The Pennsylvania State University

The Graduate School

Department of Chemistry

ENHANCING ANALYTICAL METHODS TOWARDS THE GEOCHEMICAL AND BIOGEOCHEMICAL FINGERPRINTING OF SHALE GAS SYSTEMS

A Dissertation in

Chemistry and Biogeochemistry

by

Paulina K. Piotrowski

© 2018 Paulina K. Piotrowski

Submitted in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

December 2018

The dissertation of Paulina K. Piotrowski was reviewed and approved* by the following:

Frank Dorman Associate Professor of Biochemistry and Molecular Biology Dissertation Co-Advisor Co-Chair of Committee

Thomas Mallouk Evan Pugh University Professor of Chemistry, Biochemistry and Molecular Biology, Physics, and Engineering Science and Mechanics Head of the Department of Chemistry Dissertation Co-Adviser Co-Chair of Committee

Philip Bevilacqua Professor of Chemistry and Biochemistry and Molecular Biology

Squire Booker Professor of Chemistry and Biochemistry and Molecular Biology

William Burgos Professor of Civil & Environmental Engineering

*Signatures are on file in the Graduate School

ABSTRACT

Hydraulic fracturing is a frequently utilized technique for the extraction of natural gas entrapped in shale formations. However, many have raised concerns about the environmental impacts of this process. Little is known about the organic chemistry of the flowback or produced water resulting from the hydraulic fracturing process. A better understanding of flowback and produced water chemistry and shale chemistry may facilitate environmental fingerprinting and help determine the potential impacts of contamination events from shale gas operations.

Utilizing comprehensive two-dimensional gas chromatography (GCxGC) coupled to time-of-flight mass spectrometry (TOFMS) has allowed for a better understanding of hydrocarbon origins in flowback and produced waters. Through the investigation of a series of five injection fluids and five flowback samples from a single unconventional gas well in northeastern PA by GCxGC-TOFMS, chemometric analysis by mass spectral scripting algorithms revealed that specific hydrocarbon composition remains constant during the flowback period and contains very little signature of injected fluids. Additionally, GCxGC with high-resolution timeof-flight mass spectrometry followed by Kendrick mass defect analysis of flowback from four nearby wells indicated a unique hydrocarbon pattern in each case. It was hypothesized that the hydrocarbon chemistry of the shale contributes to hydrocarbon signature of the flowback.

To further explore the hydrocarbon signature hypothesis, thermal desorption methodologies were developed as a sample introduction tool for GCxGC-TOFMS analysis of shale rock samples. The method was evaluated against Soxhlet extraction and shown to be reproducible and efficient for the hydrocarbon analysis of shale rock cores. This novel and robust characterization of Marcellus and Utica shales demonstrated the hydrocarbon differences between the two formations and improved the understanding of hydrocarbon speciation within the native rock. Additionally, thermal desorption coupled to GCxGC may provide more detailed analysis of hydrocarbons than what is currently implemented in the industry to pinpoint the most advantageous areas to exploit by hydraulic fracturing.

Lastly, biogeochemical fingerprinting of environmental contamination form shale gas operations was also explored through bioaccumulation studies in the freshwater mussel, *Elliptio complanata*. Mussels were exposed to hydraulic fracturing wastewaters and their fatty tissue was analyzed by for both organic and inorganic components by GCxGC-TOFMS and ICP-MS, respectively, that could be indicators of bioaccumulation of the produced water. Increased accumulation of strontium was observed in mussels dosed with produced water. Chemometric strategies were employed to distinguish the signatures of produced water exposure. Principle Component Analysis was used as an exploratory data analysis method and demonstrated the uptake of cyclic hydrocarbons in the fatty tissues of mussels. This study demonstrated the potential of biogeochemical fingerprinting and impact of possible environmental contamination from shale gas operations.

TABLE OF CONTENTS

LIST OF FIGURES	vii
LIST OF TABLES	X
ACKNOWLEDGEMENTS	xi
Chapter 1 Introduction	1
Hydraulic Fracturing	2
Chemical Characterization of HF Wastewaters	5
Instrumentation Overview	8
Comprehensive Two-Dimesional Gas Chromatography	8
Time-of-flight mass spectrometry	10
Experimental Reports	12
Chapter 2 Non-Targeted Chemical Characterization of a Marcellus Shale Gas Well Through GC×GC with Scripting Algorithms and High-Resolution Time-of-Flight Mass Spectrometry	18
Abstract	18
Introduction	19
Materials and Methods	21
Sample Collection	21
Sample Preparation	22
Sample Analysis	23
OA/OC	23
Data Analysis	24
Results and Discussion	25
References	35
Chapter 3 Elucidating Environmental Fingerprinting Mechanisms of Unconventional	
Gas Development Through Hydrocarbon Analysis	
Abstract	
Introduction	
Materials and Methods	41
Sample Collection	41
Sample Preparation	42
Sample Analysis	
QA/QC	43
GCxGC Data Analysis	44
Temporal Distribution Plots	45
Results and Discussion	
Hydrocarbon Homogeneity	45
Hydrocarbon Heterogeneity	46

Temporal Distribution	52
Geochemical Significance	55
Conclusions	57
References	58
Chapter 4 Applications of Thermal Desorption coupled to $GC \times GC$ -TOFMS for	
Hydrocarbon Fingerprinting of Hydraulically Fractured Shale Rocks	61
Abstract	61
Introduction	62
Materials and Methods	64
Thermal Desorption of Shale Cores	64
GCxGC-TOFMS Analysis	65
Soxhlet Extractions	65
Data Analysis	66
Results and Discussion	67
Comparison to Soxhlet Extraction	67
Method Repeatability	70
Method Efficiency	71
Comparison of Hydraulically Fractured Formations	72
Conclusions	75
References	76
Chapter 5 Determination of Bioaccumulation Potential of Hydraulic Fracturing Produced	l
Water in the Freshwater Mussel, Ellipto complanata	79
Abstract	79
Introduction	80
Materials and Methods	81
Animal Care	82
Bioaccumulation Studies	82
Chemical Characterization	84
Data Analysis	84
Results and Discussion	67
References	92
Chapter 6 Summary and Outlook	95
Appendix	99

LIST OF FIGURES

Figure 1-1: Historical energy production and future projections indicating that natural gas production will remain the fastest growing and largest energy source in the US. Adapted from U.S. Energy Information Administration, <i>Annual Energy Outlook</i>	
2018	.2
Figure 1-2: A schematic of the hydraulic fracturing process and pathways of water contamination. Adapted from Howarth et al	.4
Figure 1-3: Estimated octanol-water equilibrium coefficients (Kow) of 964 disclosed hydraulic fracturing additives with amenabilities to common analytical methodologies. Adapted from Getzinger et al	.7
Figure 1-4: The schematic of GC×GC modulation and generating two-dimensional chromatograms from raw modulated data. Adapted from Dallüge et al	.10
Figure 1-5: The schematic of a time-of-flight mass spectrometer, which determines mass- to- charge ratios of ions by measuring the time ions take to reach the detector. Adapted from Harris.	.12
Figure 2-1: A schematic of hydraulic fracturing stimulation stages. The hydraulic fracturing fluids analyzed herein originated from stimulation stages 7-10.	.22
Figure 2-2: The GC×GC total ion chromatograms of A) Stage 7 fracturing fluid with high abundance of hydrocarbons, B) Stage 8 fracturing fluid with low abundance of hydrocarbons, C) Stage 9 fracturing fluid with low abundance of hydrocarbons, and D) Stage 10 fracturing fluid with high abundance of hydrocarbons.	.22
Figure 2-3: The GC×GC total ion chromatograms of A) Day 4 flowback with low abundance of hydrocarbons, B) Day 6 flowback C) Day 8 flowback, and D) Day 10 flowback. Hydrocarbon classes were assigned through scripts that utilize class- specific fragmentation patterns. Red: n- alkanes, green: branched alkanes, purple: alkenes, white: hydronaphthalenes, salmon: cyclohexanes, orange: para substituted cyclohexanes, yellow: cyclopentanes, teal: 3-ring aromatics, blue: fluorenes. Bubble radii are scaled to the peak area. The hydrocarbon composition in Day 2 flowback (not shown) and Day 4 flowback are similar, but the composition shifts from Day 4 to Day 6, whereupon it remains approximately constant through Day 10.	.31

Figure 2-4: The GC×GC-HRT extracted ion chromatograms for A) the scale inhibitor ethylene glycol in hydraulic fracturing fluid (m/z 62.0362), B) ethylene glycol in flowback (m/z 62.0362), C) the biocide glutaraldehyde in hydraulic fracturing fluid (m/z 82.0413), D) the absence of glutaraldehyde in flowback (m/z 82.0413), E) the corrosion inhibitor cinnamaldehyde in hydraulic fracturing fluid (m/z 131.0491), and F) the absence of cinnamaldehyde in flowback (m/z 131.0491). Note, the secondary retention time was adjusted to correct for wrap-around
Figure 3-1 : The GC×GC-HRT total ion chromatograms for flowback fluids from four geographically nearby wells (samples A-D). The GC×GC separation shows sample-to-sample differentiation among the more polar components, such as cyclohexanes and hydronaphthalenes. Samples A, B and D contained hydrocarbon distributions centered between C9 and C20 <i>n</i> -alkanes, while sample C was overall more chemically complex containing longer <i>n</i> -alkanes (up to C30) and numerous aromatic compounds
Figure 3-2: The Kendrick mass defect plots generated from the total deconvoluted time- of-flight mass spectra operated at 25,000 resolving power for flowback fluids from four geographically nearby wells (samples A-D). Each well exhibits a unique hydrocarbon pattern especially with respect to heteroatomic containing ions
Figure 3-3: Temporal distribution plots for flowback fluids from four nearby wells (samples A- D). This visualization strategy combines the GC×GC separation Kendrick mass defect information to further distinguish the samples from one another. Bubble radii are scaled to ion abundance
Figure 3-4: Pie chart representation of mass spectral response assigned to hydrocarbon classes for wells A-D. Clear discrimination between the flowback fluids is observable in aromatic hydrocarbons (wells A and C) and saturated hydrocarbons (wells B and D)
Figure 4-1: A comparison of Soxhlet extraction to thermal desorption coupled to GC×GC- TOFMS. The same shale sample analyzed by thermal desorption (A) and Soxhlet extraction (B) produce nearly identical chromatograms
Figure 4-2: An assessment of repeatability for the thermal desorption coupled to GC×GC- TOFMS method. Multiple aliquots of the same shale sample produce comparable chromatograms
Figure 4-3: An assessment of efficiency for the thermal desorption coupled to GC×GC- TOFMS method. Same aliquot subjected to a second desorption shows an absence of shale components
Figure 4-4: An application for the thermal desorption coupled to GC×GC-TOFMS method. The total ion chromatogram for a core sample from the Utica Shale (A) contain a greater range of aromatic hydrocarbons as compared to a core sample of the Marcellus Shale (B) that contains mostly saturated hydrocarbons73

Figure 4-5: Pie chart representation of compositional differences between the Utica Shale (A) and Marcellus Shale (B) as determined by chromatographic peak areas	.73
Figure 5-1: The Kaplan–Meier survival curves for mussels exposed to produced water from hydraulic fracturing operations (high-dose 1:50 dilution, low-dose 1:100 dilution). Note: n = 8 for control and low-dose groups, n=9 for high-dose group	.86
Figure 5-2: The metal uptake in fatty tissue of mussels exposed to produced water from hydraulic fracturing operations. All values are presented as molar calcium ratios. Error bars represent standard deviation.	.87
Figure 5-3: The GCxGC-TOFMS total ion chromatograms of fatty tissue of mussels exposed to produced water from hydraulic fracturing operations. A) control group, B) low-dose, C) high- dose. The mussels exposed to produced water show an increase in cyclic hydrocarbons as highlighted in the white oval .	.89
Figure 5-4: The GCxGC-TOFMS total ion chromatograms of the produced water utilized for dosing. The produced water is dominated by saturated hydrocarbons, including n-alkanes, branched alkanes, and cyclic saturated hydrocarbons.	.90
Figure 5-5: The principle component analysis score plot of GCxGC-TOFMS peak lists from fatty tissue of mussels exposed to produced water from hydraulic fracturing operations. Analytes were subjected to Kruskal-Wallis ANOVA and p<0.005 was required for PCA modeling, shading represents the 95% confidence interval	.91

LIST OF TABLES

Table 2-1: The percent chemical class compositions for the GC amenable fraction of hydraulic fracturing fluids	27
Table 2-2: Ratios of the hydrocarbon classes in injected fracturing fluids and flowback samples	33
Table 3-1: Gas Productivity Data .	56
Table 4-1: Quantitative comparison of Soxhlet Extraction and Thermal Desorption of Marcellus Shale core	69
Table 4-2: Sulfur containing compounds identified in shale core samples by thermal desorption followed by GC×GC-TOFMS analysis	75

ACKNOWLEDGEMENTS

I have been fortunate to be inspired and work along side so many amazing people throughout my academic journey. First I would like to pay gratitude to my undergraduate mentors, Dr. Joanne Kehlbeck and Dr. Michael Hagerman, who have provided me with endless encouragement to pursue my degrees in chemistry. Your friendship and wisdom has been greatly appreciated.

I would also like to thank Dr. Frank Dorman, for allowing me to pursue my Ph.D. in your laboratory. Thank you for taking a risk on me and my ideas and giving me the freedom to explore my interests. Thank you for all of the opportunities to build my professional network though your endless connections; your guidance had a profound impact on my academic experience. Thank you for all of your patience when my problems really were "the nut behind the injector" and when I broke innumerable pieces of glassware and defaced brand new instruments. I couldn't have asked for a better mentor.

I would like to thank the members of my thesis committees, Dr. Thomas Mallouk, Dr. Squire Booker, Dr. Phillip Bevilacqua, and Dr. William Burgos, who contributed their time and scientific expertise to help guide my progress through graduate school. I'm especially grateful to the Biogeochemistry dual-title program at Penn State University for funding my thesis research.

I would also like to thank all of my research collaborators who, not only allowed my research to be more meaningful, but also helped me gain breadth as an analytical chemist. Specifically, I would like to acknowledge Erica Barth-Naftilan and Dr. James Saiers at Yale University for allowing me to be involved in their groundwater study project that ultimately lead to two of my own first author publications. Most importantly I'd like to thank the incredible team of scientists working on shale research at Penn State University with who I had the pleasure working with: Dr. William Burgos, Dr. Nathaniel Warner, and Dr. David Yoxtheimer. I would

especially like to acknowledge Travis Tasker for being the best research partner I could have asked for; thank you for all of the discussions, manuscript and proposal edits, and fun car rides to collect samples.

I would also like to acknowledge my colleagues in the separation science community. To the amazing team of scientist at LECO Corporation, thank you so much for your guidance and instrumental support. I am greatly appreciative of my experiences to work with prototype instruments and software. I look forward to being your lifetime collaborator! I would also like to thank my colleagues in the Dorman Lab for being an incredible support system throughout my time in graduate school.

Most importantly, I would like to thank my family for their endless love and support and the opportunities they have provided me to pursue my dreams. To my parents and sister, thank you for all of your sacrifices and everything you have done for me to let me get to place I am today; words cannot express my gratitude to you. To my husband, Jared, thank you so much for being by my side throughout my journey of becoming a chemist. Thank you for always pushing me to be better and for being the best support system, even if you were the worst lab partner I've ever had.

Chapter 1

Introduction

While global energy demands are continually rising, environmental concerns bolster the need to move from coal to cleaner burning fuels. While ideally renewable energy would replace fossil fuels, the technology is not yet amenable to wide scale integration. In the last two decadenatural gas has emerged as a viable alternative to coal and recent technological advances have allowed for the economic extraction of this resource. Since 2010, natural gas has accounted for the largest share of US energy production (Figure 1-1) and projections over the next four decades indicate that natural gas production will continue to increase.¹



Figure 1-1: Historical energy production and future projections indicating that natural gas production will remain the fastest growing and largest energy source in the US. Adapted from U.S. Energy Information Administration, *Annual Energy Outlook 2018*.¹

1.1 Hydraulic Fracturing

Advances in horizontal well drilling and hydraulic fracturing (HF) technologies have allowed for economic extraction of shale gas recourses. Shale gas is entrapped in low permeability pores within the sedimentary rock, thus not amenable to conventional gas extraction practices. Unconventional (horizontal) drilling and hydraulic fracturing are designed to increase the surface area of the shale rock and allow for greater gas permeability. Hydraulic fracturing utilizes between 10,000 liters and 37 million liters of water per well, along with drilling modifiers, to generate pressures capable of fracturing the shale. Additionally, fracturing fluids contain up to 9% sand, which is used to maintain the fracture network. Chemical modifiers, which can make up 2% of the fracturing fluids, include acids, biocides, friction reducers, corrosion inhibitors, gelling agents and subsequent breakers.² There are over 1600 known organic modifiers that are used during the hydraulic fracturing process, however in general only 3-12 chemicals are used per well.^{2.3}

The process of unconventional gas development has raised a multitude of environmental concerns, especially the impact to water resources. As outlined in Figure 1-2, contamination can occur from the migration of stray gas (largely methane) or fluids associated with the unconventional gas development process.⁴ While methane may pose risk to the population and infrastructure due to flammability concerns, the less volatile compounds are likely a greater longterm threat to the environment and human health. Improperly sealed well casings, surface spills, and centralized treatment facilities pose the greatest risk to water resources.⁵⁻¹⁵ Spills from unconventional oil and gas development have remained constant at 4-6% from 2006-2014 and typically occur due to equipment failures or human error.¹⁶ In Pennsylvania, an average spill releases 500 L of brine to the environment.¹⁶ However, fingerprinting contamination from unconventional oil and gas development is not straightforward as there is a lack of analytical methodologies that provide evidence of causation for environmental contamination events associated with shale gas development.¹⁷ Therefore, to adequately assess contamination events from shale gas development, basic research is needed to develop analytical methods capable of fully characterizing the fluids associated with unconventional gas development, and also potentially correlating a specific fingerprint to a point of source or sources.



