) interface. The method takes advantage of orthogonal separation methods to improve selectivity for pharmaceutical analytes in aqueous matrices. Chromatographic and ionization parameters remain consistent with those previously described in the LC and DMS methods above.

The DMS cell was operated with a separation voltage of 3200 V and a cell temperature of 150°C. A chemical modifier of 1.5 mol% isopropanol (IPA) was introduced into the curtain gas to enhance ion separation. For each analyte, an optimal compensation voltage (CV) was identified during preliminary DMS-MS/MS experiments and applied during initial LC×DMS-MS/MS acquisition. These CV values were embedded within the MRM transitions, enabling selective ion transmission post-chromatographic separation. Data was acquired in positive ESI mode with a dwell time of 5 ms per transition.

To evaluate whether mobile phase composition influenced CV stability due to incomplete solvent desolvation or gas-phase clustering effects, T-infusion experiments were conducted. Pharmaceuticals mixtures were infused at a constant flow rate via a T-junction while varying mobile phase compositions (corresponding to points along the LC gradient) were delivered from the LC system. CV values were monitored under these varying conditions to assess whether shifts occurred as a function of eluent strength or polarity. This allowed for validation that the CVs used during LC×DMS-MS/MS were impacted by the varying mobile compositions ultimately leading to varying signal intensity if not correctly tuned.

Raw data was processed using custom Python scripts. Extracted ion chromatograms were normalized to account for compound specific ionization efficiency and smoothed using Gaussian filter to reduce baseline noise. The data was compiled into two-dimensional matrices of retention time (RT) versus compensation voltage (CV) and generated as a contour plot to visualize analyte separation across both dimensions.

4.3 Results & Discussion

4.3.1 LC-MS/MS Separation

The liquid chromatographic separations across mixtures 1 to 3 demonstrates a strong overall performance by the Ascentis Express F5 column in retaining and resolving structurally diverse pharmaceutical compounds. In Figure 4.1, compounds in mixture 1 elute between 4 and 8.5 minutes, with excellent baseline resolution observed for most analytes. Highly polar compounds such as fluconazole and oxytetracycline elute early, while more non-polar (lipophilic) molecules like sildenafil, avanafil and diltiazem are retained longer. While most analytes are resolved, there is some co-elution at *ca.* 7.25 minutes between sildenafil and

avanafil. In such a case, the additional dimension of separation using the DMS could be beneficial.

In contrast, Figure 4.1 reveals a more poorly resolved chromatogram for mixture 2. Here, seven analytes elute within the 5.5 – 6.7-minute range, including omeprazole, lorazepam, clonazepam, alprazolam, bisoprolol, rosuvastatin and diazepam. Although the column maintained sharp peak shapes, several of these analytes have similar physicochemical characteristics and drug classification (*e.g.*, benzodiazepines and beta-blockers), and consequently LC RTs.¹³³ For example, the elution of econazole and miconazole, imidazole derivatives that are used as antifungal medications,¹³⁵ near the end of the gradient (~11-13 min) reflects their strong lipophilicity and aromatic nature.

Figure 4.1 shows good resolution for most compounds in mixture 3, ranging from 4.5 to 9.5 minutes. Polar compounds like lisinopril and olmesartan elute early, while statins such as simvastatin, lovastatin and atorvastatin are retained until the later stages of the gradient. However, peak overlap is an issue for compounds such as irbesartan, ramipril, enalapril and perindopril, which co-elute under the LC conditions employed. These results highlight a key limitation of LC-only approaches: structurally similar compounds with comparable hydrophobicity often coelute, even when using a moderately selective stationary phase like pentafluorophenyl (F5). In conventional 1D liquid chromatography (LC), improving separations between co-eluting compounds often involves lengthening the gradient (*i.e.*, slowing down the rate at which the mobile phase composition changes). While this can enhance resolution, it also increases analysis time. An alternative and more efficient approach is to introduce a second, orthogonal separation dimension, such as differential ion mobility (DMS),

which separates ions based on their behaviour in an electric field rather than interaction with a stationary phase. In the context of LC×DMS-MS/MS, using DMS adds separation power without increasing the total run time, because DMS operates on a millisecond timescale and occurs after LC but before mass spectrometric analysis. Such a workflow makes it possible to maintain a relatively fast LC gradient and still achieve enhanced resolution in the 2D space. Instead of increasing the length of the LC run, DMS can differentiate ions based on shape, charge distribution and ion-neutral clustering behaviour, effectively resolving compounds that overlap in the LC dimension. Therefore, combining LC with DMS allows us to resolve more compounds in the same amount of time as a standard 1D LC run, while increasing compound identification abilities and preserving or improving separation performance.

It is important to note the peak tailing observed in Figure 4.1, as this reflects common behaviour when analyzing pharmaceutical compounds using F5 columns. These columns promote strong π - π and dipole-dipole interactions between the stationary phase and analytes, particularly those with aromatic or highly polar functional groups, which are common in pharmaceuticals.

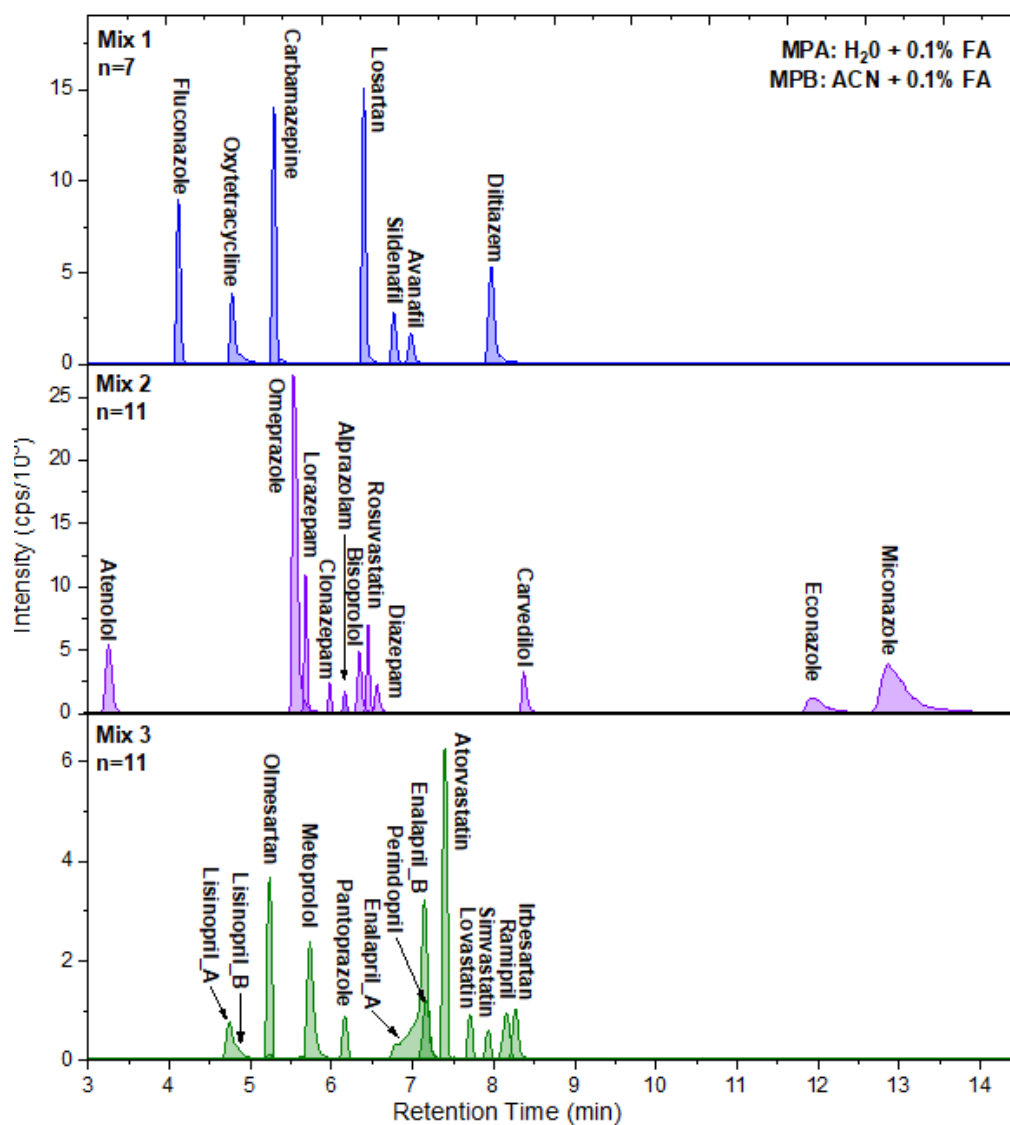


Figure 4-1. LC separation of three pharmaceutical mixtures on an Ascentis Express F5 column using a water-acetonitrile gradient with 0.1% formic acid.

4.3.2 DMS-MS/MS Separation

The DMS results provided in Figure 4.2 show the separation of the three mixtures at $SV = 3200$ V using compensation voltage (CV) as the differentiating feature. Mixture 1 analytes span a wide CV range from -22 to 0 V. Polar molecules like carbamazepine and oxytetracycline transmit at more negative CVs, indicating a higher degree of clustering behaviour or stronger interactions with the modifier (1.5 mol% IPA). More hydrophobic or

conformationally rigid molecules such as sildenafil and diltiazem transmit closer to $CV = 0$ V, a behaviour consistent with minimal clustering and lower differential mobility response. Notably, many of the compounds are not baseline resolved, which reinforces the need for an alternative approach to differentiate overlapping species. A technique such as multiple reaction monitoring can be beneficial to differentiate species because it allows for the selective detection and quantification of a targeted compound in a complex mixture. In MRM mode, the mass spectrometer is programmed to isolate a specific precursor (protonated in our case), fragment in the collision cell, and then monitor for one or more characteristic product ions. By focusing only on these predefined transitions, MRM offers high sensitivity and selectivity, making it useful for identifying trace-level compounds such as pharmaceutical in environmental or biological samples.