Figure 1-2: A schematic of the hydraulic fracturing process and pathways of water contamination. Adapted from Howarth et al.⁴

1.2 Chemical Characterization of HF Wastewaters

The last decade has seen a large increase in peer-reviewed research on the characterization of hydraulic fracturing wastewaters. Overall, characterizations can be divided into three categories: analysis of inorganic species,¹⁸⁻³² analysis of hydraulic fracturing additives,^{5,26,33-43} and analysis of geogenic organic compounds.^{5,6,7,44-49} Of these three categories inorganic analysis has received the greatest attention, which is likely due to the high quantities of dissolved solids, often metal salts, in HF wastewaters.¹⁸⁻³² The high salinity of HF wastewaters can vastly vary between and within shale basins but typically ranges between 200,000 – 400,000 ppm.³¹ The fluids returning to the surface also contain high radioactivity (gross alpha 55–123,000 pCi/L), which is of particular interest due to health concerns.^{19,24,25,29,31,32,33} The naturally occurring radioactive material (NORM) has also been shown to be a particularly stable indicator of shale formations for fingerprinting purposes, as have the stable isotopes of strontium and boron.²⁹⁻³¹

The characterization of organic compounds in HF wastewaters is a continuously developing field of study. The high salinity matrix along with the proprietary nature of hydraulic fracturing additives presents challenges in sample preparation and analysis.^{3,46} HF wastewaters have total organic carbon values ranging from 1.2 to 5,804 mg/L with average concentrations of 346 mg/L.³³ Since the organic fraction is much more dilute than the inorganic species, sample preparation steps must be undertaken to exclude the more concentrated interferents.^{3,43-50} In LC-MS analysis this high salinity matrix would result in suppression of ionization, thus hindering the detection of analytes present in the sample.³ Traditional analytical methodologies that rely on the detection and quantification of known targeted analytes are insufficient, as little is known about the proprietary and unreported compounds used in HF operations.

Non-target analysis is also merited for the characterization of HF wastewaters as recent

research suggests that sorption and molecular transformations occur in subsurface conditions. Sorption of organic compounds to exposed rock formations could affect transport in returning wastewaters and could be dependent on the organic carbon-water partitioning coefficient (K_{ow}).³³ Hydraulic fracturing additives with high Kow values will be expected to sorb to organic-rich shales and be retained by the formation (Figure 1-3).^{15,33} To date, several publications have described laboratory experiments documenting the transformations of common HF additives.^{33,39,40,44,51,52} Multiadditive synthetic hydraulic fracturing fluids reacted under high temperature and pressure conditions (HPT) suggested that HPT along with shale mineralogy might facilitate the degradation of HF additives.³³ Additionally, glutaraldehyde, a common hydraulic fracturing biocide, was found to transform with increasing temperature and pressure into polymerized products that were ineffective in controlling sulfate-reducing bacterial growth.³⁹ Nonylphenol ethoxylates, a class of surfactants and corrosion inhibitors in oilfield operations, has been shown to degrade under HPT to nonylphenol, a known endocrine disrupting compound, which carries toxicity implications in the HF wastewaters.⁴⁰ A few studies have also shown evidence of subsurface halogenations in field samples through oxidative treatments, radical substitution, nucleophilic substitution, or electrophilic addition of non-halogenated aliphatic compound in the presence of halides and free halogen species.^{44,51,52}



Figure 1-3: Estimated octanol-water equilibrium coefficients (Kow) of 964 disclosed hydraulic fracturing additives with amenabilities to common analytical methodologies. Adapted from Getzinger et al.¹⁵

Chromatography coupled with mass spectrometry is the paramount analytical tool for the characterization of organic compounds in HF wastewaters. Recent reports on the analysis and chemistry of organic components of HF wastewaters are summarized by Oetjan et al.³ and Luek et al.⁵³ Overall, it can be concluded that the analysis of geogenic compounds, the hydrocarbons native to the shale rocks which are extracted during the HF process, are more suitable to gas chromatographic analysis.^{5,6,7,44-49} Alternatively, as seen in Figure 1-3, functionalization and the subsequent increase in K_{ow} of HF additives makes them more suitable to liquid chromatography analysis.¹⁵ Getzinger and colleagues report that 75% of known HF additives are amenable to LC-MS analysis while only 15% are amenable to GC-MS analysis.¹⁵ As such, most characterizations of HF wastewaters have been performed by LC-MS and ethoxylated surfactants are the most commonly detected class of compounds.^{5,26,33-43} While GC-MS analysis has been applied to a

number of studies aiming to characterize HF wastewaters, the complexity of the samples has resulted in limited identification of the hydrocarbons present in the fluids.^{7,46,47} The work presented in this dissertation is aimed to better characterize the geogenic hydrocarbons in shale gas systems.^{48,49,54} As such, comprehensive two-dimensional gas chromatography (GC×GC) coupled to time-of-flight mass spectrometry (TOFMS) was the primary analytical approach utilized herein. These techniques, outlined in the following text, helped overcome challenges associated with complex sample matrices.

1.3 Instrumentation Overview

1.3.1 Comprehensive Two-Dimensional Gas Chromatography

Gas chromatography (GC) is the paramount tool for the analysis of hydrocarbons, but to date the technique does not possess adequate peak capacity for a complete characterization of the naturally occurring hydrocarbons in shale.^{7,46,47} As a result, previous analyses of wastewaters from shale gas systems by GC have resulted in chromatograms with unresolved complex mixtures (UCMs).^{7,46,47} The presence of an UCM can greatly complicate analysis leading to the omission of constituents that may have important implications for fingerprinting or water reuse and treatment. Comprehensive two-dimensional gas chromatography (GC×GC) overcomes the peak capacity limitations described above and features higher detection sensitivity when used with cryogenic modulation than conventional GC analysis.⁵⁵

GC×GC utilizes two columns of different stationary phase connected in series to achieve enhanced selectivity and peak capacity.⁵⁵ Typically, the first dimension column is a conventional, long non-polar column and the second dimension is a short column with alternate selectivity or chemical functionalization. The peak capacity of the complete two-dimensional separation is the product of the peak capacities of each column.⁵⁵ Additionally, the complementary selectivities of the two columns often allow for the sample matrix to be separated from the analytes of interest and homologous series separate into diagonal patterns on the two dimensional plane. The two columns are connected at the modulator which serves to:

- i. Continuously collect small fractions of the effluent from the first dimension column while maintaining the previous separation,
- ii. Focus the effluent of the first dimension column into a small band,
- iii. Re-inject the focused band onto the second dimension column.⁵⁵

The three steps described above occur on the order of 1-10 seconds and are repeated continuously throughout the chromatographic analysis. Figure 1-4 outlines the modulation process and demonstrates the reconstruction of the data into two-dimensional chromatograms. Briefly, the chromatograms acquired over each 1-10 second modulation period are de-multiplexed and plotted individually. Subsequently, peaks that were split over multiple modulation periods are recombined and projected onto contour plots.⁵⁵



Figure 1-4: The schematic of GC×GC modulation and generating two-dimensional chromatograms from raw modulated data. Adapted from Dallüge et al.⁵⁵

1.3.2 Time-of-flight mass spectrometry

Time-of-flight mass spectrometry (TOFMS) is the most useful detector for GC×GC-MS analysis. In non-targeted analysis, the use of mass spectrometry greatly aids in identification of unknowns through evaluation of mass spectral fragmentation patterns. However, not all mass analyzers are can be adapted for GC×GC applications. The narrow peak widths (ca. 100-200 msec) generated by modulation in GC×GC require a high acquisition speed detector.⁵⁵ TOFMS is capable of acquiring a large mass range simultaneously without scanning as would be the case with quadruple mass analyzers, for example.⁵⁶

Time-of-flight mass spectrometry determines mass-to-charge ratios of ions by measuring the time ions take to reach the detector.⁵⁶ As demonstrated in Figure 1-5, ions are accelerated into an drift region under vacuum where no additional magnetic or electric fields are applied. The same kinetic energy is applied to each ion and therefore lighter ions will drift with greater velocity, reaching the detector faster than heavier ions. While ideally, the same kinetic energy is applied to all ions, the position of ions in relation to the accelerating backplate in the ion source influences the initial kinetic energy.⁵⁶ To overcome this limitation, an electrostatic mirror or reflectron is implemented to equalize the kinetic energies of ions. In a reflectron, ions are stopped and reflected through a series of increasing electric potentials. Ions with higher initial kinetic energies of ions with the same masses. Thereby, ions of the same mass reach the detector at the same time, regardless of differences in initial kinetic energies.⁵⁶ The use of reflectrons is also advantageous as it increases the resolving power of the mass spectrometer. By increasing the flight path, discrimination is possible between small mass differences, which enable the measurement of masses with mass accuracies of < 0.001 Da.⁵⁶



Figure 1-5: The schematic of a time-of-flight mass spectrometer, which determines mass-tocharge ratios of ions by measuring the time ions take to reach the detector. Adapted from Harris.⁵⁶

1.4 Experimental Reports

The following chapters demonstrate efforts in enhancing analytical methods towards the geochemical and biogeochemical fingerprinting of shale gas systems. Chapters 2 and 3 focus on the characterization of hydrocarbons in wastewaters from shale gas systems.^{48,49} Chapter 2, which is published in the journal *Fuel*, investigates the chemistry of a single well is studied to compare the chemical differences and transformations between injected fluids and those which return back to the surface as wastewater.⁴⁸ The hydrocarbons present in the wastewater are distinct from those injected during hydraulic fracturing and the distribution of returned hydrocarbons remains similar over the 10 days the well was monitored.⁴⁸ My contribution towards this work includes establishing relationships to acquire the samples, sample preparation and analysis, and writing the

manuscript. Chapter 3, which is published in the journal Analytical Chemistry, studies four wells in close geographic proximity to elucidate different chemical signatures between the four wells.⁴⁹ The consistent hydrocarbon signature of a single well⁴⁸ and the distinct hydrocarbon signature of nearly wells⁴⁹ shows promise in utilizing hydrocarbon distributions in fingerprinting of environmental contamination. My contribution towards this work includes establishing relationships to acquire the samples, sample preparation and analysis, and writing the manuscript. In Chapter 4, a robust analytical method to study the hydrocarbons native to the shale rocks is presented that utilizes thermal desorption coupled to GC×GC-TOFMS.⁵⁴ This methodology can provide insight into utilizing hydrocarbon differences towards point-source identification or determining the areas of a well to target by hydraulic fracturing. This manuscript has been submitted to Journal of Chromatography A and my contributions include thermal desorption method development, Soxhlet extraction, data analysis, and manuscript preparation. Lastly, Chapter 5 explores the biogeochemical fingerprinting of potential contamination from shale gas development through bioaccumulation studies of freshwater mussels dosed with wastewaters from shale gas operations in an effort to mimic possible waterway contamination.⁵⁷ This manuscript is in preparation and my contribution to this work include experimental design, mussel care, sample extraction and analysis for organic pollutants and statistical analysis.

1.5 References

Energy Information Administration. US Department of Energy. Annual Energy Outlook 2018. <u>https://www.eia.gov/outlooks/aeo/pdf/AEO2018.pdf</u>. (Accessed April 20, 2018)
 Frac Focus Chemical Disclosure Registry. <u>https://fracfocus.org</u>. (Accessed August 20, 2017)

(3) Oetjen, K.; Giddings, C. G. S.; McLaughlin, M.; Nell, M.; Blotevogel, J.; Helbling, D. E.; Mueller, D.; Higgins, C. P. Emerging Analytical Methods for the Characterization and Quantification of Organic Contaminants in Flowback and Produced Water. *Trends in Environmental Analytical Chemistry*. 2017, pp 12–23.

(4) Howarth, R. W.; Ingraffea, A. Natural Gas: Should Fracking Stop? *Nature*. 2011, pp 271–273.

(5) Llewellyn, G. T.; Dorman, F.; Westland, J. L.; Yoxtheimer, D.; Grieve, P.; Sowers, T.; Humston-Fulmer, E.; Brantley, S. L. Evaluating a Groundwater Supply Contamination Incident Attributed to Marcellus Shale Gas Development. *Proc. Natl. Acad. Sci.* **2015**, *112* (20), 6325–6330.

(6) Drollette, B. D.; Hoelzer, K.; Warner, N. R.; Darrah, T. H.; Karatum, O.; O'Connor, M. P.; Nelson, R. K.; Fernandez, L. A.; Reddy, C. M.; Vengosh, A.; et al. Elevated Levels of Diesel Range Organic Compounds in Groundwater near Marcellus Gas Operations Are Derived from Surface Activities. *Proc. Natl. Acad. Sci.* **2015**, *112* (43), 13184–13189.

(7) Cozzarelli, I. M.; Skalak, K. J.; Kent, D. B.; Engle, M. A.; Benthem, A.; Mumford, A. C.; Haase, K.; Farag, A.; Harper, D.; Nagel, S. C.; et al. Environmental Signatures and Effects of an Oil and Gas Wastewater Spill in the Williston Basin, North Dakota. *Sci. Total Environ.* **2017**, *579*, 1781–1793.

(8) Digiulio, D. C.; Jackson, R. B. Impact to Underground Sources of Drinking Water and Domestic Wells from Production Well Stimulation and Completion Practices in the Pavillion, Wyoming, Field. *Environ. Sci. Technol.* **2016**, *50* (8), 4524–4536.

(9) Gross, S. A.; Avens, H. J.; Banducci, A. M.; Sahmel, J.; Panko, J. M.; Tvermoes, B. E. Analysis of BTEX Groundwater Concentrations from Surface Spills Associated with Hydraulic Fracturing Operations. *J. Air Waste Manag. Assoc.* **2013**, *63* (4), 424–432.

(10) Hildenbrand, Z. L.; Carlton, D. D.; Fontenot, B. E.; Meik, J. M.; Walton, J. L.; Thacker, J. B.; Korlie, S.; Shelor, C. P.; Kadjo, A. F.; Clark, A.; et al. Temporal Variation in Groundwater Quality in the Permian Basin of Texas, a Region of Increasing Unconventional Oil and Gas Development. *Sci. Total Environ.* **2016**, *562*, 906–913.

(11) Hildenbrand, Z. L.; Mach, P. M.; McBride, E. M.; Dorreyatim, M. N.; Taylor, J. T.; Carlton, D. D.; Meik, J. M.; Fontenot, B. E.; Wright, K. C.; Schug, K. A.; et al. Point Source Attribution of Ambient Contamination Events near Unconventional Oil and Gas Development. Sci. Total Environ. 2016, 573, 382–388.

(12) Kassotis, C. D.; Iwanowicz, L. R.; Akob, D. M.; Cozzarelli, I. M.; Mumford, A. C.; Orem, W. H.; Nagel, S. C. Endocrine Disrupting Activities of Surface Water Associated with a West Virginia Oil and Gas Industry Wastewater Disposal Site. *Sci. Total Environ.* **2016**, *557– 558*, 901–910.

(13) Orem, W.; Varonka, M.; Crosby, L.; Haase, K.; Loftin, K.; Hladik, M.; Akob, D. M.; Tatu, C.; Mumford, A.; Jaeschke, J.; et al. Organic Geochemistry and Toxicology of a Stream Impacted by Unconventional Oil and Gas Wastewater Disposal Operations. *Appl. Geochemistry* **2017**, *80*, 155–167.

(14) Burgos, W. D.; Castillo-Meza, L.; Tasker, T. L.; Geeza, T. J.; Drohan, P. J.; Liu, X.; Landis, J. D.; Blotevogel, J.; McLaughlin, M.; Borch, T.; et al. Watershed-Scale Impacts from Surface Water Disposal of Oil and Gas Wastewater in Western Pennsylvania. *Environ. Sci. Technol.* **2017**, *51* (15), 8851–8860.

(15) Getzinger, G. J.; O'Connor, M. P.; Hoelzer, K.; Drollette, B. D.; Karatum, O.; Deshusses, M. A.; Ferguson, P. L.; Elsner, M.; Plata, D. L. Natural Gas Residual Fluids: Sources, Endpoints, and Organic Chemical Composition after Centralized Waste Treatment in Pennsylvania. *Environ. Sci. Technol.* **2015**, *49* (14), 8347–8355.

(16) Patterson, L. A.; Konschnik, K. E.; Wiseman, H.; Fargione, J.; Maloney, K. O.; Kiesecker, J.; Nicot, J. P.; Baruch-Mordo, S.; Entrekin, S.; Trainor, A.; et al. Unconventional Oil and Gas Spills: Risks, Mitigation Priorities, and State Reporting Requirements. *Environ. Sci. Technol.* **2017**, *51* (5), 2563–2573.

(17) King, J. C.; Bryan, J. L.; Clark, M. Factual Causation: The Missing Link in Hydraulic Fracture--Groundwater Contamination Litigation. *Duke Environ. Law Policy Forum* **2012**, *22* (2), 341.

(18) Shih, J.-S.; Saiers, J. E.; Anisfeld, S. C.; Chu, Z.; Muehlenbachs, L. A.; Olmstead, S. M. Characterization and Analysis of Liquid Waste from Marcellus Shale Gas Development. *Environ. Sci. Technol.* **2015**, *49* (16), 9557–9565.

(19) Rosenblum, J.; Nelson, A. W.; Ruyle, B.; Schultz, M. K.; Ryan, J. N.; Linden, K. G. Temporal Characterization of Flowback and Produced Water Quality from a Hydraulically Fractured Oil and Gas Well. *Sci. Total Environ.* **2017**, *596–597*, 369–377.

(20) Barbot, E.; Vidic, N. S.; Gregory, K. B.; Vidic, R. D. Spatial and Temporal Correlation of Water Quality Parameters of Produced Waters from Devonian-Age Shale Following Hydraulic Fracturing. *Environ. Sci. Technol.* **2013**, *47* (6), 2562–2569.

(21) Akob, D. M.; Mumford, A. C.; Orem, W.; Engle, M. A.; Klinges, J. G.; Kent, D. B.; Cozzarelli, I. M. Wastewater Disposal from Unconventional Oil and Gas Development Degrades Stream Quality at a West Virginia Injection Facility. *Environ. Sci. Technol.* **2016**, *50* (11), 5517–5525.

(22) Alley, B.; Beebe, A.; Rodgers, J.; Castle, J. W. Chemical and Physical Characterization of Produced Waters from Conventional and Unconventional Fossil Fuel Resources. *Chemosphere* **2011**, *85* (1), 74–82.

(23) Zhang, T.; Gregory, K.; Hammack, R. W.; Vidic, R. D. Co-Precipitation of Radium with Barium and Strontium Sulfate and Its Impact on the Fate of Radium during Treatment of Produced Water from Unconventional Gas Extraction. *Environ. Sci. Technol.* **2014**, *48* (8), 4596–4603.

(24) Nelson, A. W.; Eitrheim, E. S.; Knight, A. W.; May, D.; Mehrhoff, M. A.; Shannon, R.; Litman, R.; Burnett, W. C.; Forbes, T. Z.; Schultz, M. K. Understanding the Radioactive Ingrowth and Decay of Naturally Occurring Radioactive Materials in the Environment: An Analysis of Produced Fluids from the Marcellus Shale. *Environ. Health Perspect.* **2015**, *123* (7), 689–696.

(25) Nelson, A. W.; Knight, A. W.; Eitrheim, E. S.; Schultz, M. K. Monitoring Radionuclides in Subsurface Drinking Water Sources near Unconventional Drilling Operations: A Pilot Study. *J. Environ. Radioact.* **2015**, *142*, 24–28.

(26) Lester, Y.; Ferrer, I.; Thurman, E. M.; Sitterley, K. A.; Korak, J. A.; Aiken, G.; Linden, K. G. Characterization of Hydraulic Fracturing Flowback Water in Colorado: Implications for Water Treatment. *Sci. Total Environ.* **2015**, *512–513*, 637–644.

(27) Metcalf, E. *Wastewater Engineering: Treatment and Resource Recovery*. McGraw-Hill, 2014.

(28) Kim, S.; Omur-Ozbek, P.; Dhanasekar, A.; Prior, A.; Carlson, K. Temporal Analysis of Flowback and Produced Water Composition from Shale Oil and Gas Operations: Impact of Frac Fluid Characteristics. *J. Pet. Sci. Eng.* **2016**, *147*, 202–210.

(29) Warner, N. R.; Darrah, T. H.; Jackson, R. B.; Millot, R.; Kloppmann, W.; Vengosh, A. New Tracers Identify Hydraulic Fracturing Fluids and Accidental Releases from Oil and Gas Operations. *Environ. Sci. Technol.* **2014**, *48* (21), 12552–12560.

(30) Osborn, S. G.; McIntosh, J. C.; Hanor, J. S.; Biddulph, D. Iodine-129, 87Sr/86Sr, and Trace Elemental Geochemistry of Northern Appalachian Basin Brines: Evidence for Basinal-Scale Fluid Migration and Clay Mineral Diagenesis. *Am. J. Sci.* **2012**, *312* (3), 263–287.

(31) Haluszczak, L. O.; Rose, A. W.; Kump, L. R. Geochemical Evaluation of Flowback
Brine from Marcellus Gas Wells in Pennsylvania, USA. *Appl. Geochemistry* 2013, *28*, 55–61.
(32) Stewart, B. W.; Chapman, E. C.; Capo, R. C.; Johnson, J. D.; Graney, J. R.; Kirby, C. S.;

Schroeder, K. T. Origin of Brines, Salts and Carbonate from Shales of the Marcellus Formation:

Evidence from Geochemical and Sr Isotope Study of Sequentially Extracted Fluids. *Appl. Geochemistry* **2015**, *60*, 78–88.

(33) Tasker, T. L.; Piotrowski, P. K.; Dorman, F. L.; Burgos, W. D. Metal Associations in Marcellus Shale and Fate of Synthetic Hydraulic Fracturing Fluids Reacted at High Pressure and Temperature. *Environ. Eng. Sci.* **2016**, *33* (10), 753–765.

(34) Oetjen, K.; Chan, K. E.; Gulmark, K.; Christensen, J. H.; Blotevogel, J.; Borch, T.; Spear, J. R.; Cath, T. Y.; Higgins, C. P. Temporal Characterization and Statistical Analysis of Flowback and Produced Waters and Their Potential for Reuse. *Sci. Total Environ.* 2018, *619–620*, 654–664.
(35) He, Y.; Flynn, S. L.; Folkerts, E. J.; Zhang, Y.; Ruan, D.; Alessi, D. S.; Martin, J. W.; Goss, G. G. Chemical and Toxicological Characterizations of Hydraulic Fracturing Flowback and

Produced Water. Water Res. 2017, 114, 78-87.

(36) Ferrer, I.; Thurman, E. M. Chemical Constituents and Analytical Approaches for Hydraulic Fracturing Waters. *Trends in Environmental Analytical Chemistry*. 2015, pp 18–25.
(37) Ferrer, I.; Thurman, E. M. Analysis of Hydraulic Fracturing Additives by LC/Q-TOF-MS. *Anal. Bioanal. Chem.* 2015, 407 (21), 6417–6428.

(38) Kahrilas, G. A.; Blotevogel, J.; Stewart, P. S.; Borch, T. Biocides in Hydraulic Fracturing Fluids: A Critical Review of Their Usage, Mobility, Degradation, and Toxicity. *Environmental*

Science and Technology. 2015, pp 16–32.

(39) Kahrilas, G. A.; Blotevogel, J.; Corrin, E. R.; Borch, T. Downhole Transformation of the Hydraulic Fracturing Fluid Biocide Glutaraldehyde: Implications for Flowback and Produced Water Quality. *Environ. Sci. Technol.* **2016**, *50* (20), 11414–11423.

(40) Kahrilas, G.A. The downhole behavior of the chemicals of hydraulic fracturing–An insight to the nature of biocides and surfactants underground. Colorado State University. **2016.**

(41) Thurman, E. M.; Ferrer, I.; Blotevogel, J.; Borch, T. Analysis of Hydraulic Fracturing Flowback and Produced Waters Using Accurate Mass: Identification of Ethoxylated Surfactants. *Anal. Chem.* **2014**, *86* (19), 9653–9661.

(42) Thurman, E. M.; Ferrer, I.; Rosenblum, J.; Linden, K.; Ryan, J. N. Identification of Polypropylene Glycols and Polyethylene Glycol Carboxylates in Flowback and Produced Water from Hydraulic Fracturing. *J. Hazard. Mater.* **2017**, *323*, 11–17.

(43) Thacker, J.; Carlton, D.; Hildenbrand, Z.; Kadjo, A.; Schug, K. Chemical Analysis of Wastewater from Unconventional Drilling Operations. *Water* **2015**, *7* (4), 1568–1579.

(44) Hoelzer, K.; Sumner, A. J.; Karatum, O.; Nelson, R. K.; Drollette, B. D.; O'Connor, M. P.; D'Ambro, E. L.; Getzinger, G. J.; Ferguson, P. L.; Reddy, C. M.; et al. Indications of Transformation Products from Hydraulic Fracturing Additives in Shale-Gas Wastewater. *Environ. Sci. Technol.* **2016**, *50* (15), 8036–8048.

(45) Strong, L. C.; Gould, T.; Kasinkas, L.; Sadowsky, M. J.; Aksan, A.; Wackett, L. P. Biodegradation in Waters from Hydraulic Fracturing: Chemistry, Microbiology, and Engineering. *J. Environ. Eng.* **2014**, *140* (5), B4013001.

(46) Regnery, J.; Coday, B. D.; Riley, S. M.; Cath, T. Y. Solid-Phase Extraction Followed by Gas Chromatography-Mass Spectrometry for the Quantitative Analysis of Semi-Volatile Hydrocarbons in Hydraulic Fracturing Wastewaters. *Anal. Methods* **2016**, *8* (9), 2058–2068.

(47) Orem, W.; Tatu, C.; Varonka, M.; Lerch, H.; Bates, A.; Engle, M.; Crosby, L.; McIntosh, J. Organic Substances in Produced and Formation Water from Unconventional Natural Gas Extraction in Coal and Shale. *Int. J. Coal Geol.* **2014**, *126*, 20–31.

(48) Piotrowski, P. K.; Weggler, B. A.; Barth-Naftilan, E.; Kelly, C. N.; Zimmermann, R.; Saiers, J. E.; Dorman, F. L. Non-Targeted Chemical Characterization of a Marcellus Shale Gas Well through GC \times GC with Scripting Algorithms and High-Resolution Time-of-Flight Mass Spectrometry. *Fuel* **2018**, *215*, 363–369.

(49) Piotrowski, P. K.; Weggler, B. A.; Yoxtheimer, D. A.; Kelly, C. N.; Barth-Naftilan, E.; Saiers, J. E.; Dorman, F. L. Elucidating Environmental Fingerprinting Mechanisms of Unconventional Gas Development Through Hydrocarbon Analysis. *Anal. Chem.* **2018**, acs.analchem.8b00822.

(50) Kekacs, D.; Drollette, B. D.; Brooker, M.; Plata, D. L.; Mouser, P. J. Aerobic
Biodegradation of Organic Compounds in Hydraulic Fracturing Fluids. *Biodegradation* 2015, *26*(4), 271–287.

(51) Luek, J. L.; Schmitt-Kopplin, P.; Mouser, P. J.; Petty, W. T.; Richardson, S. D.; Gonsior, M. Halogenated Organic Compounds Identified in Hydraulic Fracturing Wastewaters Using Ultrahigh Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2017**, *51* (10), 5377–5385.

(52) Liberatore, H. K.; Plewa, M. J.; Wagner, E. D.; Vanbriesen, J. M.; Burnett, D. B.; Cizmas, L. H.; Richardson, S. D. Identification and Comparative Mammalian Cell Cytotoxicity of New Iodo-Phenolic Disinfection Byproducts in Chloraminated Oil and Gas Wastewaters. *Environ. Sci. Technol. Lett.* **2017**, *4* (11), 475–480.

(53) Luek, J. L.; Gonsior, M. Organic Compounds in Hydraulic Fracturing Fluids and Wastewaters: A Review. *Water Research*. 2017, 536–548.

(54) Piotrowski, P. K.; Tasker, T. L.; Burgos, W. D.; Dorman, F. L. Applications of Thermal Desorption coupled to GC×GC-TOFMS for Hydrocarbon Fingerprinting of Hydraulically Fractured Shale Rocks. Submitted for publication *in Journal of Chromatography A*.

(55) Dallüge, J.; Beens, J.; Brinkman, U. A. T. Comprehensive Two-Dimensional Gas Chromatography: A Powerful and Versatile Analytical Tool. *Journal of Chromatography A*. 2003, 69–108.

(56) Harris, D. C. *Quantitative chemical analysis*. Macmillan, 2010.

(57) Piotrowski, P. K.; Tasker, T. L.; Geeza, T.; Warner, N.; Dorman, F. L. Determination of Bioaccumulation Potential of Hydraulic Fracturing Produced Water in the Freshwater Mussel, *Ellipto complanata*. Manuscript in preparation.

Chapter 2

Non-Targeted Chemical Characterization of a Marcellus Shale Gas Well Through GC×GC with Scripting Algorithms and High-Resolution Time-of-Flight Mass Spectrometry

2.1 Abstract

A non-targeted study of hydraulic fracturing fluids and corresponding flowback fluids allows for the understanding of the origin of wastewater constituents and provides insight into chemical signatures that may inform wastewater management practices for unconventional gas development. The source water for the hydraulic fracturing fluids, the actual hydraulic fracturing fluids used in four stimulation stages, and four flowback samples were obtained from a single unconventional gas well located in northeastern, PA. The chemical complexity of these fluids required high-resolution non-targeted methodologies. Analyses were therefore performed by GC×GC-TOFMS with the use of mass spectral scripting algorithms to expedite data analysis while maintaining a discovery approach. Our results indicate that during the flowback period hydrocarbon concentrations increase with time. The relative chemical composition remains nearly constant, which is hypothesized to be representative of the hydrocarbons present in the native shale that were extracted during the hydraulic fracturing process. Additionally, a comparison of fracturing fluids and flowback with high-resolution time-of-flight mass spectrometry inferred the fate of three common organic modifiers: ethylene glycol, glutaraldehyde, and cinnamaldehyde. It was determined that ethylene glycol is removed from the well within the first four days of flowback, while polymerization reactions are primary mechanisms of glutaraldehyde and cinnamaldehyde transformation

2.2 Introduction

Over the last decade, advancements in horizontal drilling have led to the exploitation of US natural gas resources through high-volume hydraulic fracturing.¹ During the fracturing process, large volumes of water and additives (between 10,000 liters and 37 million liters of water per well) are injected to create pressures capable of fracturing the low-permeability shale, liberating natural gas entrapped in the geologic formations.¹ Fracturing fluids are composed of 90% water, approximately 9% sand, and up to 2% chemical modifiers, which include acids, biocides, friction reducers, gelling agents and subsequent breakers.² The wastewaters that return to the surface as flowback or produced water contain traces of injected fluids but are largely dominated by components native to the shale formation, including hydrocarbon distributions resembling the complexity of crude oil.³ The chemical complexity of these wastewaters presents a challenge in water treatment; therefore, most flowback and produced waters are disposed of in Class II injection wells, which have been associated with seismic activity.⁴ Development of improved strategies for the treatment and re-use of oil-and-gas wastewaters relies on complete characterization of the chemical composition of these waters and on a better understanding of the controls on this chemistry.

While the chemical analysis of flowback and produced waters has gained significant attention in the past few years, the analysis of fracturing fluids and flowback from the same well has yet to be reported in peer-reviewed literature due to proprietary formulations protected by industry.³⁻²¹ Previous studies of flowback focus primarily on major ion and trace metal chemistry, with comparatively few focusing on organic components and changes in organic composition over time.³⁻²¹ Since the chemistry of fracturing fluids and flowback is complex, containing a large number of organic chemicals that have largely gone unresolved and unidentified, a non-targeted

approach must be utilized with special emphasis given to components native to the shale formations, as these constituents are least understood.

Gas chromatography (GC) is a vital tool for the analysis of hydrocarbons, but to date the technique has been underutilized for the analysis of flowback fluids. A literature review produced only six studies which have utilized GC towards the characterization of wastewaters associated with unconventional gas development; however, the reported data sets are largely incomplete because the methods used were capable of detecting only a small subset of chemicals present within the fluids.^{3,18-22} The limited application of GC stems from challenges associated with the technique, as one-dimensional gas chromatography does not possess adequate peak capacity for a complete characterization of the naturally occurring hydrocarbons in shale. As a result, previous analyses of flowback by GC have resulted in unresolved complex mixtures (UCMs).^{3,18-22} The presence of an UCM can greatly complicate analysis leading to the omission of constituents that may have important implications for fingerprinting or water reuse and treatment.

Comprehensive two-dimensional gas chromatography (GC×GC) is an established technique that overcomes the peak capacity limitations described above and also allows for a higher detection sensitivity, when performed with cryogenic modulation, relative to conventional GC analysis.²³⁻²⁵ This approach has previously been utilized to detect two contamination events associated with unconventional gas development: the presence of 2-butoxyethanol and elevated diesel range organic compounds in groundwater.^{20,22} Additionally, GC×GC was utilized to infer fracturing fluid halogenations through the analysis of flowback fluids.²⁵ Hoelzer et al. tentatively identified compound sources by comparison with additive disclosure.²⁵ However, their study did not analyze the hydraulic fracturing fluids used in each of the six wells reported, and the retention index methodology used to verify compound identity was likely of limited utility due to GC

stationary phase mismatches from the NIST reported indices that were used for comparison in their experiments.

The study presented here is the first to report the analysis of GC amenable compounds from injected hydraulic fracturing fluids and the associated flowback from a single unconventional gas well located in northeastern Pennsylvania. GC×GC presents a promising nontargeted method towards the characterization of wastewaters associated with unconventional gas development and may provide more complete insight into hydrocarbon speciation. The objectives of this study were: (1) to examine the organic chemical differences between hydraulic fracturing fluids and flowback from the same well, (2) to investigate the temporal organic chemical differences in flowback fluids during a 10-day period, and (3) to explore data reduction strategies for GC×GC data of hydraulic fracturing wastewaters.

2.3 Materials and Methods

2.3.1 Sample Collection

Hydraulic fracturing fluids and flowback were collected during hydraulic stimulation of a horizontal well completed in summer 2015 within the Marcellus Shale of Susquehanna County, PA. Hydraulic fracturing fluids were collected during stimulation of stages 7-10 of the well (Figure 2-1), which are the fluids used to target discrete sections of the lateral well. Subsequently, the flowback fluids from the same gas well were collected every other day over 10 days during the flowback period. The well was fractured using 3.8x10⁷ L of freshwater and shut-in for a period of 23 days.



Figure **2-1:** A schematic of hydraulic fracturing stimulation stages. The hydraulic fracturing fluids analyzed herein originated from stimulation stages 7-10.

Samples were collected in 500 mL amber glass jars with PTFE closures pre-cleaned according to EPA Specifications And Guidance For Contaminant Free Sample Containers.²⁶ The samples were transported back to the lab on ice via overnight shipment and kept at 4°C, in the dark, until solvent extraction or acid digestion. Solvent extractions were performed within 14 days of collection for the organic analysis and all other analyses were completed within 6 months of collection, in accordance with USEPA methodology.²⁷

2.3.2 Sample Preparation

Samples for GC×GC analysis were prepared using a modified EPA Method 3510C. The pH of the samples (200 mL) was adjusted (pH >11 and pH<2) using 10 M sodium hydroxide and 50% sulfuric acid, respectively. After each pH adjustment, the samples were serially extracted three times with 50 mL of dichloromethane (Avantor Performance Material, JT Baker, Center Valley, PA, Ultra-resi analyzed grade). All extracts were combined and concentrated to a final volume of 500 μ L in a Kuderna-Danish apparatus and stored at 4°C until analysis. In the case of an emulsion, the samples were centrifuged (3,700 rpm; 3 min) to break the emulsion.

2.3.3 Sample Analysis

The extracts were analyzed by comprehensive two-dimensional gas chromatography coupled to a time of flight mass spectrometer (LECO Pegasus 4D GC×GC-TOFMS and LECO Pegasus 4D GC×GC-HRT). The GC×GC-TOFMS and HRT were operated with a 250°C injector temperature, and 2 ml/min helium carrier gas flow rate. The first dimension column was a Restek Rtx-DHA100 (100 m \times 0.25 mm \times 0.5 µm), which is considered an 'industry-standard' for detailed hydrocarbon analysis and ensured an efficient separation of saturated hydrocarbons, including branched and cyclic species based on carbon chain length. The second dimension column was a Restek Rxi-17SilMS (2.0 m \times 0.25 mm \times 0.25 μ m), which was used to selectively retain more functionalized compounds in the second dimension though π - π interactions between the stationary phase of the column and the unsaturations of the analytes. The first dimension oven was programmed to maintain 40°C for 0.2 minutes after injection and increase at 1.5°C/min to 315°C. The modulator temperature was offset at 15°C and operated with a 2.5 second modulation period. The second dimension oven was offset by 5° C. The peak capacity of this method was calculated to be 9511 using the LECO Simply GC×GCTM tool. The mass spectrometer operated with a 700 second acquisition delay, 250°C ion source temperature, and -70eV ionization energy. The collected mass range was 30-550 amu at 200 Hz with a positive mass defect offset of 50 mu/100u. The GC×GC-HRT instrument was operated at a mass resolution of 25,000.

2.3.4 QA/QC

Sample integrity was evaluated and maintained through the use of surrogate standards. The samples were extracted alongside a laboratory blank consisting of 18.2 M Ω (MilliQ) water which was subjected to the same procedures and analysis as the samples, described above. The blank was free of hydrocarbon contaminants, though trace quantities of common laboratory contaminants such as toluene were observed. Compounds found in the laboratory blanks were not reported if they were also found in sample extracts. Additionally, samples were analyzed in triplicate to ensure reproducibility. In experiments involving the HRT, external mass calibration of the mass spectrometer was performed using perfluorotributylamine (PFTBA). The mass accuracy tolerance of PFTBA was set a 1 ppm for m/z 68.9947, 130.9915, 218.9851, and 413.9770. Additionally, PFTBA was infused during the acquisition and subsequently used as an internal mass calibrant in all spectra. For all compound assignments, the accurate mass measurement was within 5 ppm of the theoretical value.