Figure 4.2 illustrates a dense CV distribution for the second mixture, especially in the mid and low CV ranges (-20 to -10 V and -5 to 0 eV). Compounds like lorazepam, clonazepam, econazole and miconazole display overlapping differential mobility profiles, likely due to similar aromaticity, molecular weight, and dipole moments. Carvedilol and bisoprolol were nearly indistinguishable in DMS space, highlighting the risk of coelution and non-detection with a singular dimension of separation. However, DMS effectively resolved rosuvastatin and diazepam despite their shared bulk physicochemical properties, indicating that subtle differences in gas-phase conformation, charge distribution and ion-neutral clustering behaviour can strongly influence differential mobility and enhance separation when LC resolution is limited.

In Figure 4.2, DMS proves particularly insightful for identifying trends for similar classes of pharmaceuticals in complex mixtures. Compounds like lisinopril, olmesartan, and perindopril (antihypertensive compounds, ARBs) are seen co-eluting around the -20 to -15 eV region. Although, in cases such as enalapril and perindopril, DMS separations are successful where chromatography methods were not. This may be attributed to their structural similarity and logP values of 2.1 and 2.9 for enalapril and perindopril, respectively.^{136,137} However, their difference in logP proves to be insufficient in order to achieve clear separation in reversed-phase LC, especially given their similar core structure and functional groups. The use of logP as a defining physicochemical identifier can be beneficial in chromatography because it gives us information about how lipophilic or hydrophilic a molecule is. This is a factor of how readily the molecule will partition between an aqueous and lipophilic phase. In contrast, DMS separates compounds based on gas-phase properties such as molecular shape, charge distribution, and ion-neutral clustering behaviour, which are more sensitive to subtle conformational differences. As such, even compounds with similar retention times in LC can exhibit distinct compensation voltages in DMS, making it a powerful orthogonal technique for resolving isomeric or closely related pharmaceutical compounds like enalapril and perindopril. In some cases, such as lisinopril and irbesartan, multiple peaks are observed because different conformations or ion-neutral clustering behaviour exist in the gas-phase. These clusters involve varying numbers of orientations of neutral molecules (*e.g.*, solvent adducts) bound to the ion, and each form responds differently to the asymmetric field, resulting in distinct features in the spectrum. Limitations of DMS are apparent with statins: lovastatin and simvastatin co-elute in the gas phase, consistent with their nearly identical structures.

Atorvastatin is slightly resolved from lovastatin and simvastatin in both the CV and RT space, likely due to its significant difference in structure, charge distribution and hydrophobicity (logP) of 6.36 compared to 4.5 and 4.68 for lovastatin and simvastatin, respectively.^{138–140} Overall, the DMS data complements cases where LC alone proves to be inadequate at times.

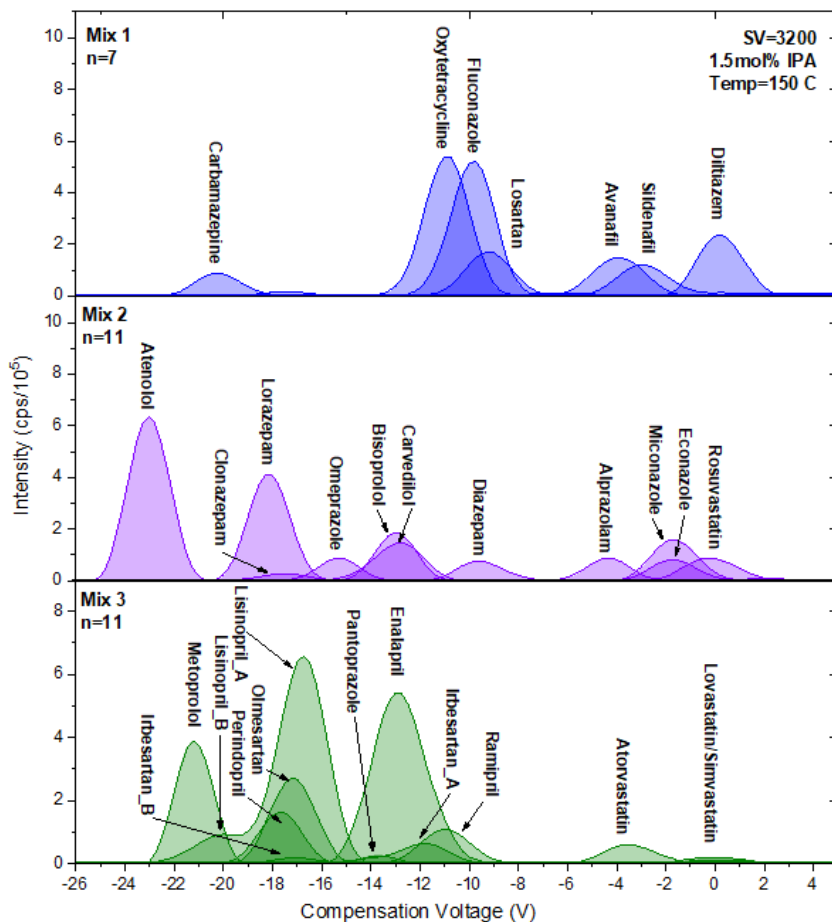


Figure 4-2. DMS separation of three pharmaceutical mixtures showing compensation voltage distributions at SV = 3200 V and 1.5 mol% IPA.

4.3.3 2D LC×DMS-MS/MS Separation

Two-dimensional LC×DMS-MS/MS analysis was performed for three pharmaceutical mixtures to evaluate the combined resolving power of retention time (RT) and compensation voltage (CV) separation. Figures 4.3 – 4.5 illustrate the RT × CV contour plots for mixtures 1,

2 and 3, respectively. These plots demonstrate the benefits of orthogonal analysis with LC and DMS, highlighting the improvements in resolution compared to one-dimensional separations alone.

For mixture 1 (Figure 4.3), most compounds are well-resolved in both dimensions. Notably, sildenafil and avanafil, which displayed partial coelution in the DMS domain, were clearly distinguished by their RT values, avoiding major peak interference. Oxytetracycline and fluconazole were also well-separated chromatographically, despite their similar polarity and aromaticity. The combination of moderate LC selectivity and DMS separation allowed for full resolution of the mixture, with each compound exhibiting a distinct, localized signal in the 2D separation space.

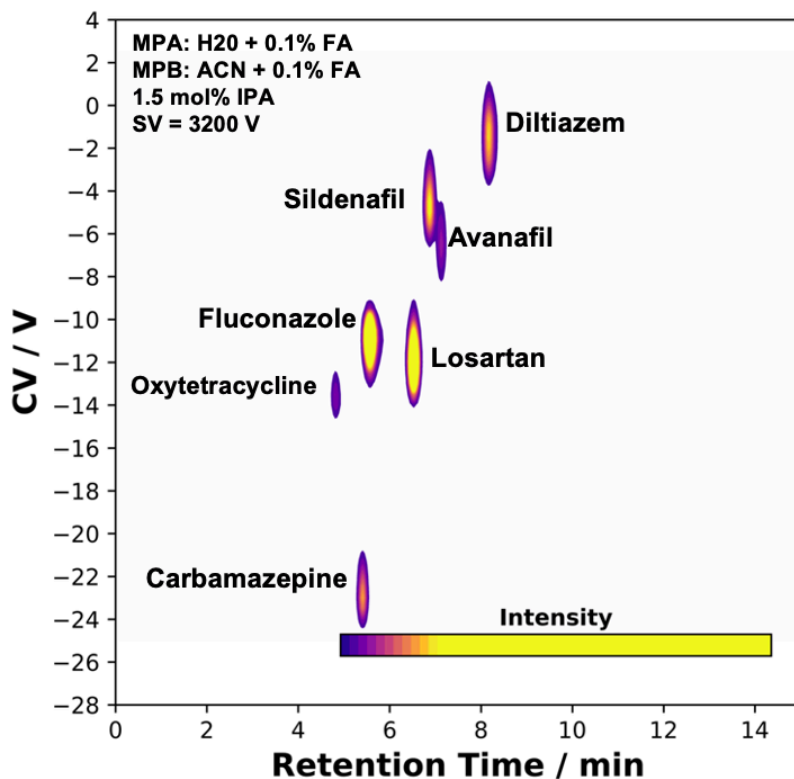


Figure 4-3. Two-dimensional LC×DMS-MS/MS contour plot for mixture 1, showing the retention time versus compensation voltage separation under 1.5 mol% IPA and SV = 3200 V. All compounds are distinguishable in the combined 2D LC×DMS-MS space.

Mixture 2 (Figure 4.4), presented a more complex scenario, featuring eleven compounds, several of which coeluted chromatographically. The RT overlap observed for compounds in the range RT = 5.5 - 6.5 min was largely resolved in CV space, with analytes occupying the range of -24 V (atenolol) to 0 V (rosuvastatin). This behaviour demonstrates the strength of the DMS technique for separating conformationally similar analytes. Conversely, carvedilol and bisoprolol, though separated in RT, showed significant CV overlap, indicating possible structural similarities affecting gas-phase behaviour. In the case of alprazolam and rosuvastatin, we observed partial overlap in both the LC and DMS dimensions; these results emphasize that although DMS provides strong orthogonality to LC, it may not fully compensate for coelution in every case.

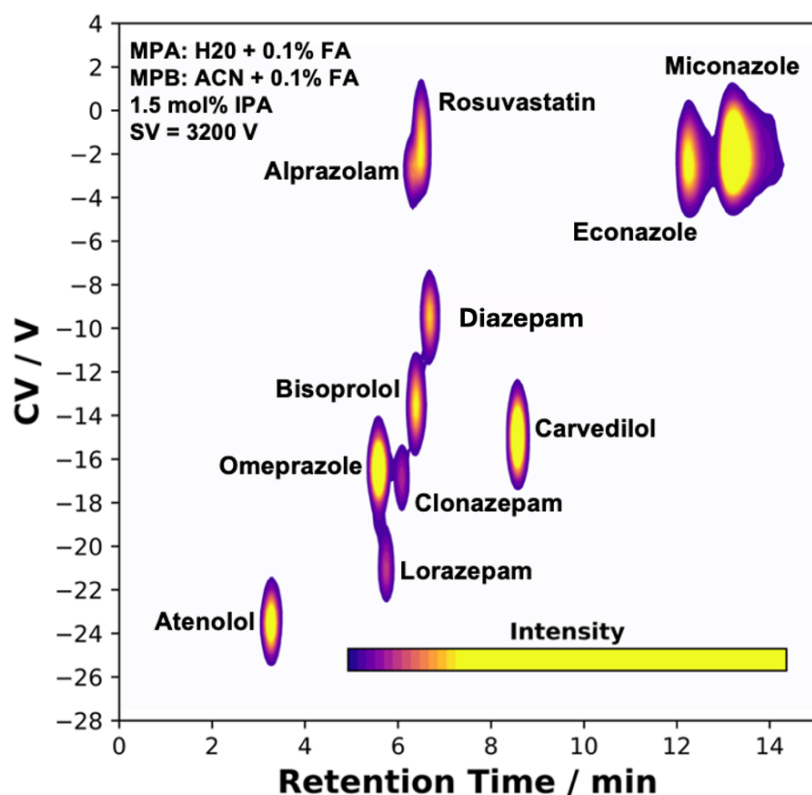


Figure 4-4. Two-dimensional LC×DMS contour plot for mixture 2, illustrating separation of eleven pharmaceutical compounds across retention time and compensation voltage. Most analytes are well-resolved, with partial overlap between alprazolam and rosuvastatin.

For mixture 3 (Figure 4.5), the two-dimensional approach proved especially valuable for resolving a dense group of antihypertensive agents. Interestingly, lisinopril, olmesartan and enalapril which exhibited a single RT feature, exhibited two features (labeled Lisinopril_A, Lisinopril_B, Olmesartan_A, Olmesartan_B, Enalapril_A, Enalapril_B) at CVs from −14 V and −23 V. This suggests the possible presence of conformers or prototropic isomers in the gas phase ensemble of protonated lisinopril, olmesartan and enalapril. Coelution observed between enalapril and perindopril in the LC space was partially resolved in CV space, with each compound adopting a slightly different differential ion mobility profile. Contrastingly, enalapril and ramipril, closely related ACE inhibitors, were well separated along the RT axis

despite having similar CVs. The separation of statins proved to be challenging; lovastatin, simvastatin, and atorvastatin are blended in the LC dimension and the DMS dimension, but we can observe partial resolution in the 2D space. The signal response for pantoprazole and irbesartan_B were too low to be displayed alongside the higher intensity peaks in the plot. Their signals were already weak in the one-dimensional analyses, making them difficult to visualize in the context of the full dataset.

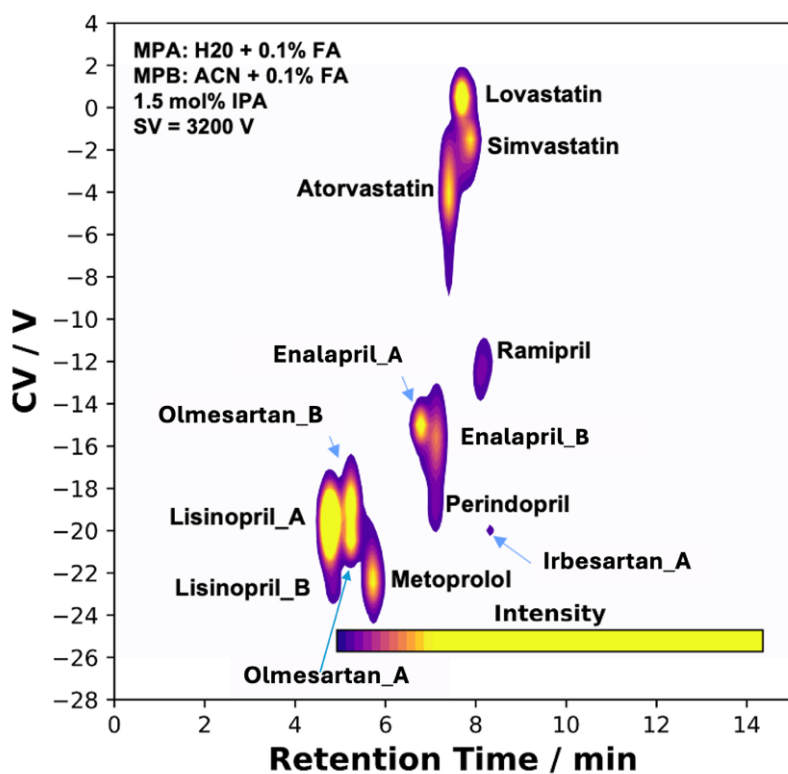


Figure 4-5. Two-dimensional LCxMS/MS contour plot for mixture 3, showing separation of eleven pharmaceuticals including multiple antihypertensives (ARBs) and statins. Coelution of lovastatin and simvastatin is observed, while other compounds are clearly distinguished across both dimensions.

Figure 4.6. illustrates the correlation between LC retention time and DMS compensation voltage for a diverse set of pharmaceuticals, grouped by therapeutic class. Drug classes such as statins (dark blue) and PDE5s (light blue) exhibit tight clustering in the upper regions of the

plot, indicating consistent behaviour across both separation dimensions. Similarly, ACE inhibitors (light green), PPIs (purple) and benzodiazepines (orange) cluster in the central region, suggesting that compounds within each class share physicochemical properties influencing both LC and DMS behaviour. Two of the three antifungals (brown) are notably well-separated from other classes, showing longer retention times and relatively low CVs. Other classes such as ARBs (dark green) and beta blockers (red) show some correlation in the LC and DMS dimensions, however this is a looser correlation than groups previously mentioned. Miscellaneous groups such as antiepileptics (pink), calcium channel blockers (grey) and antibiotics (yellow) do not show irregular placement within the 2D plot, but not enough data is available at this time to comment on their correlation behaviour. These patterns demonstrate that LC×DMS-MS/MS separations effectively highlight trends from structurally or functionally related drugs (See Table 4.2) indicating potential utility for class-based screening in complex pharmaceutical mixtures.

Table 4-2. Summary of therapeutic classes, common clinical use and specific compounds for the chemical classes investigated in this study.

Statins	Lower LDL/cholesterol; reduce cardiovascular events (primary/secondary prevention).	Atorvastatin, Lovastatin, Rosuvastatin, Simvastatin
ACE Inhibitors	Hypertension; heart failure; post-MI care; kidney protection (e.g., diabetic nephropathy).	Enalapril, Lisinopril, Perindopril, Ramipril
ARBs	Hypertension; heart failure/CKD when ACE-I not tolerated; kidney/CV protection.	Losartan, Irbesartan, Olmesartan
Beta Blockers	Lower BP/heart rate; angina; arrhythmia rate control	Atenolol, Bisoprolol, Carvedilol, Metoprolol

Benzodiazepines	Anxiety; seizure control; acute sedation; muscle spasm.	Alprazolam, Clonazepam, Diazepam, Lorazepam
PPIs	GERD/acid reflux; peptic ulcers; H. pylori regimens; NSAID ulcer prevention.	Omeprazole, Pantoprazole
Antifungal (azoles)	Yeast and dermatophyte infections (e.g., candidiasis, tinea); some systemic fungal infections.	Fluconazole, Econazole, Miconazole
Calcium Channel Blockers	Angina; rate control in atrial arrhythmias; hypertension.	Diltiazem
Antiepileptics	Seizure disorders; trigeminal neuralgia; bipolar adjunct.	Carbamazepine
Antibiotics	Broad-spectrum bacterial infections (e.g., respiratory/skin; acne; atypicals).	Oxytetracycline
PDE5 Inhibitors	Erectile dysfunction; pulmonary arterial hypertension.	Avanafil, Sildenafil