2.3.5 Data Analysis

Data processing was performed using LECO ChromaTOF[®] software version 4.50.8.0. The baseline level was set to be just above the noise, and the automated peak finding was performed utilizing mass deconvolution algorithms for all peaks with a signal-to-noise ratio (S/N) greater than 75. Peak assignments were based on a forward search in the NIST14 library for peaks with a spectral similarity score of 700. A peak broadening correction was applied in the second dimension with earlier second dimension peak widths of 100 ms transitioning to 200 ms at first dimension retention time of 2845 seconds. GC×GC subpeaks required a S/N greater than 6 and a spectral similarity score of 750 to recombine. Compound classifications were performed using the scripting feature contained in the ChromaTOF[®] software using algorithms written in Microsoft[®] VBScript language previously described by Weggler et al.^{28,29} Briefly, the scripts utilize characteristic mass spectral fragmentation patterns such as isotope ratios, mass losses, and distinctive mass-to-charge (m/z) ratios for each compound class. The subsequent data are visualized by a bubble plot generated in ChromaTOF[®] where bubble position corresponds to the analytes retention time and the bubble radii are scaled to the peak area, with each color representing a unique compound class, as determined by mass spectral features. For peaks classified by more than one group, the peak bubble is striped with the colors of the corresponding groups.
2.4 Results and Discussion

Non-targeted comprehensive two-dimensional gas chromatographic (GC×GC) methodologies were employed to characterize an unconventional gas well from northeastern PA. These analyses revealed several new findings in regards to hydrocarbon speciation. In a first pass analysis; it is very clear that the composition of the hydraulic fracturing fluids is considerably different from that of the subsequent flowback. The chemical signature of this industrial process can be further traced back through the analysis of the source water used in the formulation of they hydraulic fracturing fluids. In the well characterized herein, the hydraulic fracturing fluids were composed of 100% fresh water sourced from the Susquehanna River. We collected a sample of the source water used in these fracturing fluids at the riverbank nearby a water intake pump that was used to provide water to the fracturing site. The GC×GC-TOFMS characterization revealed that the river water was free from any major contaminants, including hydrocarbons, at levels above the detection limits of the analysis, which are ca 1.0-10 ng/L in sample. The peaks visible in the GC×GC chromatogram correspond to common laboratory contaminants such as hexadecanoic acid and octadecanoic acid, likely fingerprint residues from sample collection/handling practices.³⁰ Additionally, toluene was detected in the sample, as in the laboratory blank, due to the frequent handling of this solvent in the laboratory. Inorganic characterization of the river water was unremarkable and mostly dominated by calcium, sodium, chloride, and sulfate. These data were consistent with measurements made at the most proximal USGS site, located 14 km downstream from our sampling location.³¹

The GC×GC analysis of the fracturing fluids injected during stages 7-10 (Figure 2-2) showed various hydrocarbon distributions that may reflect the presence of hydraulic fracturing fluid additives, namely the light petroleum distillates used as friction reducers, which may be attributed to the saturated hydrocarbons observed in the GC×GC chromatograms.² Stage 7

fracturing fluids contained complex hydrocarbon distributions, with 3-ring aromatics being the dominant class (28.7%) when compared to branched alkanes mostly found in Stage 8 (40.6%) and Stage 9 (44.6%) (Table 2-1). The Stage 10 sample also contained a complex hydrocarbon distribution that appeared to be much different from Stage 7, as it contained a lighter, earlier eluting fraction that was dominated by n-alkanes (33.0%). The chemical composition of Stages 8 and 9 was difficult to determine, as the low abundance of peaks lead to low S/N of the mass spectral data. Therefore, only 65% and 62% of the chemical composition could be determined in the Stage 8 and Stage 9 samples, respectively (Table 2-1). The changing hydrocarbon compositions may be a result of stage-dependent formulations that are paired specifically to the discrete geology targeted in that stage of hydraulic fracturing. However, chemical additive disclosure databases, such as FracFocus (www.fracfocus.org), only reveal the complete list of chemical modifiers used during the completion of the well, therefore it cannot be concluded whether the changing chemistry of the injected fluids is a consequence of stage-dependent formulations.² Alternatively, the hydrocarbon variability throughout the injected fluid samples may reflect on-site handling practices such as the reuse of tanks that previously stored flowback or produced waters. Critically, we note that this study only addressed GC amenable components of hydraulic fracturing fluids, which only represent a subset of chemical modifiers used during hydraulic fracturing.¹³

	Stage 7	Stage 8	Stage 9	Stage 10
n-Alkanes	20.0	10.8	9.9	33.0
Branched Alkanes	26.4	29.9	34.7	29.9
Alkenes	5.7	2.6	1.6	5.2
Cyclohexanes	11.5	6.3	5.2	12.2
Para Substituted Cyclohexanes	2.7	3.7	2.4	7.0
3-Ring Aromatics	28.7	2.2	0.9	1.3
Phthalate	0.9	3.1	3.5	1.1
Fluorenes	2.4	5.8	3.8	5.6
Hydronaphthalenes	0	0.4	0.3	3.7
Biphenyl	1.1	0.2	0.2	1.0
Cyclopentanes	0.3	0.2	0.2	0.9

 Table 2-1: The percent chemical class compositions for the GC amenable fraction of hydraulic fracturing fluids



Figure **2-2:** The GC×GC total ion chromatograms of A) Stage 7 fracturing fluid with high abundance of hydrocarbons, B) Stage 8 fracturing fluid with low abundance of hydrocarbons, C) Stage 9 fracturing fluid with low abundance of hydrocarbons, and D) Stage 10 fracturing fluid with high abundance of hydrocarbons.

The flowback fluids were very chemically complex, as determined from the GC×GC-TOFMS chromatograms, with over 6000 peaks found though the data processing procedures described above. The interpretation of such large data sets is an active area of chemometric research, as visualization of multiple dimensional data is challenging. Here, we explored data reduction strategies through algorithms that probe class-specific fragmentation mass-to-charge ratios, which allowed for the construction of figures that not only display a two dimensional separation, but also incorporate evaluated mass spectral information (Figure 2-3). Additionally, the use of bubble-size to indicate ion abundance retains semi-quantitative information, thus providing four-dimensions of information: first dimension retention time, second dimension retention time, interpreted mass spectral fragmentation, and abundance relative to other peaks in that chromatogram. This data reduction methodology still allowed for a non-targeted approach to the analysis of complex GC×GC-TOFMS data and categorized over 6000 peaks in less than one hour of processing time.

The analysis of flowback by $GC \times GC$ with subsequent chemical class assignments via scripting (Figure 2-3) revealed a hydrocarbon pattern unlike that observed in the fracturing fluids. During the first four days of flowback (sampled at day 2 and day 4), the $GC \times GC$ chromatograms (Figure 3) were largely uncomplicated with regards to major organic constituents, and were dominated by C_6 - C_{30} linear and branched, saturated alkanes (58.5%). Surprisingly, the chemically complex hydrocarbons in the fracturing fluids (Figure 2-2) were not present in the Day 2 or Day 4 flowback, possibly due to in-well dilution, sorption, or interactions with other fracturing components such as breakers.²⁵ A comparison of all of the tentatively identified components of the hydraulic fracturing fluids and subsequent flowback revealed only a few instances of compound overlap between the two samples with nearly all matched compounds being hydrocarbons that can be attributed to the hydrotreated light distillate chemical modifier.² Additionally, the GC amenable chemical modifiers used in the fracturing fluid, as listed in the FracFocus disclosure² for the well discussed in this study, were investigated with $GC \times GC$ coupled to a high-resolution time-of-flight mass spectrometer. The accurate masses, with a 5 ppm mass accuracy tolerance, were extracted for the scale inhibitor ethylene glycol, biocide glutaraldehyde, and corrosion inhibitor cinnamaldehyde (Figure 2-4). All three additives were found during all analyzed fracturing fluid stages. However, glutaraldehyde and cinnamaldehyde were not detectable in any of the analyzed flowback samples (Figure 2-4). Kahrilas et al. reported modeled down-bore transformations of glutaraldehyde and concluded that polymerizations are a dominant mechanism.³² Additionally, the absence of the corrosion inhibitor cinnamaldehyde may also be a result of similar down-bore transformations. Previous reports indicate that the corrosion

inhibiting properties of cinnamaldehyde are due to polymerizations that form a protective film on the surface of the steel casing.³³ The formation of larger, polymer molecules with lower vapor pressures that are not amenable to gas chromatographic analysis may explain the absence of glutaraldehyde and cinnamaldehyde transformation products in this study. The scale inhibitor ethylene glycol was detected during the first four days of the flowback period but was not detectable in the day 6, 8, and 10 samples (Figure 2-4). It is probable that this additive was flushed out of the well during those first four days of flowback, which is also consistent with a shift in the hydrocarbon chemistry that persists throughout the remainder of the flowback period.



Figure **2-3:** The GC×GC total ion chromatograms of A) Day 4 flowback with low abundance of hydrocarbons, B) Day 6 flowback C) Day 8 flowback, and D) Day 10 flowback. Hydrocarbon classes were assigned through scripts that utilize class-specific fragmentation patterns. Red: n-alkanes, green: branched alkanes, purple: alkenes, white: hydronaphthalenes, salmon: cyclohexanes, orange: para substituted cyclohexanes, yellow: cyclopentanes, teal: 3-ring aromatics, blue: fluorenes. Bubble radii are scaled to the peak area. The hydrocarbon composition in Day 2 flowback (not shown) and Day 4 flowback are similar, but the composition shifts from Day 4 to Day 6, whereupon it remains approximately constant through Day 10.



Figure 2-4: The GC×GC-HRT extracted ion chromatograms for A) the scale inhibitor ethylene glycol in hydraulic fracturing fluid (m/z 62.0362), B) ethylene glycol in flowback (m/z 62.0362), C) the biocide glutaraldehyde in hydraulic fracturing fluid (m/z 82.0413), D) the absence of glutaraldehyde in flowback (m/z 82.0413), E) the corrosion inhibitor cinnamaldehyde in hydraulic fracturing fluid (m/z 131.0491), and F) the absence of cinnamaldehyde in flowback (m/z 131.0491). Note, the secondary retention time was adjusted to correct for wrap-around.

At day 6 of flowback, a distinctive hydrocarbon distribution was observed. Throughout the flowback period, this hydrocarbon distribution increased in concentration with the overall chemical pattern remaining constant centered between the C_{10} and C_{16} n-alkanes. In addition to the overall chromatographic separation, chemical class-specific information extracted from the mass spectral scripting algorithms corroborate the compositional similarities in the day 6, 8 and 10 flowback samples with each chemical class demonstrating similar abundance and localization within the chromatograms. These chemical similarities were further investigated in order to associate the hydrocarbon source of these samples. A ratio comparison of compound classes allowed for a compositional assessment while normalizing for the concentration differences within these samples. The percent peak areas calculated from the scripting algorithm chemical classes were used for computing ratios. Only classes with greater than 1% abundance in the chromatograms were chosen. The ratios of the three most abundant classes in flowback were compared: saturated hydrocarbons to para substituted cyclohexanes and fluorenes. In the case of day 6, 8, and 10 flowback, the ratios of hydrocarbon classes remained relatively constant as compared to the other samples (Table 2-2), which suggested that the hydrocarbon source is the same for each sample, even though the actual concentration of hydrocarbons increased with time during the flowback period. Additionally, since the ratios of the hydrocarbons did not match that of the injected fluid, it is unlikely that the major source of the hydrocarbon in flowback originates from chemical modifiers used during hydraulic fracturing.

Sample	saturated/fluorenes	saturated/para substituted cyclohexanes
Stage 7 Frac Fluid	19.3	16.9
Stage 8 Frac Fluid	7.0	11.1
Stage 9 Frac Fluid	11.8	18.5
Stage 10 Frac Fluid	11.2	9.0
Day 4 Flowback	18.8	*
Day 6 Flowback	1.5	3.3
Day 8 Flowback	1.2	3.1
Day 10 Flowback	1.0	3.6

Table 2-2: Ratios of the hydrocarbon classes in injected fracturing fluids and flowback samples.

Note: *no para substituted cyclohexanes were detected in this sample

These data suggest that the observed hydrocarbons are likely a consequence of the formation geology and represent the hydrocarbon signature of the native shale. Physical mixing processes of the hydraulic fracturing fluids with formation fluids that have equilibrated with the shale rock over geologic time may pose as a mechanism for the hydrocarbon signatures observed herein. However, the inorganic characterization suggests that the hydrocarbon signature of these flowback samples is not solely an artifact of formation fluid dilution with the hydraulic fracturing fluids. The consistent ratio of the concentrations of Cl⁻/Br⁻ in all of the flowback samples of 128±7, suggests that the proportion of formation fluid to hydraulic fracturing fluid remains relatively constant during the flowback period. Nevertheless, the concentration of hydrocarbons

increases during this period suggesting another mechanism of hydrocarbon extraction is occurring in the subsurface. The later flowback fluids (day 6-10) spent more within the shale, and, owing to this greater equilibration time, the hydrocarbons present within the late-stage flowback more closely resembled those occurring naturally in the shale. Although the Marcellus is only commercially viable for gas extraction, the shale formation is organic rich and heavier hydrocarbons in flowback have been previously documented.^{3,18} However, contrary to previous characterizations, which reported much greater changes in hydrocarbon compositions with time, the data presented here show relatively little temporal variation. This is likely due to the high sampling frequency of the flowback fluids, which is unique to this study. It is believed that once the period of high TOC is reduced, the consistent hydrocarbon distribution will be diminished in the low-TOC produced waters.³⁴ Nevertheless, this consistency of hydrocarbon distributions within flowback may represent a potential fingerprinting mechanism since the formation geology may vary slightly for each unconventional well.²⁵

2.5 Conclusion

Hydraulic fracturing has transformed the United States economy in the last decade. However, the technology is highly contentious and debated due, in part, to lack of knowledge on subsurface chemistry and environmental impacts. To our knowledge, this study is the first-of-itskind to analyze the chemistry of injected hydraulic fracturing fluids and flowback from the same unconventional well utilizing a discovery approach, and aims to provide a simplified approach to characterizing samples associated with shale gas systems. The results presented in this report imply that the hydrocarbon chemistry of hydraulic fracturing fluids do not largely affect hydrocarbons present in flowback, the chemistry of which seems to be more reflective of interactions with the organic-rich shale. Because studies of down-bore transformations of chemicals introduced in hydraulic fracturing fluids are limited,^{25,32} understanding additive fate is crucial in developing sustainable treatment practices of these wastewaters. Herein, we presented

the fate of three hydraulic fracturing additives; ethylene glycol, glutaraldehyde, and

cinnamaldehyde. Understanding the conservancy or transformation of these components may

enable more informed treatment processes. These results provide evidence of subsurface

reactions, as hydrocarbons introduced in the hydraulic fracturing fluids were indistinguishable in

flowback. Additionally, the flowback characterization aided by data reduction strategies of mass

spectral scripting algorithms allowed for a rapid chemical class determination methodology that

showed consistent hydrocarbon composition of flowback, thus indicating a potential

fingerprinting mechanism for environmental contamination events from unconventional gas

development.

2.6 References

(1) Howarth, R. W.; Ingraffea, A. Natural Gas: Should Fracking Stop? *Nature*. 2011, 271–273.

(2) Frac Focus Chemical Disclosure Registry. <u>https://fracfocus.org</u>. (Accessed May 20, 2016).

(3) Orem, W.; Tatu, C.; Varonka, M.; Lerch, H.; Bates, A.; Engle, M.; Crosby, L.; McIntosh, J. Organic substances in produced and formation water from unconventional natural gas extraction in coal and shale. *International Journal of Coal Geology*. **2014**, *126*, 20-31.

(4) McGarr, A.; Bekins, B.; Burkardt, N.; Dewey, J.; Earle, P.; Ellsworth, W.; Ge, S.; Hickman, S.; Holland, A.; Majer, E.; Rubinstein, J. Coping with earthquakes induced by fluid injection. *Science*. **2015**, *347* (6224), 830-831.

(5) Haluszczak, L.O.; Rose, A.W.; Kump, L.R. Geochemical evaluation of flowback brine from Marcellus gas wells in Pennsylvania, USA. *Applied Geochemistry*. **2013**, *28*, 55-61.

(6) Barbot, E.; Vidic, N.S.; Gregory, K.B.; Vidic, R.D. Spatial and temporal correlation of water quality parameters of produced waters from Devonian-age shale following hydraulic fracturing. *Environmental Science & Technology*. **2013**, *47* (6), 2562-2569.

(7) Abualfaraj, N.; Gurian, P.L.; Olson, M.S. Characterization of Marcellus shale flowback water. *Environmental Engineering Science*. **2014**, *31* (9), 514-524.

(8) Lester, Y.; Ferrer, I.; Thurman, E. M.; Sitterley, K. A.; Korak, J. A.; Aiken, G.; Linden, K. G. Characterization of hydraulic fracturing flowback water in Colorado: Implications for water treatment. *Science of The Total Environment*. **2015**, *512*, 637-644.

(9) Thurman, E. M.; Ferrer, I.; Blotevogel, J.; Borch, T. Analysis of hydraulic fracturing flowback and produced waters using accurate mass: identification of ethoxylated surfactants. *Analytical Chemistry*. **2014**, *86* (19), 9653-9661.

(10) Ferrer, I.; Thurman, E. M. Chemical constituents and analytical approaches for hydraulic fracturing waters. *Trends in Environmental Analytical Chemistry.* **2015**, *5*, 18-25.

(11) Ferrer, I.; Thurman, E.M. Analysis of hydraulic fracturing additives by LC/Q-TOF-MS. *Analytical and Bioanalytical Chemistry.* **2015**, *407* (21), 6417-6428.

(12) Thurman, E.M.; Ferrer, I.; Rosenblum, J.; Linden, K.; Ryan, J.N. Identification of polypropylene glycols and polyethylene glycol carboxylates in flowback and produced water from hydraulic fracturing. *Journal of Hazardous Materials*. **2016**.

(13) Getzinger, G. J.; O'Connor, M. P.; Hoelzer, K.; Drollette, B. D.; Karatum, O.; Deshusses, M. A.; Ferguson, P. L., Plata, D. L. Natural Gas Residual Fluids: Sources, Endpoints, and Organic Chemical Composition after Centralized Waste Treatment in Pennsylvania. *Environmental Sci- ence & Technology*. **2015**, *49*, 8347-8355.

(14) Akob, D. M.; Cozzarelli, I. M.; Dunlap, D. S.; Rowan, E. L.; Lorah, M. M. Organic and inorganic composition and microbiology of produced waters from Pennsylvania shale gas wells. *Applied Geochemistry*. **2015**, *60*, 116-125.

(15) Kahrilas, G. A.; Blotevogel, J.; Stewart, P. S.; Borch, T. Biocides in hydraulic fracturing fluids: a critical review of their usage, mobility, degradation, and toxicity. *Environmental Science* & *Technology*. **2014**, *49* (1), 16-32.

(16) Shih, J.S.; Saiers, J.E.; Anisfeld, S.C.; Chu, Z.; Muehlenbachs, L.A.; Olmstead, S.M. Characterization and analysis of liquid waste from Marcellus Shale gas development. *Environmental Science & Technology*. **2015**, *49* (16), 9557-9565.