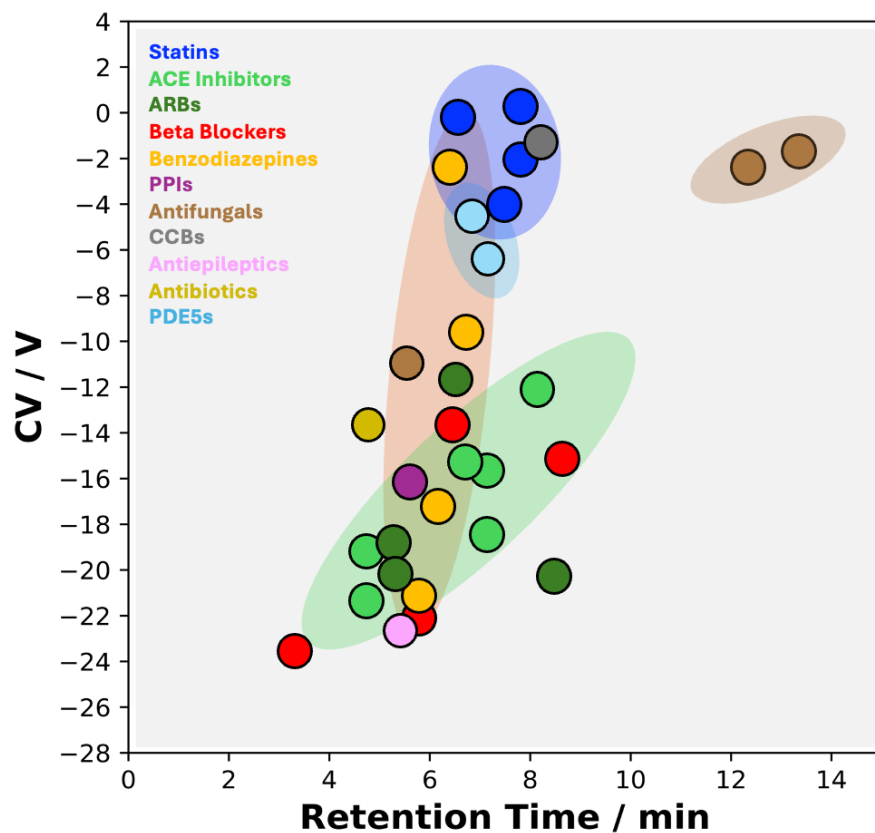


Figure 4-6. Correlation between retention time and compensation voltage for various drug classes via LC×DMS-MS/MS.

To assess overall separation performance, retention time (RT), compensation voltage (CV) and full width half max (FWHM) values from three independently run pharmaceutical mixtures were consolidated into a single resolution matrix. This approach assumes that each compound FWHM remains consistent across mixtures, a simplification made to enable resolution calculations, despite the potential for ionization efficiency and matrix effects to slightly alter peak width and height in complex samples. Resolving power was quantified as the percentage of peak pairs with resolution values greater than or equal to 1.5, using the equation:

$$\textbf{Resolving Power} (\%) = \left(1 - \frac{U}{T - D}\right) \times 100 \quad \text{Eq. 4-1}$$

Where U is the number of unresolved peak pairs (*i.e.*, those with resolution $0 \leq U \leq 1.5$), T is the total number of values in the resolution matrix, and D is the number of diagonal entries corresponding to self-comparisons. For the LC×DMS dataset, the matrix contained 1156 values in total ($T = 1156$) with 34 peaks ($D = 34$), resulting in 1122 valid comparisons after excluding the diagonal. Among these, 72 peak pairs had resolution values $0 \leq U \leq 1.5$ ($U = 72$), yielding a resolving power of 93.6% according to the above equation. The same calculation was applied to LC-MS alone and DMS alone, which produced resolving powers of 91.4% and 56.5% respectively. Although, the DMS dimension alone contributed a greater number of unresolved peak pairs overall, it uniquely enabled the separation of a few isomeric species that coeluted in the LC dimension (*e.g.* Lisinopril, Enalapril, Olmesartan and Irbesartan). Consequently, the combined LC×DMS method not only provided the highest overall resolving power but also enhanced selectivity by enabling the differentiation of

structurally similar compounds (*e.g.* Lisinopril, Olmesartan, Enalapril) not otherwise resolvable by LC-MS alone.

The success of this approach highlights its potential for targeted screening workflows in complex pharmaceutical or environmental matrices. Table 4.3 shows Canadian studies conducted on various water matrices with their respective LOD, LOQ and chosen instrumental techniques.

Table 4-3. Reported limits of detection (LOD) and quantification (LOQ) for pharmaceuticals in various Canadian water matrices using LC-MS/MS techniques

Matrix/ Sample Type	Typical LOD (ng L ⁻¹)	Typical LOQ (ng L ⁻¹)	Instrumentation & Sample Preparation
Source water (95 First Nations) ¹⁴¹	0.24-3.1	2-20	Direct injection/SPE + LC-MS/MS
Tap water (Eastern Canada) ¹⁴²	0.03-0.1	0.09-1.5	Offline SPE + UHPLC-MS/MS
Surface water (Eastern Canada) ¹⁴²	0.1-0.5	0.3-1.5	Offline SPE + UHPLC-MS/MS
Wastewater Influent (Eastern Canada) ¹⁴²	0.5-1	1.5-3	Offline SPE + UHPLC-MS/MS
Wastewater Effluent (Eastern Canada) ¹⁴²	0.15-1	0.45-3	Offline SPE + UHPLC-MS/MS

4.4 Conclusions

This work demonstrates the effectiveness of a two-dimensional LC×DMS-MS/MS approach for the separation and detection of pharmaceutical contaminants in complex mixtures. By combining reversed-phase liquid chromatography with differential ion mobility spectrometry, enhanced resolution was achieved across both retention time and compensation voltage dimensions. The use of 1.5 mol% isopropanol as a chemical modifier and a separation voltage of SV = 3200 V provided robust and reproducible ion mobility separation conditions for all

compounds analyzed. Due to field induce dissociation, 3200 V was the greatest separation voltage that could be applied to the DMS cell while maintaining consistent and reproducible signal response of each analyte. Alternatively, separation can be improved by taking advantage of temperature effects within the DMS cell.¹⁴³ An ions differential mobility is strongly influenced by its ability to form clusters with neutral bath gas molecules.¹⁴³ According to two-temperature theory, an empirical model describing how electric field and temperature affect ion transport, lower thermal energy in the DMS cell favours more stable ion-neutral clustering.¹⁴³ This enhanced clustering increases the difference in mobility between low- and high-field conditions.¹⁴³ Therefore, amplifying differential mobility and potentially improving the separation of structurally similar analytes.¹⁴³ For these reasons we operated our DMS cell on “low” temperature mode with it set at 150 °C.¹⁴³

Across the three model mixtures, LC×DMS-MS/MS proved especially valuable in resolving compounds that coeluted in either one-dimensional method. In mixtures 1 and 2, structurally similar compounds that overlapped in compensation voltage were clearly separated in retention time space. Mixture 3, which contained a high portion of antihypertensives and statins, presented greater separation challenges using the 1D methods, but most analytes were distinguishable in the two-dimensional space. Limited resolution between isomeric statins, lovastatin and simvastatin, was observed, underscoring the challenges of using LC alone to separate compounds with nearly identical molecular structures. These structural similarities result in comparable retention behaviour, making it difficult for LC to fully resolve them based solely on hydrophobic interactions or polarity differences.

Overall, this multidimensional approach enabled greater selectivity and analytical confidence in compound identification. The combined resolution capacity, along with moderate analysis times and method complexity, demonstrates the potential of LC×DMS-MS/MS as a powerful tool for targeted screening of pharmaceutical contaminants in environmental and biological matrices. These findings also highlight the potential for using DMS in future analytical workflows to improve separation of complex mixtures. When combined with other techniques like LC, DMS can help expand the range of compounds that can be confidently identified and resolved. Practical applications include screening pharmaceutical impurities, resolving isobaric drug metabolites and improving confidence in detection for clinical or forensic samples.

Chapter 5. Conclusions and Future Work

The purpose of this thesis was to address two complementary challenges in the analysis of emerging micropollutants. First, we sought to improve our understanding of gas-phase fragmentation pathways of perfluoroalkyl sulfonates (PFSAs), a subclass of PFAS, in order to rationalize the formation of commonly observed product ions. This is a critical step towards building accurate and predictive MRM transition libraries, especially given the widespread presence of PFAS with unknown or unverified transitions.

Second, we aimed to develop a complementary two-dimensional liquid chromatography-differential ion mobility mass spectrometry (LC×MDS-MS/MS) workflow for the separation of co-eluting pharmaceutical contaminants in complex aqueous matrices. Improving separation not only enhances quantitation accuracy but also helps clarify how physicochemical properties influence analytical behaviour, such as retention time (RT), collision cross section (CCS) and DMS response. These classed-related behaviours provide another avenue for the identification of unknowns.

In Chapter 3, I present both experimental and computational investigations of PFSA fragmentation to gain mechanistic insights into their gas-phase ion chemistry. In a previous study of per- and polyfluorocarboxylic acids (PFCAs) conducted by Lee et al. (2024) the formation of FCO_2^- was observed unexpectedly during experimental analysis. This prompted a further investigation into FCO_2^- formation, energetic barriers to reaction, mechanism of product formation and product structures. The work of Lee et al. served as a key motivation for my own investigation of the gas-phase ion behaviour of PFSAs. Throughout the project, experimental data revealed formation of the m/z 80 and m/z 99 fragment ions, consistent with

MRM transitions reported by other recent studies of PFSA using LC-MS/MS instrumentation.^{144,145} The repeated detection of SO_3^- and FSO_3^- fragments in experimental spectra raised questions about their formation mechanisms. To address this, I conducted computational investigations aimed at mapping possible reaction pathways and determining the energy barriers associated with each process.

Experimental breakdown curves of PFSA across varying chain lengths revealed that nearly isoenergetic pathways are accessible for the formation of key fragment ions. While SO_3^- formation is consistent with a direct bond cleavage mechanism, via impulsive dissociation, FSO_3^- formation requires a more complex pathway and structural rearrangement. This rearrangement is inferred from the PFSA composition: FSO_3^- contains an S–F bond, not present in the original sulfonate structure, indicating that a fluorine abstraction or migration event must occur. Rearrangement mechanisms of this type generally require the system to overcome a well-defined transition state, implying higher energetic demands compared to the impulsive dissociation as concluded for the SO_3^- .

This interpretation is supported by the shape and slope of the breakdown curves: the collision energy required to generate SO_3^- and FSO_3^- increased linearly with PFSA chain length, suggesting that the energetic threshold for these pathways remains consistent across the series. Using PFPeS as a representative case, computational reaction pathway analysis was conducted to elucidate possible FSO_3^- formation mechanisms and to quantify the associated barriers. These results provide mechanistic insight into the recurring but previously unexplained diagnostic fragment, m/z 99.

The computational study revealed six closed-shell and seven-open-shell fragmentation channels for PFPeS. Concerted AB and EF pathways, which involve synchronous C–S and C–F bond cleavage form linear and kinked backbone reactant conformations respectively, showed activation barriers 12-15 kJ/mol higher than their non-concerted counterparts, underscoring the effect of a non-covalent dispersion-stabilizing interaction that forms in the transition states (See Figure 3.12). All A-precursor mechanism proceeded with a linear conformation, whereas E-initiated routes utilized a kinked backbone structure (See Figure 3.5). The fragmentation pathways modeled in this work revealed that both A- and E-type mechanisms share the benefit of a lowered activation barrier when undergoing a sulfonate rearrangement through a transition state reminiscent of a diamond geometry (See Figure 3.12). This finding contrasts with our initial hypothesis of a concerted elimination (AB, EF) being the most probable course of FSO_3^- formation. With this information, further exploration of the sulfonate rearrangement was applied to PFSA's of shorter and longer chain lengths (PFPS, PFBS, PFHxS and PFHpS) to see if this lowered activation energy held true despite the length of the CF_2 backbone. Altering the perfluoroalkyl chain by one or two CF_2 units introduced only small variations in the relative activation barriers, though a minor oscillation in the first transition state energy was observed (See Figure 3.21). This variation may reflect subtle differences in the backbone orientation; however, further investigation is needed to determine whether this represents a real structural effect or is within the uncertainty of the computational method. I conclude that the non-concerted mechanisms initiating with the sulfonate rearrangement rival the concerted elimination mechanisms as the lowest energy pathway to FSO_3^- formation regardless of chain PFSA length. While this conclusion is supported by

current experimental results and computational data, it is possible that future studies, particularly those employing alternative experimental approaches or higher-level theory, may provide additional insights or lead to revised interpretations. To build on these findings, I further explored product ions outside the perfluorinated terminal alkene. Using the β -perfluoropentane sulfonate intermediate, we investigated mechanisms to form both cis and trans internal alkenes, which proved to be lower in energy than the terminal alkene, owing to hyperconjugation and stereoelectronic effects (See Figure 3.4). Alternative open-shell pathways were also investigated, but these were found to be less favourable than the six closed-shell pathways investigated due to their elevated barrier heights, suggesting a lower probability of formation under experimental conditions (See Figure 3.13).

To support the theoretical assignments, collision cross sections (CCS) were measured for the deprotonated PFPeS ion using travelling-wave ion mobility spectrometry (TWIMS). The experimentally determined CCS ($139.99 \pm 0.21 \text{ \AA}^2$) showed excellent agreement with both the calculated value for the linear structure ($141.09 \pm 1.26 \text{ \AA}^2$) and the literature value of $142.20 \text{ \AA}^2$. This consistency across computational, experimental, and literature values validates the assigned linear geometry and highlights the utility of TWIMS as a structural tool for characterizing PFAS ions. To further determine the identities of neutral fragments, which cannot be directly detected by MS, future studies could employ infrared (IR) spectroscopy as a complementary technique. In conventional MS-based workflows, neutral species produced during fragmentation are lost to the system because they are not guided by electric fields and cannot be detected unless ionized. Although post-fragmentation ionization using a UV laser is theoretically possible, this approach is limited in the case of PFAS, as these compounds

typically lack the ability to absorb energy in the UV range and thus cannot be efficiently excited or ionized by UV photons. Even when post-fragmentation ionization is feasible, capturing and analyzing neutral species presents significant experimental challenges due to their short lifetime and low ionization efficiency within the mass spectrometer.

As an alternative, IR spectroscopy, particularly techniques like IR photodissociation (IRPD) of fragment ions, offer a non-destructive and structurally informative means of probing the identity or neutral losses indirectly. By comparing experimental IR spectra with DFT-calculated vibrational signatures, these approaches can help assign fragmentation pathways and resolve ambiguities that cannot be addressed through mass analysis alone.

In Chapter 4, a two-dimensional LC×DMS-MS/MS workflow was developed to enhance the analytical resolution of pharmaceutical mixtures in aqueous samples. This technique, which combined reverse-phase liquid chromatography with differential ion mobility spectrometry, achieved improved resolution across three representative mixtures as compared to the one-dimensional analyses alone. Quantitative assessment of resolving power, calculated as the percentage of peak pairs with resolution values greater than or equal to 1.5, showed that the combined LC×DMS method achieved 93.6% resolving power, outperforming LC-MS (91.4%) and DMS alone (56.5%). While DMS alone contributed more unresolved peak pairs overall, it enabled separation of isomeric species not distinguishable by LC, underscoring the added value of an orthogonal separation dimension for enhanced selectivity and identification.

Interestingly, we found that structurally or functionally similar compounds consistently clustered in the 2D LC×DMS space. Statins, ACE inhibitors and benzodiazepines each formed distinct groupings, indicating similar separation patterns for such classes across both the LC

and DMS dimensions. These patterns reflect differences in gas-phase clustering behaviour and polarity between varying groups of pharmaceuticals, highlighting potential use for characterization using non-target analysis, however, future efforts are needed to explore this concept. The use of CV-labelled multiple reaction monitoring (MRM) enabled full two-dimensional data acquisition on a tandem triple-quadrupole instrument, showing that the approach is compatible with routine environmental analyses and does not require high-resolution instrumentation, though it requires pre-existing knowledge of each ions DMS behaviour and resultant CVs.

The 2D LC×DMS-MS/MS method was optimized for a relatively small number of analytes and will likely require refinement to address additional micropollutant classes. Moreover, some analytes also showed field-induced fragmentation at high dispersion voltages, which will impact LoDs and LoQs for those species. To address this challenge, future work should incorporate the use of a protective gas such as CO₂, as it may help reduce field-induced fragmentation for labile compounds and coupling this with LC×DMS-MS/MS would enable full ionogram acquisition at higher separation voltage, and possibly baseline separation of more compounds.

Finally, applying this method to real environmental matrices such as surface water, wastewater and biosolids, will help evaluate its robustness under realistic conditions. Because LC×DMS-MS/MS accommodates both anionic and cationic species, it also holds potential for broader applications such as monitoring disinfection by-products, personal-care products and other emerging contaminants.

Together, these investigations demonstrate how integrated experimental and computational approaches can advance our ability to detect and characterize diverse classes of micropollutants. By elucidating fragmentation mechanism of PFSA's and developing a multidimensional workflow for pharmaceuticals, this work contributes to the growing toolkit for addressing analytical challenges posed by complex environmental mixtures. The findings highlight the importance of understanding the gas-phase ion chemistry of PFSA's and the value of orthogonal separations in revealing previously overlooked links between structure and experimental behaviour. Moving forward, continued refinement of these strategies and their application to real-world matrices will be essential to enabling more accurate, sensitive and comprehensive monitoring of contaminants in environmental and public health contexts.

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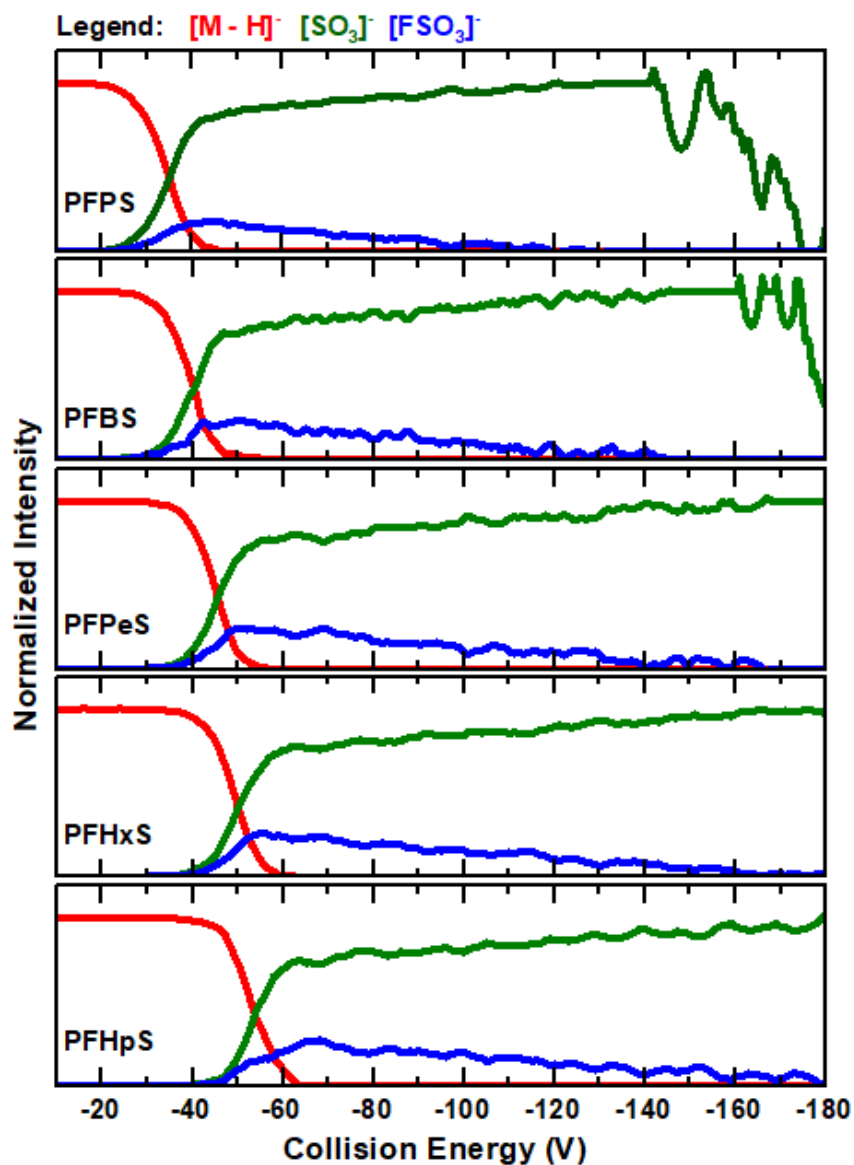
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Appendix A: Supplementary Information (Chapter 3)