(17) Ferrar, K.J.; Michanowicz, D.R.; Christen, C.L.; Mulcahy, N.; Malone, S.L.; Sharma, R.K. Assessment of effluent contaminants from three facilities discharging Marcellus Shale wastewater to surface waters in Pennsylvania. *Environmental Science & Technology*. **2013**, *47* (7), 3472-3481.

(18) Regnery, J.; Coday, B.D.; Riley, S.M.; Cath, T.Y. Solid-phase extraction followed by gas chromatography-mass spectrometry for the quantitative analysis of semi-volatile hydrocarbons in hydraulic fracturing wastewaters. *Analytical Methods*. **2016**, *8* (9), 2058-2068.

(19) Strong, L.C.; Gould, T.; Kasinkas, L.; Sadowsky, M.J.; Aksan, A.; Wackett, L.P. Biodegradation in waters from hydraulic fracturing: chemistry, microbiology, and engineering. *Journal of Environmental Engineering*. **2013**, *140* (5), B4013001.

(20) Drollette, B. D.; Hoelzer, K.; Warner, N. R.; Darrah, T. H.; Karatum, O.; O'Connor, M. P.; Nelson, R. K.; Fernandez, L. A.; Reddy, C. M.; Vengosh, A.; et al. Elevated Levels of Diesel Range Organic Compounds in Groundwater near Marcellus Gas Operations Are Derived from Surface Activities. *Proc. Natl. Acad. Sci.* **2015**, *112* (43), 13184–13189.

(21) Thacker, J.B.; Carlton, D.D.; Hildenbrand, Z.L.; Kadjo, A.F.; Schug, K.A. Chemical analysis of wastewater from unconventional drilling operations. *Water*. **2015**, *7* (4), 1568-1579.

(22) Llewellyn, G.T.; Dorman, F.; Westland, J.L.; Yoxtheimer, D.; Grieve, P.; Sowers, T.; Humston-Fulmer, E.; Brantley, S.L. Evaluating a groundwater supply contamination incident attributed to Marcellus Shale gas development. *Proceedings of the National Academy of Sciences*. **2015**, *112* (20), 6325-6330.

(23) Liu, Z.; Phillips, J.B. High-speed gas chromatography using an on-column thermal desorption modulator. *Journal of Microcolumn Separations*. **1989**, *1* (5), 249-256.

(24) Blomberg, J.; Schoenmakers, P. J.; Beens, J.; Tijssen, R. Comprehensive twodimensional gas chromatography (GC× GC) and its applicability to the characterization of complex (petrochemical) mixtures. *Journal of High-Resolution Chromatography*. **1997**, *20* (10), 539-544.

(25) Hoelzer, K.; Sumner, A.J.; Karatum, O.; Nelson, R.K.; Drollette, B.D.; O'Connor, M.P.; D'Ambro, E.L.; Getzinger, G.J.; Ferguson, P.L.; Reddy, C.M.; Elsner, M.; Plata, D.L. Indications of transformation products from hydraulic fracturing additives in shale-gas wastewater. *Environmental Science & Technology*. **2016**, *50* (15), 8036–8048.

(26) United States Environmental Protection Agency, Specifications And Guidance For Contaminant-Free Sample Containers, **1992.**

(27) United States Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, **1983.**

(28) Welthagen, W.; Schnelle-Kreis, J.; Zimmermann, R. Search Criteria and Rules for Comprehensive Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry Analysis of Airborne Particulate Matter. In *Journal of Chromatography A*; 2003; Vol. 1019, pp 233–249.

(29) Weggler, B. A.; Groger, T.; Zimmermann, R. Advanced scripting for the automated profiling of two-dimensional gas chromatography-time-of-flight mass spectrometry data from combustion aerosol *J. Chromatog. A.* **2014**, *1364*, 241-248.

(30) Michalski, S.; Shaler, R.; Dorman, F. L. The evaluation of fatty acid ratios in latent fingermarks by gas chromatography/mass spectrometry (GC/MS) analysis. *Journal of Forensic Sciences.* **2013**, *58*. 215-220.

(31) U.S. Geological Survey, **2013**, National Water Information System data available on the World Wide Web (USGS Water Data for the Nation), accessed September 10, 2016, at URL http://wdr.water.usgs.gov/wy2013/pdfs/01502771.2013.pdf.

(32) Kahrilas, G.A.; Blotevogel, J.; Corrin, E.R.; Borch, T. Downhole Transformation of the Hydraulic Fracturing Fluid Biocide Glutaraldehyde: Implications for Flowback and Produced Water Quality. *Environmental Science & Technology*. **2016**, *50* (20), 11414-11423.

(33) Growcock, F.B. Inhibition of Steel Corrosion in HCl by Derivatives of Cinnamaldehyde: Part I. Corrosion Inhibition Model. *Corrosion*. **1989**, *45* (12), 1003-1007.

Chapter 3

Elucidating Environmental Fingerprinting Mechanisms of Unconventional Gas Development Through Hydrocarbon Analysis

3.1 Abstract

Hydraulic fracturing is an increasingly common technique for the extraction of natural gas entrapped in shale formations. This technique has been highly criticized due to the possibility of environmental contamination, underscoring the need for method development to identify chemical factors that could be utilized in point-source identification of environmental contamination events. Here, we utilize comprehensive two-dimensional gas chromatography (GC×GC) coupled to high-resolution time-of-flight (HRT) mass spectrometry, which offers a unique instrumental combination allowing for petroleomics hydrocarbon fingerprinting. Four flowback fluids from Marcellus shale gas wells in geographic proximity were analyzed for differentiating factors that could be exploited in environmental forensics investigations of shale gas impacts. Kendrick mass defect (KMD) plots of these flowback fluids illustrated well-to-well differences in heteroatomic substituted hydrocarbons, while GC×GC separations showed variance in cyclic hydrocarbons and polyaromatic hydrocarbons (PAHs) among the four wells. Additionally, generating plots that combine GC×GC separation with KMD established a novel data-rich visualization technique that further differentiated the samples.

3.2 Introduction

Unconventional oil and gas resources, formed in extremely low permeability shales, have been economically exploited for two decades through advances in horizontal drilling and subsequent hydraulic fracturing.¹ The hydrocarbons in these formations are extracted by injecting water with a suite of chemical modifiers at high pressures capable of fracturing the shale along with sand used as a proppant to prevent fracture collapse.² Hydrocarbons flows to the surface through the production casing along with wastewaters that are connate waters and by-products of the hydraulic fracturing process.¹ These wastewaters, called flowback (initial return of water prior to production) or produced waters (generated throughout the productive lifetime of the well) are chemically complex, containing traces of chemical modifiers,³⁻⁸ transformation products,⁹⁻¹¹ and components native to the shale itself.¹¹⁻¹⁴ The hydrocarbon speciation of flowback fluids may resemble the complexity of light crude oils.¹¹⁻¹⁴

Recent studies have raised concerns about the impact of hydraulic fracturing wastewaters on the environment, namely water resources.¹⁵ There have been incidents where poorly constructed well casings, surface spills, and migration of drilling fluids, fracturing fluids, or stray gas have contributed to water contamination.^{1,7} However, analytical methods for fingerprinting environmental contamination have focused largely on using stable and radioactive isotope tracers, which are not a common techniques in the commercial analytical community.¹⁶⁻¹⁷ Fingerprinting contamination events through organic speciation has only been demonstrated in a few reports. Thurman et al. proposed the use of LC-Q-ToF for fingerprinting contamination using polyethoxylate alcohols,³ polypropylene glycols,⁴ and polyethylene glycols,⁴ as these surfactants remain unchanged throughout the hydraulic fracturing process.¹⁸ However, these methodologies have yet to be applied in environmental forensic investigations. Alternatively, semi-volatile compounds have been used as tracers for environmental contamination. Comprehensive twodimensional gas chromatography (GC×GC) coupled to time-of-flight mass spectrometry (TOFMS) has been used to investigate two contamination events associated with shale gas development: the presence of 2-butoxyethanol and elevated diesel range organic compounds in groundwater.^{7,19} Additionally, a spill of oil and gas wastewaters into surface waters in North Dakota was assessed by GC-MS, in addition to a suite of inorganic parameters.²⁰ The chromatograms obtained from the affected area indicated a presence of hydrocarbons, many of which could not be resolved using the one-dimensional separations employed.²⁰ Hydrocarbon speciation may be a powerful fingerprinting mechanism of shale gas related environmental contamination events, as a recent study utilized GC×GC-TOFMS to investigate hydraulic fracturing fluids and subsequent flowback from the same well, and demonstrated that the chemical signatures from injected fluids are lost in the flowback chemistry and the hydrocarbon chemistry remains constant during the flowback period.¹³

Hydrocarbon speciation is an established mechanism in petroleomic fingerprinting and is typically performed using ultrahigh resolution mass spectrometry.²¹⁻²² According to our literature search, According to our literature search, only a single study investigated the chemical composition of hydraulic fracturing wastewaters using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR).⁹ However, this report gave no discussion of hydrocarbon speciation and instead focused on the identification of numerous halogenated species. Seven studies of hydraulic fracturing wastewaters have employed high-resolution mass spectrometry with all but one using liquid chromatography separations as a front end for the analysis.^{3-6,8-9,23} Moreover, only one study to date utilizing high-resolution mass spectrometry coupled with gas chromatography for the analysis of hydraulic fracturing wastewaters in which a targeted GC×GC separation was used to infer the transformations of hydraulic fracturing fluid additives.¹³ Recently, Byer et al. demonstrated that the combination of GC×GC separations and high-

resolution time-of-flight mass spectrometry (HRT) provides a comparable analysis to FT-ICR and allows for the distinction of C_3 and SH_4 that previously could only be resolved with FT-ICR.²⁴

The study presented here is the first discussion of hydrocarbon chemistry in flowback fluids originating from four proximally located Marcellus shale gas wells with known well operators. The use of GC×GC with high-resolution time-of-flight mass spectrometry enabled a petroleomic approach for the study of hydrocarbon speciation through the use of Kendrick mass defect plots. Here, we tie together GC×GC retention with Kendrick mass defect to present a novel visualization approach for these data to illustrate compound class elution fingerprints that may aid in environmental forensic cases of contamination events from shale gas development.

3.3 Materials and Methods

3.3.1 Sample Collection

Flowback fluids were collected during the hydraulic stimulation of four horizontal wells completed in summer-fall 2015 within the Marcellus Shale of Susquehanna County, PA. Of the four wells, three were fractured by Baker Hughes while one was fractured by Halliburton (Sample B).² Samples A-C were fractured with 100% fresh water from the Susquehanna River, while sample D contained 2.59% recycled fluids. Samples were collected in 500 mL amber glass jars with PTFE closures pre-cleaned according to EPA Specifications and Guidance for Contaminant Free Sample Containers.²⁶ The samples were transported back to the lab on ice via overnight shipment and kept at 4°C, in the dark, until solvent extraction. Solvent extractions were performed within 14 days of receiving the samples, in accordance with USEPA methodology.²⁷

3.3.2 Sample Preparation

Samples were prepared for GC analysis through liquid-liquid extraction using a modified USEPA Method 3510C.²⁸ 200mL of flowback fluid was serially extracted with dichloromethane (Avantor Performance Material, JT Baker, Center Valley, PA, Ultra-resi analyzed grade). The samples were acid-preserved with HCl to pH<2; no other pH adjustments were made prior to extraction 3 times with 50 mL of dichloromethane. The pH was subsequently adjusted to >11 with 10 M sodium hydroxide followed by three additional extractions with 50 mL of dichloromethane were combined and concentrated to a final volume of 500 μ L in a Kuderna-Danish apparatus and stored at 4°C until analysis. Emulsions, which occurred in all four samples, were broken by centrifugation (2,500 rpm; 2 min).

3.3.3 Sample Analysis

The extracts were analyzed by comprehensive two-dimensional gas chromatography coupled to a high-resolution time of flight mass spectrometer (LECO Pegasus 4D GC×GC-HRT, St. Joseph, MI). The GC×GC -HRT was operated with a 250 °C injector temperature, and 2 mL/min helium flow rate. The first dimension column was a Restek Rtx-DHA100 (100 m × 0.25 mm × 0.5 µm) (Bellefonte, PA), which efficiently separates saturated hydrocarbons, including saturated and aromatic hydrocarbons by vapor pressure and dispersive interactions. The second dimension column was a Restek Rxi-17SilMS (2.0 m × 0.25 mm × 0.25 µm), which was selected to retain functionalized compounds in the second dimension though π - π interactions between the 50% diphenyl stationary phase of the column and the π electrons in the analytes. The first dimension oven was programmed to maintain 40 °C for 0.2 minutes after a 1µL 1:100 split injection and increase at 1.5 °C/min to 315 °C. The modulator temperature was offset at 15 °C and operated with a 2.5 second modulation period. The second dimension oven was offset by 5 °C. The mass spectrometer operated with a 700 second acquisition delay, 250 °C ion source temperature, and -70eV ionization energy. The collected mass range was 30-550 amu at 200 Hz at a mass resolution of 25,000.

High-resolution mass spectrometry allows for the utilization of Kendrick mass defect as an analytical tool in hydrocarbon speciation.²⁵ The International Union of Pure and Applied Chemistry (IUPAC) defines the exact mass of carbon to equal 12.00000 Da. The Kendrick scale normalizes the mass of a CH_2 unit to the mass of 14.00000 Da. This scaling allows for a facile identification of hydrocarbon homologous series by exploiting their common mass defect, as defined by the difference between the exact Kendrick mass and the nominal Kendrick mass (Equation 3-1).²⁵

Kendrick mass = accurate mass m/z ×
$$\left(\frac{14.00000}{14.01565}\right)$$
 (3-1)

Kendrick mass defect = nominal Kendrick mass - Kendrick mass

Homologous series of hydrocarbons differing by the presence of heteroatoms (N, O, S) in their structure or different ring and double bond substitutions will have unique Kendrick mass defect values. A Kendrick mass defect plot (KMD) thereby visualizes the complete mass spectra of hydraulic fracturing flowback fluids by plotting the Kendrick mass defect against the nominal Kendrick mass, which allows the horizontal alignment of each hydrocarbon series.

3.3.4 QA/QC

All data were subjected to stringent quality assessments. Surrogate standards were used to assess extraction efficiency. The recovery of the surrogate standard *p*-terphenyl-D14 (spiked at 200 pg/ μ L in final extract) exceeds 60% in all samples, indicating acceptable extraction efficiency, as it falls within the EPA recovery guideline of 33-141% for water samples.²⁹ *p*-terphenyl-D14 was used as the most representative surrogate for this study, as it is closest in chemical functionality to the range of identified compounds discussed in later sections. A laboratory blank consisting of MilliQ water (Millipore, Billerica, MA) was assessed alongside the

samples, and was free of any major contaminants including hydrocarbons within limits of detection (ca 1.0-10 ng/L sample). Toluene was identified in the laboratory blank, likely due to the large-scale handing of the solvent in our laboratory and was excluded from further consideration. To evaluate injection repeatability, all samples were analyzed at least three times. However, due to high-resolution instrument availability limitations, composition repeatability was formally assessed using a nominal mass GC×GC-TOFMS system (LECO Pegasus 4D GC×GC TOFMS, St. Joseph, MI). The high-resolution time-of-flight mass spectrometer was mass calibrated externally using perfluorotributylamine (PFTBA). The mass accuracy tolerance of PFTBA was set a 1 ppm for m/z 68.9947, 130.9915, 218.9851, and 413.9770. Additionally, PFTBA was infused during the acquisition and subsequently used as an internal mass calibrant in all spectra. For all analyses, the mass tolerance was set to the larger of 5 ppm or 0.005 Da. This was implemented to account for discrepancy in the relative mass tolerance at lower masses where the ppm scale constrains to only one or two possible formulas.

3.3.5 GC×GC Data Analysis

Data processing was performed using a beta-test version of LECO ChromaTOF[®] for HRT software, version 1.92.28.0. Automated peak finding was performed utilizing highresolution deconvolution algorithms for all peaks with a signal-to-noise ratio (S/N) greater than 100. To ease mass spectral library searching, PFTBA background ions were removed from spectra. Peak assignments were based on a forward search in the NIST 2014 library for peaks with a spectral similarity score of 700. GC×GC subpeaks required a similarity score of 500 to recombine. Data were visualized through Kendrick mass defect plots generated in ChromaTOF[®], which plot the Kendrick mass defect (y-axis) against the nominal mass (x-axis). This visualization strategy creates one simplified plot to represent all of the high-resolution mass spectral data with easy identification of homologous series that align in a single row with each point representing an addition of a CH₂ unit. Hydrocarbons with different ring and double bond equivalent (RDBE) and heteroatomic substitutions have unique Kendrick mass defects and thus align in unique horizontal patterns. Additionally, the use of coloration in the plot can help distinguish individual compound classes and bubble radius corresponds to the intensity of the ion in the summed deconvoluted spectra.

3.3.6 Temporal Distribution Plots

The Spectral Analysis Tools built into the ChromaTOF[®] software generate Kendrick mass defect plots using only mass spectral information. A direct link to chromatographic information, such as elution profiles,^{30,31} has yet to be reported with two-dimensional chromatography. To generate temporal distribution plots based on Kendrick mass defect, peak lists and the deconvoluted mass spectra were exported in .csv format after a basic processing step in ChromaTOF[®]. Subsequently, this data were processed using Eigenvectors MATLAB v.R2016b. In house developed algorithms were applied to calculate the KMD for each fragment. Screening for fixed KMD values allowed for a sophisticated search of specific fragments or fragmentation patterns. Combination with knowledge based rules, as described in Weggler et. al,³² Welthagen et. al,³³ and Vogt et.al³⁴ delivered refined results and allow for group type analysis of the GC×GC -HRT data in less than 20 seconds per sample. The figures generated through this process, termed temporal distribution plots, were used to demonstrate hydrocarbon fingerprints based on Kendrick mass defect.

3.4 Results and Discussion

3.4.1 Hydrocarbon Homogeneity

The analysis of four Marcellus shale gas wells in close geographic proximity revealed several similarities among the samples. Through the assessment of hydrocarbon distributions by GC×GC separations (Figure 3-1), samples A, B, and D all look remarkably similar. Each of these three samples contains a combination of alkane, monocyclic and bicyclic hydrocarbons, centered between C₉ and C₂₀ *n*-alkanes, as determined by the retention times of an alkane standard. Sample C was overall more chemically complex containing longer *n*-alkanes (up to C₃₀) and numerous aromatic compounds. The observed hydrocarbon ranges are to be expected for samples originating from hydraulic fracturing processes^{11,13} and are quite comparable to flowback fluids in the Fayetteville Formation.¹¹ The hydrocarbons in samples A-D are mostly shale-derived, exhibiting the chemical signature of the rock itself, with a small subset of chemical additives and transformation products.^{11,13} However, even in the small geographic region studied, well-to-well differentiation is quite possible upon more detailed data analysis. It should be noted that the discussion presented herein is not aimed at the identification of individual components in flowback samples, but rather presents a broader analysis focusing on uncovering patterns and anomalies that can be exploited in point-source identification of environmental contamination events in regions with shale gas development.