A- 1. Normalized collision-induced dissociation (CID) breakdown curves for five perfluoroalkyl sulfonates (PFPS, PFBS, PFPeS, PFHxS, PFHpS), showing loss of precursor ion ($[M-H]^-$, red) and formation of SO_3^- (green) and FSO_3^- (blue) product ions as a function of collision energy.

A-2.

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Appendix B. Supplementary Information (Chapter 4)

B- 1. Composition of Pharmaceutical Mixtures Used for LC×DMS-MS/MS Analysis

Suppliers:

1. Aaron Chemicals (CA, USA)
2. Aurum Pharmatech (TX, USA)
3. Cayman Chemical Company (MI, USA)

Mixture 1 (n=7)	Mixture 2 (n=11)	Mixture 3 (n=11)
Avanafil ²	Alprazolam ³	Atorvastatin ²
Carbamazepine ¹	Atenolol ¹	Enalapril ²
Diltiazem ¹	Bisoprolol ²	Irbesartan ²
Fluconazole ²	Carvedilol ²	Lisinopril ²
Losartan ²	Clonazepam ³	Lovastatin ²
Oxytetracycline ¹	Diazepam ³	Metoprolol ²
Sildenafil ²	Econazole ²	Olmesartan ²
-	Lorazepam ³	Pantoprazole ²
-	Miconazole ²	Perindopril ²
-	Omeprazole ²	Ramipril ²
-	Rosuvastatin ²	Simvastatin ²

B- 2. Optimized LC×DMS-MS/MS Parameters for Selected Pharmaceuticals in Mixture 1

Q1 Mass (Da)	Q3 Mass (Da)	Dwell Time (ms)	ID	DP (volts)	CE (volts)	CoV (eV)
485	155	5	Avanafil_Q101	130	62	-7.75
485	155	5	Avanafil_Q102	130	62	-7.25
485	155	5	Avanafil_Q103	130	62	-6.75
485	155	5	Avanafil_Q104	130	62	-6.25
485	155	5	Avanafil_Q105	130	62	-5.75
485	155	5	Avanafil_Q106	130	62	-5.25
485	155	5	Avanafil_Q107	130	62	-4.75
485	155	5	Avanafil_Q108	130	62	-4.25
485	155	5	Avanafil_Q109	130	62	-3.75
485	155	5	Avanafil_Q110	130	62	-3.25
485	155	5	Avanafil_Q111	130	62	-2.75
485	155	5	Avanafil_Q112	130	62	-2.25
237	194	5	Carbamazepine_Q101	65	30	-23.35
237	194	5	Carbamazepine_Q102	65	30	-22.85
237	194	5	Carbamazepine_Q103	65	30	-22.35
237	194	5	Carbamazepine_Q104	65	30	-21.85

237	194	5	Carbamazepine_Q105	65	30	-21.35
237	194	5	Carbamazepine_Q106	65	30	-20.85
237	194	5	Carbamazepine_Q107	65	30	-20.35
237	194	5	Carbamazepine_Q108	65	30	-19.85
237	194	5	Carbamazepine_Q109	65	30	-19.35
237	194	5	Carbamazepine_Q110	65	30	-18.85
237	194	5	Carbamazepine_Q111	65	30	-18.35
237	194	5	Carbamazepine_Q112	65	30	-17.85
332	231	5	Ciprofloxacin_Q101	90	55	-16.15
332	231	5	Ciprofloxacin_Q102	90	55	-15.65
332	231	5	Ciprofloxacin_Q103	90	55	-15.15
332	231	5	Ciprofloxacin_Q104	90	55	-14.65
332	231	5	Ciprofloxacin_Q105	90	55	-14.15
332	231	5	Ciprofloxacin_Q106	90	55	-13.65
332	231	5	Ciprofloxacin_Q107	90	55	-13.15
332	231	5	Ciprofloxacin_Q108	90	55	-12.65
332	231	5	Ciprofloxacin_Q109	90	55	-12.15
332	231	5	Ciprofloxacin_Q110	90	55	-11.65
332	231	5	Ciprofloxacin_Q111	90	55	-11.15
332	231	5	Ciprofloxacin_Q112	90	55	-10.65
415	178	5	Diltiazem_Q101	100	37	-3.15
415	178	5	Diltiazem_Q102	100	37	-2.65
415	178	5	Diltiazem_Q103	100	37	-2.15
415	178	5	Diltiazem_Q104	100	37	-1.65
415	178	5	Diltiazem_Q105	100	37	-1.15
415	178	5	Diltiazem_Q106	100	37	-0.65
415	178	5	Diltiazem_Q107	100	37	-0.15
415	178	5	Diltiazem_Q108	100	37	0.35
415	178	5	Diltiazem_Q109	100	37	0.85
415	178	5	Diltiazem_Q110	100	37	1.35
415	178	5	Diltiazem_Q111	100	37	1.85
415	178	5	Diltiazem_Q112	100	37	2.35
307	220	5	Fluconazole_Q101	90	30	-11.75
307	220	5	Fluconazole_Q102	90	30	-11.25
307	220	5	Fluconazole_Q103	90	30	-10.75
307	220	5	Fluconazole_Q104	90	30	-10.25
307	220	5	Fluconazole_Q105	90	30	-9.75
307	220	5	Fluconazole_Q106	90	30	-9.25
307	220	5	Fluconazole_Q107	90	30	-8.75

307	220	5	Fluconazole_Q108	90	30	-8.25
307	220	5	Fluconazole_Q109	90	30	-7.75
307	220	5	Fluconazole_Q110	90	30	-7.25
307	220	5	Fluconazole_Q111	90	30	-6.75
307	220	5	Fluconazole_Q112	90	30	-6.25
423	207	5	Losartan_Q101	100	39	-12.95
423	207	5	Losartan_Q102	100	39	-12.45
423	207	5	Losartan_Q103	100	39	-11.95
423	207	5	Losartan_Q104	100	39	-11.45
423	207	5	Losartan_Q105	100	39	-10.95
423	207	5	Losartan_Q106	100	39	-10.45
423	207	5	Losartan_Q107	100	39	-9.95
423	207	5	Losartan_Q108	100	39	-9.45
423	207	5	Losartan_Q109	100	39	-8.95
423	207	5	Losartan_Q110	100	39	-8.45
423	207	5	Losartan_Q111	100	39	-7.95
423	207	5	Losartan_Q112	100	39	-7.45
461	426	5	Oxytetracycline_Q101	84	27	-13.75
461	426	5	Oxytetracycline_Q102	84	27	-13.25
461	426	5	Oxytetracycline_Q103	84	27	-12.75
461	426	5	Oxytetracycline_Q104	84	27	-12.25
461	426	5	Oxytetracycline_Q105	84	27	-11.75
461	426	5	Oxytetracycline_Q106	84	27	-11.25
461	426	5	Oxytetracycline_Q107	84	27	-10.75
461	426	5	Oxytetracycline_Q108	84	27	-10.25
461	426	5	Oxytetracycline_Q109	84	27	-9.75
461	426	5	Oxytetracycline_Q110	84	27	-9.25
461	426	5	Oxytetracycline_Q111	84	27	-8.75
461	426	5	Oxytetracycline_Q112	84	27	-8.25
476	58	5	Sildenafil_Q101	110	80	-5.95
476	58	5	Sildenafil_Q102	110	80	-5.45
476	58	5	Sildenafil_Q103	110	80	-4.95
476	58	5	Sildenafil_Q104	110	80	-4.45
476	58	5	Sildenafil_Q105	110	80	-3.95
476	58	5	Sildenafil_Q106	110	80	-3.45
476	58	5	Sildenafil_Q107	110	80	-2.95
476	58	5	Sildenafil_Q108	110	80	-2.45
476	58	5	Sildenafil_Q109	110	80	-1.95
476	58	5	Sildenafil_Q110	110	80	-1.45

476	58	5	Sildenafil_Q111	110	80	-0.95
476	58	5	Sildenafil_Q112	110	80	-0.45

B- 3. Optimized LC×DMS-MS/MS Parameters for Selected Pharmaceuticals in Mixture 2