3.4.2 Hydrocarbon Heterogeneity

The sedimentary nature of shale presents opportunity for sample-to-sample heterogeneity even in small geographic areas. Because all samples were collected within a week of flowback onset, the observed chemical differences cannot be the attributed to the timing of sampling events. Cyclic and heteroatomic substituted hydrocarbons are two categories where samples appear to be differentiated to the greatest degree. Differences between the four samples are best revealed by comparing compounds eluting around a retention time of 0.5 seconds in the second dimension (Figure 3-1). This region is mostly dominated by monocyclic species such as alkylated cyclohexanes. Sample B contained the lowest percentage of cyclohexane homologues among the four samples (21%), while the monocyclic class represented 30% and 29% of Samples A and D, respectively. At retention times of 0.75-1 seconds in the second dimension retention profiles are dominated by saturated bicyclic components such as hydronaphthalenes. As was observed with the monocyclic components, Sample B contained the fewest percentage of hydronaphthalene analogs (7%). However, distinction between Samples A and D becomes more obvious when analyzing the hydronaphthalene region, as 11% of Sample A and 9% of Sample D is comprised of this class of compounds. Of the four samples, Sample C is the only flowback fluid with a significant aromatic portion, with alkylated benzene analogs eluting between retention times of 1-1.5 seconds in the second dimension and naphthalenes eluting between retention times of 1.5-2 seconds in the second dimension. Sample A also contained a small fraction (<1%) of alkylated benzene species, however diaromatic compounds were absent in Samples A, B, and D.



Figure 3-1: The GC×GC-HRT total ion chromatograms for flowback fluids from four geographically nearby wells (samples A-D). The GC×GC separation shows sample-to-sample differentiation among the more polar components, such as cyclohexanes and hydronaphthalenes. Samples A, B and D contained hydrocarbon distributions centered between C₉ and C₂₀ *n*-alkanes, while sample C was overall more chemically complex containing longer *n*-alkanes (up to C₃₀) and numerous aromatic compounds.

To visualize the high-resolution mass spectral data with greater ease, Kendrick mass defect plots were constructed for each flowback sample and are shown in Figure 3-2. Each point on the Kendrick mass defect plot corresponds to a unique ion in the total deconvoluted spectrum whose formula is known from the exact mass (within 5 ppm) and is color coded according to chemical class. Samples were screened by chemical formulas calculated from the high resolution mass spectral data for compound classes common in petroleum systems including: CH, O, O₂, O₃, N, NO, NO₂, S, SO, SO₂, SO₃, SO₄. All compound classes include both the whole- and half-integer RDBE ions, where in EI ionization only whole-integer RDBE ions can correspond to

molecular formulas with half-integer RDBE ions being indicative of fragments. The use of both whole- and half-integer RDBE formulas helps negate lack of or low abundance molecular ions in EI ionization by also classifying fragment ions. More than 95% of all peaks were classified in all of the samples into the above classes; however, no compounds belonging to SO, SO₃, SO₄ classes were detected in any of the samples. The CH class represents ions without heteroatomic substitutions and therefore covers a large range of hydrocarbons including alkanes, cyclohexanes, hydronaphthalenes, and PAHs, which are common in shale organic matter.¹³ Most hydraulic fracturing additives are highly oxygenated and may present in the O and O₂ classes, which include alcohol, ether, ketone, carboxylic acid, and polyalcohol functionalities.²



Figure **3-2:** The Kendrick mass defect plots generated from the total deconvoluted time-of-flight mass spectra operated at 25,000 resolving power for flowback fluids from four geographically nearby wells (samples A-D). Each well exhibits a unique hydrocarbon pattern especially with respect to heteroatomic containing ions.

The KMD plots (Figure 3-2) show differentiation among the heteroatomic species in each sample that are not apparent in the GC×GC chromatograms. Interestingly, Sample B, which was hydraulically fractured by a different operator from the other 3 samples, did not contain any ions in the N class. While this could be attributed to differences in hydraulic fracturing fluid compositions, this is unlikely as the FracFocus disclosure of Sample B did contain nitrogenated components such as acrylamide polymers.² Additionally, the hydraulic fracturing fluid of sample B contained fewer oxygenated components when compared to Samples A, C, and D, yet still exhibited a complex pattern on O and O₂ class ions. Sample A contained homologous series of both O and O₂ classes, while Sample C only contained one homologous series of the O₂ class and

Sample D did not contain homologues of either O or O_2 classes. Since Samples A, C, and D were all hydraulically fractured using similar formulations, it is not believed that the chemical additives are contributing to the differences in the O and O_2 classes observed among these samples. Instead, we postulate that these components reflect the heterogeneity of the shale and caution the use of FracFocus or other disclosure databases to identify compound origins in flowback fluids, not only due to the proprietary nature of some additives, but also due to additive interactions with the shale. Sample C was the only sample which contained homologues of NO and S classes. Due to the high RDBE values of these ions, it can be inferred that NO and S moieties were incorporated into aromatic structures. Of the four samples, Sample D contained the fewest heteroatomic substituted species, as 99% of the peaks in this sample belonged to the CH class.

Recent research^{9,11} suggests the formation of halogenated species and disinfection byproducts (DBPs) in down-bore transformations of hydraulic fracturing fluids. Upon evaluation of the data for halogenated products, no probable formulas within 5 ppm mass accuracy could be assigned in any of the four samples herein. The analysis of the source water used in fracturing these four wells from the Susquehanna River showed no indication of halogenated DBPs.¹³ We believe that the wide spread prevalence of DBP contamination in surface waters across the United States may contribute to the occurrence of DBPs in flowback fluids.³⁵ Additionally, the wells analyzed in this study have been hydraulically fractured with either no or minimal amounts of recycled flowback or produced waters. Recycling practices include a disinfection step, usually though chlorination, which can generate DBPs through reaction with the organic matter present in the flowback and produced waters. The lack of DBPs in these samples is consistent with the fact that recycling practices were not used during the hydraulic fracturing of these wells.

3.4.3 Temporal Distribution

The combination of GC×GC with high-resolution mass spectrometry offers a complex, multi-dimensional data set that can be exploited with chemometric techniques. While Kendrick mass defect plots offer a reduced view of all mass spectra acquired during the chromatographic run, they do not offer any retention time information that may further distinguish one sample from another. Ortiz et al. utilized one-dimensional GC separations followed by FT-ICR to create compound-class specific elution profiles for oil sands processed water samples.³⁰ This study was the first to visualize chromatographic information with Kendrick mass defect.³⁰ Here, we expanded upon that technique by visualizing Kendrick mass defect against the two-dimensional chromatographic retention times. Not only does this novel and data-rich visualization technique, which we term a "temporal distribution plot", combine the high-resolution mass spectral information with the GC×GC separation, but it also serves to further distinguish sample-to-sample variability. Additionally, this combined plot can replace both a GC×GC chromatogram and a Kendrick mass defect plot by showing the data from both techniques in one comprehensive and easy to read figure.

Combination of the Kendrick mass defect, knowledge based rules (KBR) and chromatographic information allows the detailed determination of hydrocarbon fingerprints. For the four flowback samples, KBR for saturated hydrocarbons, unsaturated hydrocarbons (and saturated ring structures), decahydronaphthalenes, tetrahydronaphthalenes, benzenes and naphthalenes were applied, as described in the Materials and Methods section. Here, we focused on expanding upon the CH class of the Kendrick mass defect plots, as this class was the most prominent in the four flowback samples and contained a multitude of different hydrocarbon species. Figure 3-3 shows temporal (chromatographic) distribution these classes. As expected from the GC×GC separation, the hydrocarbon classes form structured patterns in the chromatographic space, aiding the visualization of each sample. This technique provides class– specific elution profiles that are more clearly distinguished from the total ion chromatograms (Figure 3-1) and allows for a direct qualitative and semi-quantitative comparison between samples. While Samples B and D were difficult to distinguish by looking at the total ion chromatograms (Figure 3-1), upon examining the temporal distribution plots (Figure 3-3), differentiation among the two samples is quite apparent, especially in the decahydronaphthalene class. Sample B contained two homologous series of decahydronaphthalenes while sample D contained three. Additionally, the differences between the individual samples become even more prominent by the addition of the mass spectral response (peak area). In Figure 3-4 the mass spectral response for each sample, as described in above, is depicted in a pie chart representation. This hydrocarbon fingerprint allows for a detailed discrimination between the investigated samples.



Figure **3-3:** Temporal distribution plots for flowback fluids from four nearby wells (samples A-D). This visualization strategy combines the GC×GC separation Kendrick mass defect information to further distinguish the samples from one another. Bubble radii are scaled to ion abundance.



Figure **3-4:** Pie chart representation of mass spectral response assigned to hydrocarbon classes for wells A-D. Clear discrimination between the flowback fluids is observable in aromatic hydrocarbons (wells A and C) and saturated hydrocarbons (wells B and D).

3.4.4 Geochemical Significance

As expected, the chemical complexity of well C served to distinguish this well from the remaining three. Interestingly, the hydrocarbon distribution of sample B, which was hydraulically fractured by a different operator than samples A, C, and D, did not appear to be influenced by the differences in hydraulic fracturing fluid composition, as its chemical distribution was closely related to samples A and D. Additionally, the use of recycled flowback fluids during hydraulic fracturing of Sample D did not appear to impact the chemistry of the flowback fluids upon the qualitative analysis. This is consistent with studies of hydraulic fracturing fluids and flowback flowback chemistry is mostly influenced by shale

geochemistry.¹³ The chemistry of the flowback fluids amongst these four wells seems to be more related to their geographic distribution, as wells B and D are more proximately located when compared to well A. However, the geographic trend does not hold when considering well C, as the chemistry of the flowback fluids originating from this well are the most dissimilar and yet this wellhead is located between wells B and D. Interestingly, upon examining the initial gas productivity (first 1-6 months) of these wells (Table 3-1), well B was the most productive, followed by wells C, D, and A. Since no trend is observed between the hydrocarbon present in the flowback fluids and well productivity in these samples, local variability of source rock cannot be directly correlated to the observed chemistry. The data show that the closest relationship, although unexpected, appears to be well depth. Upon considering the stratigraphy and elevation of the evaluated wells, differences that may account for the observed chemistry among the wells were uncovered. Drilling logs were used to determine the strata that were subjected to hydraulic fracturing. In the case of wells A, B, and D the true vertical depth was an average of 253±6 feet below the Cherry Valley member of the Marcellus shale. On the contrary, the true vertical depth of well C was shallower than that of the other three wells, only reaching 199 feet below the Cherry Valley member of the Marcellus shale. This stratigraphic variation may explain the hydrocarbon differentiation between these wells as the shale that was exploited in wells A, B, and D would be more stratigraphically correlated than well C. However, these proposed trends may only be valid due to the small geographic grouping of the wells evaluated herein, and should be further evaluated using larger sample sizes.

	Gas (Mcf/day)		
Well A	3806.1		
Well B	8786.3		
Well C	7334.8		
Well D	6475.4		

3.5 Conclusion

GC×GC with high-resolution time-of-flight mass spectrometry was applied to flowback fluids from four Marcellus shale gas wells in close proximity to each other. This petroleomics approach characterized the hydrocarbon heterogeneity within a small geographic area offering insight into factors that differentiate the chemistry of flowback fluids. Cyclic and heteroatomic substituted hydrocarbons showed the greatest degree of differentiation between the four samples, even though these components were less abundant than alkanes. GC×GC-HRT offered superior resolution of these components through the high peak capacity of GC×GC separation and the resolving power of the HRT that highlighted the heteroatomic species. This work highlights that the composition of the hydraulic fracturing fluids was not a large differentiating factor of flowback composition, as seen in the heteroatomic classes in the Kendrick mass defect plots. Additionally, generating plots that combine GC×GC separation with KMD established a novel data-rich visualization technique that further differentiated the samples and suggested that hydrocarbon distributions in flowback fluids were related to the depth of the wells as opposed to the wellhead location, at least within this sample set. Due to high well pad density and the proprietary nature of hydraulic fracturing additives the fingerprinting of environmental contamination events in shale gas systems is challenging. Thus, determining factors of differentiation among flowback fluids, especially in small geographic areas such as done in this study, can lead to more informed environmental forensic investigations of shale gas impacts by identifying factors that can better determine point-source of contamination.

3.6 References

(34) Howarth, R. W.; Ingraffea, A. Natural Gas: Should Fracking Stop? *Nature*. 2011, pp 271–273.

(35) Frac Focus Chemical Disclosure Registry. <u>https://fracfocus.org</u>. (Accessed August 20, 2017).

(36) Thurman, E. M.; Ferrer, I.; Blotevogel, J.; Borch, T. Analysis of Hydraulic Fracturing Flowback and Produced Waters Using Accurate Mass: Identification of Ethoxylated Surfactants. *Anal. Chem.* **2014**, *86* (19), 9653–9661.

(37) Thurman, E. M.; Ferrer, I.; Rosenblum, J.; Linden, K.; Ryan, J. N. Identification of Polypropylene Glycols and Polyethylene Glycol Carboxylates in Flowback and Produced Water from Hydraulic Fracturing. *J. Hazard. Mater.* **2017**, *323*, 11–17.

(38) Ferrer, I.; Thurman, E. M. Analysis of Hydraulic Fracturing Additives by LC/Q-TOF-MS. *Anal. Bioanal. Chem.* **2015**, *407* (21), 6417–6428.

(39) Lester, Y.; Ferrer, I.; Thurman, E. M.; Sitterley, K. A.; Korak, J. A.; Aiken, G.; Linden, K. G. Characterization of Hydraulic Fracturing Flowback Water in Colorado: Implications for Water Treatment. *Sci. Total Environ.* **2015**, *512–513*, 637–644.

(40) Llewellyn, G. T.; Dorman, F.; Westland, J. L.; Yoxtheimer, D.; Grieve, P.; Sowers, T.; Humston-Fulmer, E.; Brantley, S. L. Evaluating a Groundwater Supply Contamination Incident Attributed to Marcellus Shale Gas Development. *Proc. Natl. Acad. Sci.* **2015**, *112* (20), 6325–6330.

(41) Thacker, J.; Carlton, D.; Hildenbrand, Z.; Kadjo, A.; Schug, K. Chemical Analysis of Wastewater from Unconventional Drilling Operations. *Water* **2015**, *7* (4), 1568–1579.

(42) Luek, J. L.; Schmitt-Kopplin, P.; Mouser, P. J.; Petty, W. T.; Richardson, S. D.; Gonsior, M. Halogenated Organic Compounds Identified in Hydraulic Fracturing Wastewaters Using Ultrahigh Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2017**, *51* (10), 5377–5385.

(43) Kahrilas, G. A.; Blotevogel, J.; Stewart, P. S.; Borch, T. Biocides in Hydraulic Fracturing Fluids: A Critical Review of Their Usage, Mobility, Degradation, and Toxicity. *Environ. Sci. Technol.* **2014**, *49* (1), 16-32.

(44) Hoelzer, K.; Sumner, A. J.; Karatum, O.; Nelson, R. K.; Drollette, B. D.; O'Connor, M. P.; D'Ambro, E. L.; Getzinger, G. J.; Ferguson, P. L.; Reddy, C. M.; et al. Indications of Transformation Products from Hydraulic Fracturing Additives in Shale-Gas Wastewater. *Environ. Sci. Technol.* 2016, *50* (15), 8036–8048.

(45) Orem, W.; Tatu, C.; Varonka, M.; Lerch, H.; Bates, A.; Engle, M.; Crosby, L.; McIntosh, J. Organic Substances in Produced and Formation Water from Unconventional Natural Gas Extraction in Coal and Shale. *Int. J. Coal Geol.* **2014**, *126*, 20–31.

(46) Piotrowski, P. K.; Weggler, B. A.; Barth-Naftilan, E.; Kelly, C. N.; Zimmermann, R.; Saiers, J. E.; Dorman, F. L. Non-Targeted Chemical Characterization of a Marcellus Shale Gas Well through GC \times GC with Scripting Algorithms and High-Resolution Time-of-Flight Mass Spectrometry. *Fuel* **2018**, *215*, 363–369.

(47) Regnery, J.; Coday, B. D.; Riley, S. M.; Cath, T. Y. Solid-Phase Extraction Followed by Gas Chromatography-Mass Spectrometry for the Quantitative Analysis of Semi-Volatile Hydrocarbons in Hydraulic Fracturing Wastewaters. *Anal. Methods* **2016**, *8* (9), 2058–2068.

Butkovskyi, A.; Bruning, H.; Kools, S. A.; Rijnaarts, H. H.; Van Wezel, A. P. Organic
Pollutants in Shale Gas Flowback and Produced Waters: Identification, Potential Ecological
Impact, and Implications for Treatment Strategies. *Environ. Sci. Technol.* 2017, *51*(9), 4740-4754.

(49) Warner, N. R.; Christie, C. A.; Jackson, R. B.; Vengosh, A. Impacts of shale gas wastewater disposal on water quality in western Pennsylvania. *Environ. Sci. Technol.* **2013**, *47*(20), 11849-11857.

(50) Lauer, N. E.; Harkness, J. S.; Vengosh, A. Brine spills associated with unconventional oil development in North Dakota. *Environ. Sci. Technol.* **2016**, *50*(10), 5389-5397.

(51) Tasker, T. L.; Piotrowski, P. K.; Dorman, F. L.; Burgos, W. D. Metal Associations in Marcellus Shale and Fate of Synthetic Hydraulic Fracturing Fluids Reacted at High Pressure and Temperature. *Environ. Eng. Sci.* **2016**, *33* (10), 753–765.

(52) Drollette, B. D.; Hoelzer, K.; Warner, N. R.; Darrah, T. H.; Karatum, O.; O'Connor, M. P.; Nelson, R. K.; Fernandez, L. A.; Reddy, C. M.; Vengosh, A.; et al. Elevated Levels of Diesel Range Organic Compounds in Groundwater near Marcellus Gas Operations Are Derived from Surface Activities. *Proc. Natl. Acad. Sci.* **2015**, *112* (43), 13184–13189.

(53) Cozzarelli, I. M.; Skalak, K. J.; Kent, D. B.; Engle, M. A.; Benthem, A.; Mumford, A. C.; Haase, K.; Farag, A.; Harper, D.; Nagel, S. C.; et al. Environmental Signatures and Effects of an Oil and Gas Wastewater Spill in the Williston Basin, North Dakota. *Sci. Total Environ.* **2017**, *579*, 1781–1793.

(54) Marshall, A.G.; Rodgers, R.P. Petroleomics: the next grand challenge for chemical analysis. *Accounts Chem. Res.* **2004**, *37*(1), 53-59.

(55) Marshall, A. G.; Rodgers, R. P. Petroleomics: Chemistry of the underworld. *P. Natl. Acad. Sci. USA.* **2008**, *105*(47), 18090-18095.

(56) He, Y.; Flynn, S. L.; Folkerts, E. J.; Zhang, Y.; Ruan, D.; Alessi, D. S.; Martin, J. W.; Goss, G. G. Chemical and Toxicological Characterizations of Hydraulic Fracturing Flowback and Produced Water. *Water Res.* **2017**, *114*, 78–87.

(57) Byer, J. D.; Siek, K.; Jobst, K. Distinguishing the C3 vs SH4 Mass Split by Comprehensive Two-Dimensional Gas Chromatography–High Resolution Time-of-Flight Mass Spectrometry. *Anal. Chem*, **2016**, *88*(12), 6101-6104.

(58) Kendrick, E. A Mass Scale Based on CH2 = 14.0000 for High Resolution Mass Spectrometry of Organic Compounds. *Anal. Chem*, **1963**, *35*(13), 2146-2154.

(59) United States Environmental Protection Agency, Specifications And Guidance For Contaminant-Free Sample Containers, **1992.**

(60) United States Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, **1983.**

(61) United States Environmental Protection Agency. Method 3510C (SW-846): Separatory Funnel Liquid-Liquid Extraction, **1996**, Revision 3.

(62) United States Environmental Protection Agency. Method 625 (SW-846): Base/Neutrals and Acids. **1984.**

(63) Ortiz, X.; Jobst, K.J.; Reiner, E.J.; Backus, S.M.; Peru, K.M.; McMartin, D.W.; O'Sullivan, G.; Taguchi, V.Y; Headley, J.V. Characterization of naphthenic acids by gas chromatography-Fourier transform ion cyclotron resonance mass spectrometry *Anal. Chem*, **2014**, *86*(15), 7666-7673.

(64) Harvey, P. M.; Shellie, R. A. Data reduction in comprehensive two-dimensional gas chromatography for rapid and repeatable automated data analysis. *Anal. Chem*, **2012**, *84*(15), 6501-6507.

(65) Weggler, B. A.; Groger, T.; Zimmermann, R. Advanced scripting for the automated profiling of two-dimensional gas chromatography-time-of-flight mass spectrometry data from combustion aerosol *J. Chromatog. A.* **2014**, *1364*, 241-248.

(66) Welthagen, W.; Shellie, R. A.; Spranger, J.; Ristow, M.; Zimmermann, R.; Fiehn, O. Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC ×

GC-TOF) for high resolution metabolomics: biomarker discovery on spleen tissue extracts of obese NZO compared to lean C57BL/6 mice. *Metabolomics*, **2005**, *1*(1), 65-73.

(67) Vogt, L.; Gröger, T.; Zimmermann, R. Automated compound classification for ambient aerosol sample separations using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *J. Chromatog. A.* **2007**, *1150*(1), 2-12.

(68) United States Environmental Protection Agency. Controlling Disinfection By-Products and Microbial Contaminants in Drinking Water. 2001.

(69) Gas Production Reports.

https://www.paoilandgasreporting.state.pa.us/publicreports/Modules/Welcome/Agreement.aspx. (Accessed January 20, 2018).
Chapter 4

Applications of Thermal Desorption coupled to GC×GC-TOFMS for Hydrocarbon Fingerprinting of Hydraulically Fractured Shale Rocks

4.1 Abstract

Development of shale gas resources through the use of hydraulic fracturing has raised a multitude of environmental concerns and motivated research towards the understanding of shale gas systems. Previous research has demonstrated the potential of utilizing hydrocarbon distributions towards the fingerprinting of a potential environmental contamination event arising from shale gas operations. However, to apply hydrocarbon distributions from shale gas wells towards point-source identification and apportionment, a better understanding of hydrocarbon origins must be achieved. Here we present an efficient and repeatable thermal desorption method, as a sample introduction methodology for GC×GC analysis of shale rock samples that results in comparable chromatograms to those produced by solvent extraction. This novel and robust characterization technique of shale cores from Marcellus and Utica formations by thermal desorption followed by GC×GC enables the understanding of hydrocarbon speciation within the native rock with minimal sample preparation time and solvent use. The detailed shale chemistry gives insight into utilizing hydrocarbon differences towards point-source identification methodologies of environmental contamination events associated with unconventional gas development. Additionally, this analytical technique may provide a more detailed analysis of hydrocarbons than what is currently implemented in the industry to pinpoint the most advantageous areas to exploit by hydraulic fracturing, yet avoiding undesirable areas such as those with a high abundance of sulfur containing compounds.

4.2 Introduction

Political and socioeconomic conditions coupled with advances in horizontal drilling have enabled the exploitation of shale gas resources through hydraulic fracturing.¹ The rapid development of shale gas has raised a multitude of environmental concerns especially with regards to water resources, which can be impacted through surface spills of hydraulic fracturing fluids and wastewaters as well as insecure well casings.²⁻⁸ After hydraulic fracturing, the initial wastewater returning to the surface is known as flowback water while the wastewater generated throughout the well production life is called produced waters. Both wastewaters can be chemically complex with high salinity and complex distributions of organic compounds.⁹⁻¹²

The hydrocarbons that are found in produced waters are hypothesized to largely originate from the shale rocks and utilizing hydrocarbons towards the fingerprinting of contamination from shale gas development has been proposed.¹⁰⁻¹² To date, studies have employed comprehensive two-dimensional gas chromatography (GC×GC) coupled to time-of-flight mass spectrometry (TOFMS) to study hydrocarbon signatures in shale gas related systems. Analyses of hydraulic fracturing fluids and subsequent flowback water from the same well demonstrated that the chemical signatures from materials added to the injected fluids are lost in the flowback chemistry yet the hydrocarbon speciation remained consistent during the flowback period.¹¹ Additionally, analyses of flowback water from nearby wells indicated a unique hydrocarbon pattern in each case.¹² However, to apply hydrocarbon distributions from shale gas wells towards point-source identification and apportionment, a better understanding of hydrocarbon origins must be achieved. Additionally, the development of analytical methodologies that may allow for more rapid characterization by reducing time of sample preparation and materials required would be very advantageous. Thermal desorption as a sample introduction technique for GC×GC analysis has typically been applied to the analysis of volatile and semi-volatile compounds pre-sorbed to solid-phase adsorbents. Thermal desorption directly from solid samples has yet to become widespread.¹³ During thermal desorption, heat is applied to extract the volatile and semi-volatile analytes (\leq n-C40) from a solid sample matrix.¹⁴ The extraction occurs under a high flow of an inert gas and the analytes undergo cryo-trapping prior to transfer onto the GC column. The thermal desorption technique enhances detection limits by 3 to 4 orders of magnitude and can achieve 95% or better desorption efficiencies as compared to the 30-80% extraction efficiencies achieved by solvent extraction methods. This methodology can also be considered "green chemistry," as it eliminates solvent use and the human and environmental health hazards associated with solvent handling and disposal.

No reports of direct hydrocarbon analyses from hydraulically fractured shales have been reported. Previously published characterizations of shale hydrocarbons have focused on oil producing formations with targeted methodologies to establish hydrocarbon thermal maturity.¹⁵⁻¹⁷ A commercial instrument, Rock-Eval, (Vinci Technologies, France) has been previously reported for the analysis of petroleum source rocks by thermal desorption.¹⁵ The Rock-Eval methodology measures the total amount of hydrocarbons desorbed from a crushed rock sample by pyrolysis at 358°C followed by a fast GC-FID analysis.¹⁵ While this methodology produced the first thermal desorption chromatograms of shale that mirrored chromatograms of solvent extracted samples, the fast GC-FID analysis results in large unresolved complex mixtures (UCM) that only provide a crude total carbon measurement and lack information about the detailed chemistry of the shale.¹⁶⁻¹⁷ Interestingly, no studies of shale hydrocarbons have coupled thermal desorption to comprehensive two-dimensional chromatography, an area of study expanded upon herein.

This study describes the coupling of thermal desorption to GC×GC with applications to the characterization of shale rock cores from hydraulically fractured formations. A robust, repeatable,

and efficient method was developed and applied to two shale formations - Marcellus Shale and Utica/Point Pleasant Shale. Unique hydrocarbon speciation for each formation is demonstrated, likely a consequence of the sedimentary nature of shale and lends itself to spatial variation that reflects the conditions present during formation. This hydrocarbon signature may create a location-specific fingerprint that could have potential in point-source identification techniques. Thermal Desorption coupled to GC×GC-TOFMS may also provide a more detailed analysis of hydrocarbons than Rock-Eval methods to pinpoint the most advantageous areas to exploit by hydraulic fracturing, yet avoiding areas with a high abundance of sulfur containing compounds that may lead to soured gas.

4.3 Materials and Methods

4.3.1 Thermal Desorption of Shale Cores

Shale cores from the Marcellus and Utica formations were obtained from the Pennsylvania Core Repository in Harrisburg, PA (EGSP1) and the Ohio Core Repository in Columbus, OH (Core # 2936 and 3372). All method development was performed on a Marcellus Shale core obtained from the Eastern Gas Shales Project (EGSP) due to material availability. The obtained cores were pulverized to mesh < 300 µm and approximately 2 mg of material was weighed into micro vial inserts. A small plug of deactivated glass wool (Restek, Bellefonte, PA) was placed on top of the micro vial insert to prevent any transfer of solid material into the GC inlet. All thermal desorption experiments were carried out on a GERSTEL Thermal Desorption Unit (TDU) connected directly to a GERSTEL Cooled Injection System (CIS) (GERSTEL Inc., Linthicum, MD). The GERSTEL Multipurpose Sampler (MPS-2) was used to directly inject 1 µL of SV Internal Standard (Restek, Bellefonte, PA) at a concentration of 200 pg on-column directly into the TDU to allow for semi-quantitative comparisons.

The thermal desorption of hydrocarbons present in the shale cores was carried out under helium at 50 mL/min and programmed initially at 30°C followed by a 12°C/sec ramp to 350°C with a 5 min hold. The desorbed analytes were cryogenically focused in the CIS, which was programmed to -120°C for 0.2 min followed by a 12°C/sec ramp to 275°C with a 5 min hold.

4.3.2 GC×GC-TOFMS Analysis

The samples were analyzed by comprehensive two-dimensional gas chromatography coupled to a time of flight mass spectrometer (LECO Pegasus 4D GC×GC-TOFMS, St. Joseph, MI) using previously reported methodologies which have been optimized for hydrocarbons present in hydraulic fracturing wastewaters.^{11,12} Briefly, the first dimension column was a Restek Rtx-DHA100 (100 m × 0.25 mm × 0.5 μ m) coupled to a Restek Rxi-17SilMS (2.0 m × 0.25 mm × 0.25 μ m) in the second dimension. A slow temperature ramp of 2.0°C/min was used from 40°C to 315°C under a constant 2 mL/min He flow rate. The modulator temperature was offset at 15°C positive to the primary GC oven and operated with a 3 sec. modulation period. The second dimension oven was offset by 5°C to the primary GC oven. The mass spectrometer was operated with a 250°C ion source temperature, and –70 eV ionization energy. The collected mass range was 30–550 amu at 200 Hz with a positive mass defect offset of 50 mu/100u.

4.3.3 Soxhlet Extractions

For method validation purposes, the EGSP core that was used for thermal desorption method development was also Soxhlet extracted in triplicate and analyzed using GC×GC-TOFMS. A modified USEPA Method 3540C was used for the Soxhlet extraction of ~1g of the pulverized shale core along with ~3g of MgSO₄ (Avantor Performance Material, JT Baker, Center Valley, PA). The solid sample was fortified with 2-methylnaphthalene-d10 and fluoranthrene-d10 (Restek, Bellefonte, PA) at a concentration of 200 pg/ μ L in final extract as a quality control measurement of extraction efficiency. 200 mL of 50:50 actetone:dichloromethane (Avantor Performance Material, JT Baker, Center Valley, PA) was cycled every ~12 minutes for 24 hours. The extracts were concentrated to a final volume of 500 μ L in a Kuderna-Danish apparatus and stored at 4°C until analysis. The extracts were analyzed using GC×GC-TOFMS methodologies analogous to those used in thermal desorption but adapted for a 1 μ L splitless liquid injection. Quantitative analysis was performed using DRO and PAH standards (Restek Bellefonte, PA).

4.3.4 Data analysis

Data processing was performed using LECO ChromaTOF® software version 4.50.8.0. The baseline level was offset to be just above the noise (1.0), and automated peak finding was performed utilizing mass spectral deconvolution algorithms built into the ChromaTOF® software. For identification, peaks required a signal-to-noise ratio (S/N) greater than 1000. Tentative identifications were based on a forward search in the NIST14 library for peaks with a spectral similarity score of 700. GC×GC subpeaks required a S/N greater than 6 and a spectral similarity score of 500 to recombine.

4.4 Results and Discussion

Thermal desorption is a robust sample introduction technique for GC×GC analysis. The application of thermal desorption to shale core samples from formations exploited by hydraulic fracturing allows for rapid analysis of hydrocarbon chemistry with minimal sample requirements. For example, in the validation of this technique only 2 mg of homogenized shale core were utilized for thermal desorption experiments, while 1 gram of shale material was used for Soxhlet extraction. There are multiple benefits of this method in comparison to tradition solvent-based extraction methods. Thermal desorption can improve detection limits by 3 to 4 orders of magnitude which is largely attributed to the cryogenic focusing that occurs in the Cooled Injection System. Sample preparation time is minimized when utilizing thermal desorption as Soxhlet extraction followed by Kuderna-Danish concentration requires >24 h. while thermal desorption is fully automated and can be performed in minutes. Furthermore, thermal desorption implements green chemistry practices by eliminating the use of solvents, especially chlorinated solvents such as dichloromethane that pose greater concerns in human and environmental toxicity.

4.4.1 Comparison to Soxhlet Extraction

The developed thermal desorption method was compared to Soxhlet extraction to determine the feasibility of thermal desorption against the widely accepted standard extraction technique for solid samples. The GCxGC total ion chromatograms for the thermal desorption and Soxhlet extracted Marcellus Shale samples show many similarities (Figure 4-1). Overall, unsaturated hydrocarbons are the dominant organic compounds in the shale core. Both chromatograms also contain numerous alkylated benzenes, which are common in wastewaters derived from hydraulic fracturing.¹⁰⁻¹² Upon comparing the two chromatograms it can be

observed that Soxhlet extraction excludes low molecular weight alkanes, likely due to volatilization during the concentration process. Soxhlet extraction may perform better at isolating the heaviest hydrocarbons, as thermal desorption cannot achieve temperatures suitable of volatilizing these species. However, as this is approaching the limit for gas chromatography analysis without the use of high temperature columns, this loss of high molecular weight hydrocarbons is likely insignificant with regards to the ability to adequately characterize individual samples.



Figure 4-1: A comparison of Soxhlet extraction to thermal desorption coupled to GC×GC-TOFMS. The same shale sample analyzed by thermal desorption (A) and Soxhlet extraction (B) produce nearly identical chromatograms.

Quantitative comparisons of the two methodologies resulted in comparable

concentrations (Table 4-1). With the exception of pentadecane, all concentrations are on the same order of magnitude. While the concentrations of hydrocarbons are not identical from the two extraction methodologies, it should be noted that the thermal desorption concentrations were determined semi-quantitatively through the use of an internal standard while assuming a mass spectral response factor of 1. The average response factor for the reported analytes was 1.14,

therefore the assumed response factor of 1 may account for some of the discrepancy between the concentrations of Soxhlet and thermally desorbed samples. In this study, we utilized mass spectral information to confirm compound identity. However, to improve quantitative analysis in cases where compound identity is known, coupling thermal desorption to GC×GC-FID would allow for more accurate semi-quantitative results since response factor is more consistent for hydrocarbons in FID analysis.

Analyte	Soxhlet Extraction (ng/mg)	Thermal Desorption* (ng/mg)
Anthracene	1.6	2.5
Fluorene	0.1	0.8
Heptadecane	3.5	2.0
Hexadecane	47.8	55.0
Nonadecane	1.1	1.0
Octadecane	0.5	0.8
Pentadecane	1.8	0.1
Pyrene	0.2	0.6
Tetradecane	7.7	11.0
Tridecane	15.5	34.4

Table **4-1:** Quantitative comparison of Soxhlet Extraction and Thermal Desorption of Marcellus Shale core.

* Thermal desorption concentrations were determined semi-quantitatively through the use of an internal standard while assuming a response factor of 1

A large difference between the thermally desorbed and Soxhlet extracted chromatograms (Figure 4-1) is the presence of contaminants arising from the use of the solvents in the extraction process. Specifically, the presence of acetone condensation products is observed around retention time 800, 1.25 seconds. These contaminants originated from the use of acetone as a co-extraction solvent during the Soxhlet process and undergo self-condensation via Aldol reaction and Aldol condensation mechanisms during the Kuderna-Danish evaporation steps [18]. The use of thermal desorption eliminates this contamination pathway, thus resulting in chromatograms that are easier to interpret.

4.4.2 Method Repeatability

The repeatability of the thermal desorption method was assessed by making replicate injections of the EGSP1 core. The GC×GC total ion chromatograms for two sample replicates are shown in Figure 4-2. The two chromatograms are comparable indicating the presence of alkane and mono-aromatic hydrocarbons. Each sample contained 1-2 mg of shale material and chromatographic discrepancies may be accounted for when considering mass of sample. For example, Sample 1 in Figure 4-2 shows more abundant longer chain length alkanes at primary retention time of 5500-6000 seconds than Sample 2. However, Sample 1 contained 2.0 mg of shale material while Sample 2 only contained 1.2 mg. With less shale material, the sensitivity of the analysis is compromised thus resulting in chromatographic differences. However, with more precise and repeatable sample quantities this challenge can be overcome. An average relative standard deviation for the analytes reported in Table 1 was calculated to be 13.6% for three replicates, normalized to mass of shale. This variation can be a result of heterogeneity in the shale material; however, it is comparable with other thermal desorption applications such as stir bar sorptive extraction (SBSE).¹⁹



Figure 4-2: An assessment of repeatability for the thermal desorption coupled to $GC \times GC$ -TOFMS method. Multiple aliquots of the same shale sample produce comparable chromatograms.

4.4.3 Method Efficiency

The efficiency of the thermal desorption method was assessed by desorbing the same shale material multiple times and is shown in Figure 4-3. Thermal desorption techniques typically achieve >95% desorption efficiencies as compared to the 30-80% extraction efficiencies achieved by solvent extraction methods.²⁰ Desorption efficiency was calculated as follows:

$$\frac{C_o - C_f}{C_o}$$

where C_o is the analyte concentration during the first desorption and C_f is the analyte concentration during the second desorption. Average desorption efficiency was calculated to be 96.8% for the analytes reported in Table 4-1, indicating that this methodology is applicable for shale core analysis. Comparatively, surrogate recoveries for the Soxhlet extracted samples were only 73% for floranthrene-d10 and 59% for 2-methylmapthalene-d10, indicating that Soxhlet extraction may have resulted in residues of not extracted hydrocarbon material.