Q1 Mass (Da)	Q3 Mass (Da)	Dwell Time (ms)	ID	DP (volts)	CE (volts)	CoV (eV)
310	206	5	Alprazolam_Q101	130	61	-7.7
310	206	5	Alprazolam_Q102	130	61	-7.2
310	206	5	Alprazolam_Q103	130	61	-6.7
310	206	5	Alprazolam_Q104	130	61	-6.2
310	206	5	Alprazolam_Q105	130	61	-5.7
310	206	5	Alprazolam_Q106	130	61	-5.2
310	206	5	Alprazolam_Q107	130	61	-4.7
310	206	5	Alprazolam_Q108	130	61	-4.2
310	206	5	Alprazolam_Q109	130	61	-3.7
310	206	5	Alprazolam_Q110	130	61	-3.2
310	206	5	Alprazolam_Q111	130	61	-2.7
310	206	5	Alprazolam_Q112	130	61	-2.2
267	145	5	Atenolol_Q101	70	40	-26
267	145	5	Atenolol_Q102	70	40	-25.5
267	145	5	Atenolol_Q103	70	40	-25
267	145	5	Atenolol_Q104	70	40	-24.5
267	145	5	Atenolol_Q105	70	40	-24
267	145	5	Atenolol_Q106	70	40	-23.5
267	145	5	Atenolol_Q107	70	40	-23
267	145	5	Atenolol_Q108	70	40	-22.5
267	145	5	Atenolol_Q109	70	40	-22
267	145	5	Atenolol_Q110	70	40	-21.5
267	145	5	Atenolol_Q111	70	40	-21
267	145	5	Atenolol_Q112	70	40	-20.5
326	116	5	Bisoprolol_Q101	90	26	-16.3
326	116	5	Bisoprolol_Q102	90	26	-15.8
326	116	5	Bisoprolol_Q103	90	26	-15.3
326	116	5	Bisoprolol_Q104	90	26	-14.8
326	116	5	Bisoprolol_Q105	90	26	-14.3
326	116	5	Bisoprolol_Q106	90	26	-13.8
326	116	5	Bisoprolol_Q107	90	26	-13.3
326	116	5	Bisoprolol_Q108	90	26	-12.8
326	116	5	Bisoprolol_Q109	90	26	-12.3

326	116	5	Bisoprolol_Q110	90	26	-11.8
326	116	5	Bisoprolol_Q111	90	26	-11.3
326	116	5	Bisoprolol_Q112	90	26	-10.8
407	224	5	Carvedilol_Q101	90	32	-16.4
407	224	5	Carvedilol_Q102	90	32	-15.9
407	224	5	Carvedilol_Q103	90	32	-15.4
407	224	5	Carvedilol_Q104	90	32	-14.9
407	224	5	Carvedilol_Q105	90	32	-14.4
407	224	5	Carvedilol_Q106	90	32	-13.9
407	224	5	Carvedilol_Q107	90	32	-13.4
407	224	5	Carvedilol_Q108	90	32	-12.9
407	224	5	Carvedilol_Q109	90	32	-12.4
407	224	5	Carvedilol_Q110	90	32	-11.9
407	224	5	Carvedilol_Q111	90	32	-11.4
407	224	5	Carvedilol_Q112	90	32	-10.9
317	271	5	Clonazepam_Q101	90	41	-21.7
317	271	5	Clonazepam_Q102	90	41	-21.2
317	271	5	Clonazepam_Q103	90	41	-20.7
317	271	5	Clonazepam_Q104	90	41	-20.2
317	271	5	Clonazepam_Q105	90	41	-19.7
317	271	5	Clonazepam_Q106	90	41	-19.2
317	271	5	Clonazepam_Q107	90	41	-18.7
317	271	5	Clonazepam_Q108	90	41	-18.2
317	271	5	Clonazepam_Q109	90	41	-17.7
317	271	5	Clonazepam_Q110	90	41	-17.2
317	271	5	Clonazepam_Q111	90	41	-16.7
317	271	5	Clonazepam_Q112	90	41	-16.2
285	154	5	Diazepam_Q101	90	39	-12.5
285	154	5	Diazepam_Q102	90	39	-12
285	154	5	Diazepam_Q103	90	39	-11.5
285	154	5	Diazepam_Q104	90	39	-11
285	154	5	Diazepam_Q105	90	39	-10.5
285	154	5	Diazepam_Q106	90	39	-10
285	154	5	Diazepam_Q107	90	39	-9.5
285	154	5	Diazepam_Q108	90	39	-9
285	154	5	Diazepam_Q109	90	39	-8.5
285	154	5	Diazepam_Q110	90	39	-8
285	154	5	Diazepam_Q111	90	39	-7.5
285	154	5	Diazepam_Q112	90	39	-7

383	89	5	Econazole_Q101	90	100	-4.8
383	89	5	Econazole_Q102	90	100	-4.3
383	89	5	Econazole_Q103	90	100	-3.8
383	89	5	Econazole_Q104	90	100	-3.3
383	89	5	Econazole_Q105	90	100	-2.8
383	89	5	Econazole_Q106	90	100	-2.3
383	89	5	Econazole_Q107	90	100	-1.8
383	89	5	Econazole_Q108	90	100	-1.3
383	89	5	Econazole_Q109	90	100	-0.8
383	89	5	Econazole_Q110	90	100	-0.3
383	89	5	Econazole_Q111	90	100	0.2
383	89	5	Econazole_Q112	90	100	0.7
321	275	5	Lorazepam_Q101	90	35	-21.8
321	275	5	Lorazepam_Q102	90	35	-21.3
321	275	5	Lorazepam_Q103	90	35	-20.8
321	275	5	Lorazepam_Q104	90	35	-20.3
321	275	5	Lorazepam_Q105	90	35	-19.8
321	275	5	Lorazepam_Q106	90	35	-19.3
321	275	5	Lorazepam_Q107	90	35	-18.8
321	275	5	Lorazepam_Q108	90	35	-18.3
321	275	5	Lorazepam_Q109	90	35	-17.8
321	275	5	Lorazepam_Q110	90	35	-17.3
321	275	5	Lorazepam_Q111	90	35	-16.8
321	275	5	Lorazepam_Q112	90	35	-16.3
417	159	5	Miconazole_Q101	90	52	-4.7
417	159	5	Miconazole_Q102	90	52	-4.2
417	159	5	Miconazole_Q103	90	52	-3.7
417	159	5	Miconazole_Q104	90	52	-3.2
417	159	5	Miconazole_Q105	90	52	-2.7
417	159	5	Miconazole_Q106	90	52	-2.2
417	159	5	Miconazole_Q107	90	52	-1.7
417	159	5	Miconazole_Q108	90	52	-1.2
417	159	5	Miconazole_Q109	90	52	-0.7
417	159	5	Miconazole_Q110	90	52	-0.2
417	159	5	Miconazole_Q111	90	52	0.3
417	159	5	Miconazole_Q112	90	52	0.8
346	136	5	Omeprazole_Q101	60	50	-19.2
346	136	5	Omeprazole_Q102	60	50	-18.7
346	136	5	Omeprazole_Q103	60	50	-18.2

346	136	5	Omeprazole_Q104	60	50	-17.7
346	136	5	Omeprazole_Q105	60	50	-17.2
346	136	5	Omeprazole_Q106	60	50	-16.7
346	136	5	Omeprazole_Q107	60	50	-16.2
346	136	5	Omeprazole_Q108	60	50	-15.7
346	136	5	Omeprazole_Q109	60	50	-15.2
346	136	5	Omeprazole_Q110	60	50	-14.7
346	136	5	Omeprazole_Q111	60	50	-14.2
346	136	5	Omeprazole_Q112	60	50	-13.7
482	258	5	Rosuvastatin_Q101	90	52	-3.5
482	258	5	Rosuvastatin_Q102	90	52	-3
482	258	5	Rosuvastatin_Q103	90	52	-2.5
482	258	5	Rosuvastatin_Q104	90	52	-2
482	258	5	Rosuvastatin_Q105	90	52	-1.5
482	258	5	Rosuvastatin_Q106	90	52	-1
482	258	5	Rosuvastatin_Q107	90	52	-0.5
482	258	5	Rosuvastatin_Q108	90	52	0
482	258	5	Rosuvastatin_Q109	90	52	0.5
482	258	5	Rosuvastatin_Q110	90	52	1
482	258	5	Rosuvastatin_Q111	90	52	1.5
482	258	5	Rosuvastatin_Q112	90	52	2

B- 4. Optimized LC×DMS-MS/MS Parameters for Selected Pharmaceuticals in Mixture 3

Q1 Mass (Da)	Q3 Mass (Da)	Dwell Time (ms)	ID	DP (volts)	CE (volts)	CoV (eV)
559	250	5	Atorvastatin_Q101	16	73	-6.45
559	250	5	Atorvastatin_Q102	16	73	-5.95
559	250	5	Atorvastatin_Q103	16	73	-5.45
559	250	5	Atorvastatin_Q104	16	73	-4.95
559	250	5	Atorvastatin_Q105	16	73	-4.45
559	250	5	Atorvastatin_Q106	16	73	-3.95
559	250	5	Atorvastatin_Q107	16	73	-3.45
559	250	5	Atorvastatin_Q108	16	73	-2.95
559	250	5	Atorvastatin_Q109	16	73	-2.45
559	250	5	Atorvastatin_Q110	16	73	-1.95
559	250	5	Atorvastatin_Q111	16	73	-1.45
559	250	5	Atorvastatin_Q112	16	73	-0.95
377	234	5	Enalapril_Q101	26	29	-17.05

377	234	5	Enalapril_Q102	26	29	-16.55
377	234	5	Enalapril_Q103	26	29	-16.05
377	234	5	Enalapril_Q104	26	29	-15.55
377	234	5	Enalapril_Q105	26	29	-15.05
377	234	5	Enalapril_Q106	26	29	-14.55
377	234	5	Enalapril_Q107	26	29	-14.05
377	234	5	Enalapril_Q108	26	29	-13.55
377	234	5	Enalapril_Q109	26	29	-13.05
377	234	5	Enalapril_Q110	26	29	-12.55
377	234	5	Enalapril_Q111	26	29	-12.05
377	234	5	Enalapril_Q112	26	29	-11.55
429	207	5	Irbesartan_A_Q101	16	35	-20.08
429	207	5	Irbesartan_A_Q102	16	35	-19.58
429	207	5	Irbesartan_A_Q103	16	35	-19.08
429	207	5	Irbesartan_A_Q104	16	35	-18.58
429	207	5	Irbesartan_A_Q105	16	35	-18.08
429	207	5	Irbesartan_A_Q106	16	35	-17.58
429	207	5	Irbesartan_A_Q107	16	35	-17.08
429	207	5	Irbesartan_A_Q108	16	35	-16.58
429	207	5	Irbesartan_A_Q109	16	35	-16.08
429	207	5	Irbesartan_A_Q110	16	35	-15.58
429	207	5	Irbesartan_A_Q111	16	35	-15.08
429	207	5	Irbesartan_A_Q112	16	35	-14.58
429	207	5	Irbesartan_B_Q101	16	35	-14.75
429	207	5	Irbesartan_B_Q102	16	35	-14.25
429	207	5	Irbesartan_B_Q103	16	35	-13.75
429	207	5	Irbesartan_B_Q104	16	35	-13.25
429	207	5	Irbesartan_B_Q105	16	35	-12.75
429	207	5	Irbesartan_B_Q106	16	35	-12.25
429	207	5	Irbesartan_B_Q107	16	35	-11.75
429	207	5	Irbesartan_B_Q108	16	35	-11.25
429	207	5	Irbesartan_B_Q109	16	35	-10.75
429	207	5	Irbesartan_B_Q110	16	35	-10.25
429	207	5	Irbesartan_B_Q111	16	35	-9.75
429	207	5	Irbesartan_B_Q112	16	35	-9.25
406	84	5	Lisinopril_A_Q101	15	68	-22.75
406	84	5	Lisinopril_A_Q102	15	68	-22.25
406	84	5	Lisinopril_A_Q103	15	68	-21.75
406	84	5	Lisinopril_A_Q104	15	68	-21.25

406	84	5	Lisinopril_A_Q105	15	68	-20.75
406	84	5	Lisinopril_A_Q106	15	68	-20.25
406	84	5	Lisinopril_A_Q107	15	68	-19.75
406	84	5	Lisinopril_A_Q108	15	68	-19.25
406	84	5	Lisinopril_A_Q109	15	68	-18.75
406	84	5	Lisinopril_A_Q110	15	68	-18.25
406	84	5	Lisinopril_A_Q111	15	68	-17.75
406	84	5	Lisinopril_A_Q112	15	68	-17.25
406	84	5	Lisinopril_B_Q101	15	68	-19.45
406	84	5	Lisinopril_B_Q102	15	68	-18.95
406	84	5	Lisinopril_B_Q103	15	68	-18.45
406	84	5	Lisinopril_B_Q104	15	68	-17.95
406	84	5	Lisinopril_B_Q105	15	68	-17.45
406	84	5	Lisinopril_B_Q106	15	68	-16.95
406	84	5	Lisinopril_B_Q107	15	68	-16.45
406	84	5	Lisinopril_B_Q108	15	68	-15.95
406	84	5	Lisinopril_B_Q109	15	68	-15.45
406	84	5	Lisinopril_B_Q110	15	68	-14.95
406	84	5	Lisinopril_B_Q111	15	68	-14.45
406	84	5	Lisinopril_B_Q112	15	68	-13.95
405	199	5	Lovastatin_Q101	13	32	-3.75
405	199	5	Lovastatin_Q102	13	32	-3.25
405	199	5	Lovastatin_Q103	13	32	-2.75
405	199	5	Lovastatin_Q104	13	32	-2.25
405	199	5	Lovastatin_Q105	13	32	-1.75
405	199	5	Lovastatin_Q106	13	32	-1.25
405	199	5	Lovastatin_Q107	13	32	-0.75
405	199	5	Lovastatin_Q108	13	32	-0.25
405	199	5	Lovastatin_Q109	13	32	0.25
405	199	5	Lovastatin_Q110	13	32	0.75
405	199	5	Lovastatin_Q111	13	32	1.25
405	199	5	Lovastatin_Q112	13	32	1.75
268	159	5	Metoprolol_Q101	20	32	-24.25
268	159	5	Metoprolol_Q102	20	32	-23.75
268	159	5	Metoprolol_Q103	20	32	-23.25
268	159	5	Metoprolol_Q104	20	32	-22.75
268	159	5	Metoprolol_Q105	20	32	-22.25
268	159	5	Metoprolol_Q106	20	32	-21.75
268	159	5	Metoprolol_Q107	20	32	-21.25

268	159	5	Metoprolol_Q108	20	32	-20.75
268	159	5	Metoprolol_Q109	20	32	-20.25
268	159	5	Metoprolol_Q110	20	32	-19.75
268	159	5	Metoprolol_Q111	20	32	-19.25
268	159	5	Metoprolol_Q112	20	32	-18.75
447	207	5	Olmesartan_Q101	20	40	-20.65
447	207	5	Olmesartan_Q102	20	40	-20.15
447	207	5	Olmesartan_Q103	20	40	-19.65
447	207	5	Olmesartan_Q104	20	40	-19.15
447	207	5	Olmesartan_Q105	20	40	-18.65
447	207	5	Olmesartan_Q106	20	40	-18.15
447	207	5	Olmesartan_Q107	20	40	-17.65
447	207	5	Olmesartan_Q108	20	40	-17.15
447	207	5	Olmesartan_Q109	20	40	-16.65
447	207	5	Olmesartan_Q110	20	40	-16.15
447	207	5	Olmesartan_Q111	20	40	-15.65
447	207	5	Olmesartan_Q112	20	40	-15.15
384	200	5	Pantoprazole_Q101	24	16	-16.7
384	200	5	Pantoprazole_Q102	24	16	-16.2
384	200	5	Pantoprazole_Q103	24	16	-15.7
384	200	5	Pantoprazole_Q104	24	16	-15.2
384	200	5	Pantoprazole_Q105	24	16	-14.7
384	200	5	Pantoprazole_Q106	24	16	-14.2
384	200	5	Pantoprazole_Q107	24	16	-13.7
384	200	5	Pantoprazole_Q108	24	16	-13.2
384	200	5	Pantoprazole_Q109	24	16	-12.7
384	200	5	Pantoprazole_Q110	24	16	-12.2
384	200	5	Pantoprazole_Q111	24	16	-11.7
384	200	5	Pantoprazole_Q112	24	16	-11.2
369	172	5	Perindopril_Q101	16	33	-21.15
369	172	5	Perindopril_Q102	16	33	-20.65
369	172	5	Perindopril_Q103	16	33	-20.15
369	172	5	Perindopril_Q104	16	33	-19.65
369	172	5	Perindopril_Q105	16	33	-19.15
369	172	5	Perindopril_Q106	16	33	-18.65
369	172	5	Perindopril_Q107	16	33	-18.15
369	172	5	Perindopril_Q108	16	33	-17.65
369	172	5	Perindopril_Q109	16	33	-17.15
369	172	5	Perindopril_Q110	16	33	-16.65

369	172	5	Perindopril_Q111	16	33	-16.15
369	172	5	Perindopril_Q112	16	33	-15.65
417	234	5	Ramipril_Q101	12	30	-14.55
417	234	5	Ramipril_Q102	12	30	-14.05
417	234	5	Ramipril_Q103	12	30	-13.55
417	234	5	Ramipril_Q104	12	30	-13.05
417	234	5	Ramipril_Q105	12	30	-12.55
417	234	5	Ramipril_Q106	12	30	-12.05
417	234	5	Ramipril_Q107	12	30	-11.55
417	234	5	Ramipril_Q108	12	30	-11.05
417	234	5	Ramipril_Q109	12	30	-10.55
417	234	5	Ramipril_Q110	12	30	-10.05
417	234	5	Ramipril_Q111	12	30	-9.55
417	234	5	Ramipril_Q112	12	30	-9.05
419	199	5	Simvastatin_Q101	24	40	-3.55
419	199	5	Simvastatin_Q102	24	40	-3.05
419	199	5	Simvastatin_Q103	24	40	-2.55
419	199	5	Simvastatin_Q104	24	40	-2.05
419	199	5	Simvastatin_Q105	24	40	-1.55
419	199	5	Simvastatin_Q106	24	40	-1.05
419	199	5	Simvastatin_Q107	24	40	-0.55
419	199	5	Simvastatin_Q108	24	40	-0.05
419	199	5	Simvastatin_Q109	24	40	0.45
419	199	5	Simvastatin_Q110	24	40	0.95
419	199	5	Simvastatin_Q111	24	40	1.45
419	199	5	Simvastatin_Q112	24	40	1.95

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