Figure 4-3: An assessment of efficiency for the thermal desorption coupled to GC×GC-TOFMS method. Same aliquot subjected to a second desorption shows an absence of shale components.

4.4.4 Comparison of Hydraulically Fractured Formations

The chemistry of Marcellus and Utica Shale cores was compared by applying thermal desorption as a sample introduction technique for GC×GC-TOFMS analysis. As shown in Figure 4-4, the total ion chromatograms for the Utica Shale and Marcellus Shale are highly differentiated. Specifically, the Utica Shale contains more aromatic species with the majority of hydrocarbons centered in the C_6 - C_{15} range, as determined by a DRO standard. The sample contains numerous homologs of alkylated benzenes with up to five carbon chain lengths. Additionally, alkylated naphthalenes are also present in the Utica Shale sample with up to three carbon-substituted homologs. In comparison, the Marcellus Shale was dominated by heavier saturated hydrocarbons centered in the C_9 - C_{30} range. Compositional differences in the two shale samples are represented in a pie chart (Figure 4-5) based on peak areas. The chemistry may reflect differences in the thermal maturity or kerogen type between the two formations. The organic compounds in Utica Shale formed from Type II kerogen (planktonic), while organic

compounds in Marcellus Shale formed from a mixture of Type II and III (humic) kerogen, which would lead to the formation of heavier hydrocarbons in the Marcellus Shale.²¹⁻²²



Figure 4-4: An application for the thermal desorption coupled to GC×GC-TOFMS method. The total ion chromatogram for a core sample from the Utica Shale (A) contain a greater range of aromatic hydrocarbons as compared to a core sample of the Marcellus Shale (B) that contains mostly saturated hydrocarbons.



Figure 4-5: Pie chart representation of compositional differences between the Utica Shale (A) and Marcellus Shale (B) as determined by chromatographic peak areas.

While the hydrocarbons extracted during thermal desorption are similar to those found in flowback and produced water from hydraulic fracturing operations, there are some differences that are interesting to mention. Extraction of hydrocarbons from core material via thermal desorption extracts a greater molecular weight distribution of hydrocarbons than what is observed in flowback and produced water.¹⁰⁻¹² This may be due to spatial variability, as detailed hydrocarbon characterizations from Ohio flowback or produced waters have yet to be reported, especially that of the Utica Shale. However, the discrepancy between hydrocarbons in the shale and those in the flowback and produced waters may also originate from fractionation that occurs during the hydraulic fracturing process. It may be likely that the chemical additives in hydraulic fracturing fluids are targeting lighter hydrocarbons, thus not extracting the heavier (>C₂₀) species. Experiments towards determining the extent of extraction during hydraulic fracturing may be warranted though the use of high-temperature, high-pressure vessels.²³⁻²⁴

Thermal Desorption coupled to GC×GC-TOFMS of shale rocks may also help to pinpoint the most advantageous areas to exploit by hydraulic fracturing. It is well known that the presence of sulfur containing compounds may lead to soured gas.²⁵ Upon analysis of the GC×GC chromatograms, several alkyl-thiophenes were identified in the Marcellus and Utica cores (Table 4-2), indicating that thermal desorption is amenable for determination of sulfur containing compounds within shale rocks. These were identified first through characteristic ions and subsequently by the complete mass spectra of each compound match to the NIST 2017 mass spectral library. In this regard the compound identifications in Table 4-2 are tentative, as reference materials were not analyzed for positive identification. Alkyl-thiophenes have very characteristic mass spectra, however, so the indication of compound class is quite clear. This technique can be highly advantageous to the oil and gas industry to minimize the potential of soured gas. For example, screening drill cuttings, a waste product of a well, by thermal desorption followed by GC×GC-TOFMS analysis could by used to determine which areas of the lateral well contain lowest concentrations of sulfur containing compounds and thus may be most suitable for hydraulic fracturing. Applying such proactive techniques can be highly cost effective to oil and gas producers by preventing problems during the production of the well. Thermal desorption coupled to GC×GC-TOFMS analysis provides much more detailed characterization of shale rocks as obtaining information with such high level of detail is not possible with the current Rock Eval methodologies.

Table **4-2:** Sulfur containing compounds identified in shale core samples by thermal desorption followed by GC×GC-TOFMS analysis.

Marcellus Shale	Utica Shale
2,8-Dimethyldibenzo(b,d)thiophene	3,4-diethyl-thiophene
	2,5-diethyl-thiophene
	2,5-dimethyl-thiophene
	2,3-dimethyl-thiophene
	3-methyl-thiophene
	2-methyl-thiophene
	2,3,4-trimethyl-thiophene
	2-(1-methylethyl)-thiophene
	2,7-dimethyl- Benzo[b]thiophene
	2-propyl-thiophene
	2-ethyl-thiophene

4.5 Conclusion

Shale gas development has raised a multitude of environmental concerns and created a need for point-source identification of environmental contamination events. The technique presented herein expands the understanding of hydrocarbon differences between two gas producing shale formations though a repeatable and efficient thermal desorption GC×GC method. The described methodology can be advantageous in both environmental forensics and in industrial practice. When applied to fingerprinting of an environmental contamination event, it may be possible to achieve point-source identification through hydrocarbon distribution matching of a contamination event to drill cuttings, a waste product of drilling the well that is often collected and stored by well operators. This technique could be utilized towards an understanding of hydrocarbon heterogeneity in both geographically similar and distinct shales on a statistically relevant sample set, as only 2 mg of sample are required for each analysis enabling the use of drill cuttings. Thermal desorption coupled to GC×GC could also allow for the detailed analysis of hydrocarbons from drill cuttings that would be able to pinpoint the most advantageous areas to exploit by hydraulic fracturing in order to reach areas with highest yield potential, yet avoiding areas that may lead to soured gas by monitoring for sulfur containing compounds. The presented framework of analytical methodologies provides a sophisticated approach towards studying shale gas systems in greater detail than through Rock Eval approaches, yet also reducing time of sample preparation and materials required.

4.6 References

(1) Energy Information Administration. US Department of Energy. Hydraulically fractured wells provide two-thirds of U.S. natural gas production.

https://www.eia.gov/todayinenergy/detail.php?id=26112 (accessed 7 March 2018)

(2) Howarth, R. W.; Ingraffea, A. Natural Gas: Should Fracking Stop? *Nature*. 2011, pp 271–273.

(3) Butkovskyi, A.; Bruning, H.; Kools, S. A. E.; Rijnaarts, H. H. M.; Van Wezel, A. P. Organic Pollutants in Shale Gas Flowback and Produced Waters: Identification, Potential Ecological Impact, and Implications for Treatment Strategies. *Environ. Sci. Technol.* **2017**, *51* (9), 4740–4754.

(4) Lauer, N. E.; Harkness, J. S.; Vengosh, A. Brine Spills Associated with Unconventional Oil Development in North Dakota. *Environ. Sci. Technol.* **2016**, *50* (10), 5389–5397.

(5) Kahrilas, G. A.; Blotevogel, J.; Stewart, P. S.; Borch, T. Biocides in Hydraulic Fracturing Fluids: A Critical Review of Their Usage, Mobility, Degradation, and Toxicity. *Environmental Science and Technology*. 2015, pp 16–32.

(6) Cozzarelli, I. M.; Skalak, K. J.; Kent, D. B.; Engle, M. A.; Benthem, A.; Mumford, A. C.; Haase, K.; Farag, A.; Harper, D.; Nagel, S. C.; et al. Environmental Signatures and Effects of an Oil and Gas Wastewater Spill in the Williston Basin, North Dakota. *Sci. Total Environ.* **2017**, *579*, 1781–1793.

(7) Llewellyn, G. T.; Dorman, F.; Westland, J. L.; Yoxtheimer, D.; Grieve, P.; Sowers, T.; Humston-Fulmer, E.; Brantley, S. L. Evaluating a Groundwater Supply Contamination Incident Attributed to Marcellus Shale Gas Development. *Proc. Natl. Acad. Sci.* **2015**, *112* (20), 6325–6330.

(8) Drollette, B. D.; Hoelzer, K.; Warner, N. R.; Darrah, T. H.; Karatum, O.; O'Connor, M. P.; Nelson, R. K.; Fernandez, L. A.; Reddy, C. M.; Vengosh, A.; et al. Elevated Levels of Diesel

Range Organic Compounds in Groundwater near Marcellus Gas Operations Are Derived from Surface Activities. *Proc. Natl. Acad. Sci.* **2015**, *112* (43), 13184–13189.

(9) Lester, Y.; Ferrer, I.; Thurman, E. M.; Sitterley, K. A.; Korak, J. A.; Aiken, G.; Linden, K. G. Characterization of Hydraulic Fracturing Flowback Water in Colorado: Implications for Water Treatment. *Sci. Total Environ.* **2015**, *512–513*, 637–644.

(10) Hoelzer, K.; Sumner, A. J.; Karatum, O.; Nelson, R. K.; Drollette, B. D.; O'Connor, M. P.; D'Ambro, E. L.; Getzinger, G. J.; Ferguson, P. L.; Reddy, C. M.; et al. Indications of Transformation Products from Hydraulic Fracturing Additives in Shale-Gas Wastewater. *Environ. Sci. Technol.* **2016**, *50* (15), 8036–8048.

(11) Piotrowski, P. K.; Weggler, B. A.; Barth-Naftilan, E.; Kelly, C. N.; Zimmermann, R.; Saiers, J. E.; Dorman, F. L. Non-Targeted Chemical Characterization of a Marcellus Shale Gas Well through GC \times GC with Scripting Algorithms and High-Resolution Time-of-Flight Mass Spectrometry. *Fuel* **2018**, *215*, 363–369.

(12) Piotrowski, P. K.; Weggler, B. A.; Yoxtheimer, D. A.; Kelly, C. N.; Barth-Naftilan, E.; Saiers, J. E.; Dorman, F. L. Elucidating Environmental Fingerprinting Mechanisms of Unconventional Gas Development Through Hydrocarbon Analysis. *Anal. Chem.* **2018**, acs.analchem.8b00822.

(13) Baltussen, E.; Cramers, C. A.; Sandra, P. J. F. Sorptive Sample Preparation - A Review. *Analytical and Bioanalytical Chemistry*. 2002, pp 3–22.

(14) C.F. Poole, The essence of chromatography, Elsevier, 2003.

(15) P.T. Crisp, J. Ellis, J.W. De Leeuw, P.A. Schenck. Flash thermal desorption as an alternative to solvent extraction for the determination of C8-C35 hydrocarbons in oil shales, Anal. Chem. **1986**, 58258-61.

(16) Jiang, C.; Chen, Z.; Mort, A.; Milovic, M.; Robinson, R.; Stewart, R.; Lavoie, D. Hydrocarbon Evaporative Loss from Shale Core Samples as Revealed by Rock-Eval and Thermal Desorption-Gas Chromatography Analysis: Its Geochemical and Geological Implications. *Mar. Pet. Geol.* **2016**, *70*, 294–303.

(17) Meredith, W.; Russell, C. A.; Cooper, M.; Snape, C. E.; Love, G. D.; Fabbri, D.; Vane, C. H. Trapping Hydropyrolysates on Silica and Their Subsequent Thermal Desorption to Facilitate Rapid Fingerprinting by GC-MS. In *Organic Geochemistry*; 2004; Vol. 35, pp 73–89.

Hu, X.; Wu, L.; Wang, Y.; Mourant, D.; Lievens, C.; Gunawan, R.; Li, C.-Z. Mediating Acid-Catalyzed Conversion of Levoglucosan into Platform Chemicals with Various Solvents. *Green Chem.* 2012, *14* (11), 3087.

(19) Yu, C.; Hu, B. Novel Combined Stir Bar Sorptive Extraction Coupled with Ultrasonic Assisted Extraction for the Determination of Brominated Flame Retardants in Environmental Samples Using High Performance Liquid Chromatography. *J. Chromatogr. A* **2007**, *1160* (1–2), 71–80.

(20) United States Environmental Protection Agency, Method 625: Base/Neutrals and Acids, SW-846, **1984**,1-42.

(21) R.T. Ryder, Assessment of Appalachian basin oil and gas resources: Utica-Lower
Paleozoic Total Petroleum System, U.S. Geological Survey Open-File Report, **2008**, 1287, 1-29.
(22) USGS Marcellus Shale Assessment Team, Information relevant to the U.S. Geological
Survey assessment of the Middle Devonian Shale of the Appalachian Basin Province, U.S.
Geological Survey Open-File Report, **2011**, 1298, 1-22.

(23) Tasker, T. L.; Piotrowski, P. K.; Dorman, F. L.; Burgos, W. D. Metal Associations in Marcellus Shale and Fate of Synthetic Hydraulic Fracturing Fluids Reacted at High Pressure and Temperature. *Environ. Eng. Sci.* **2016**, *33* (10), 753–765.

(24) Sumner, A. J.; Plata, D. L. Exploring the Hydraulic Fracturing Parameter Space: A Novel High-Pressure, High-Throughput Reactor System for Investigating Subsurface Chemical Transformations. *Environ. Sci. Process. Impacts* **2018**.

(25) Li, M.; Zhang, S.; Jiang, C.; Zhu, G.; Fowler, M.; Achal, S.; Milovic, M.; Robinson, R.; Larter, S. Two-Dimensional Gas Chromatograms as Fingerprints of Sour Gas-Associated Oils. *Org. Geochem.* **2008**, *39* (8), 1144–1149.

Chapter 5

Determination of Bioaccumulation Potential of Hydraulic Fracturing Produced Water in the Freshwater Mussel, *Ellipto complanata*

5.1 Abstract

Hydraulic fracturing has become a commonly utilized method for the extraction of shale gas resources. This process has been highly scrutinized due to the potential impacts to waster resources, which are poorly understood. This study evaluated whether exposure to hydraulic fracturing wastewater could be biomonitored in the freshwater mussel, *Ellipto complanata*. A controlled laboratory experiment was performed exposing *Ellipto complanata* to produced water from a hydraulically fractured well over an 8 week period. The fatty tissue of the mussels was assessed for bioaccumulation of both inorganic and organic pollutants by ICP and GCxGC-TOFMS, respectively. Mussels exposed to produced water showed an increase in strontium uptake and increase in cyclic hydrocarbons which may serve as mechanisms of determining impacts of shale gas in aquatic organisms.

5.2 Introduction

The extraction of hydrocarbons from low permeability formations has been performed over the last two decades through unconventional well development.¹ Typically, unconventional oil and gas wells are drilled not only laterally, but also horizontally and subjected to hydraulic fracturing. During hydraulic fracturing millions of gallons of water along with organic and inorganic fluid modifiers are pumped underground to generate pressures capable to fracturing the rock that entraps the oil and/or gas in tight pores.¹ Upon completion of hydraulic fracturing, the fluids return back to the surface as either flowback, the initial fluid that returns prior to gas production, or produced water, the fluid that returns throughout the lifetime of the well. Flowback and produced waters are highly chemically complex and are often described as high brines with dissolved organic content that contains traces of the chemical modifiers and their transformation products and hydrocarbon signatures that resemble the exploited shale rock.²⁻¹⁰

The potential toxicity of wastewaters from unconventional oil and gas extraction has raised a multitude of concerns. Instances of fluid migration from spills and blowouts, in addition to centralized waste treatment plants that are ineffective at removing contaminants from oil and gas wastewaters, have lead to a number of groundwater and surface water impacts and motivated research in impacts to aquatic organisms.⁹⁻²³ To date, studies have focused on effects of produced water spills on microbial communities and have showed population changes towards more saline resistant species.¹² Significant toxicity to hydraulic fracturing produced water has been demonstrated in zebra fish, rainbow trout, and *Daphnia magna*.^{9, 19-23} The lethal concentrations (LC₅₀) of produced water on each species were evaluated in addition to developmental, mobility, and transcriptional responses to xenobiotic metabolomics pathways.^{9, 19-23} However, theses studies did not evaluate the bioaccumulation of produced water components or metabolites thereof, which may carry potential impacts throughout the ecosystem.

Mussels have been used as indicators of water quality for decades as the animals are filter feeders that are well known to bioaccumulate environmental pollutants.²⁴⁻²⁷ Most notably, NOAA has been monitoring the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in mussels since the 1980s.²⁷ While most research pertaining to bioaccumulation of environmental pollutants has focused on marine mussels, a few reports have studied freshwater mussels, which possess the same filter feeder qualities as the marine counterparts.²⁴⁻²⁵ The extensive habitat range of freshwater mussels, especially in respect to overlap with areas of unconventional oil and gas development, makes these organisms ideal candidates for studying the impacts of unconventional oil and gas development in surface waters. Previous studies have demonstrated the utility of mussels as indicators of stream impacts in areas of unconventional hydrocarbon extraction, with Pilote et al. reporting the accumulation of metals in the fatty tissue of caged mussels in the Athabasca River.²⁵

The bioaccumulation potential of hydraulic fracturing produced water in the freshwater mussel, *Ellipto complanata*, was assessed in this study. Mussels were grown in a controlled laboratory environment and dosed with produced water from a hydraulically fractured well in the Utica Shale play. The fatty tissue of the mussels was analyzed for both organic and inorganic components that could be indicators of bioaccumulation of the produced water. Statistical analyses were implemented to help differentiate the impacts of produced water exposure on mussel fatty tissue chemistry.

5.3 Materials and Methods

5.3.1 Animal Care

Ellipto complanata were obtained from Carolina Biological Supply Company (Burlington, NC) and kept in 50 L aquaria with University Park, PA tap water that was conditioned for 14 days. 8-9 mussels were kept per tank and water was maintained 21-22 °C with pH 7.2-8.2. Mussels were fed daily with 5 mL of algae food source (Carolina Biological Supply Company, Burlington, NC).

5.3.2 Bioaccumulation Studies

Produced water was obtained from a hydraulically fractured Utica Shale well located in southeastern Ohio. Bioaccumulation and toxicity was assessed for three treatments: control, low-dose, and high-dose. The control tank contained only conditioned tap water while the low-dose contained a 1:100 dilution of produced water, and the high-dose contained a 1:50 dilution of produced water. The dilutions were determined based on the total organic content (TOC) measurements of the produced water and matched to those reported by streams impacted by unconventional gas development to best mimic the environmental conditions mussels may be exposed to.¹⁷⁻¹⁸ To account for evaporative losses, conditioned tap water was added every 14 days to maintain the 50 L water level. The tanks were re-dosed with produced water after 4 weeks. The experiment was conducted for 8 weeks. Deceased mussels were immediately removed from the tanks and stored at -20 °C.

5.3.3 Chemical Characterization

The raw produced water was characterized for organic and inorganic chemistry by GCxGC-TOFMS and ICP-OES, respectively. The sample was prepared for organic analysis by liquid-liquid extraction by a modified USEPA Method 3510C.²⁸ 384.19 g of produced water were extracted 3 times with 25 mL of dichloromethane and concentrated to 1 mL using a Kuderna-Danish apparatus. The sample was analyzed using GCxGC-TOFMS (LECO Pegasus 4D, St. Joseph, MI), which was operated with a 250°C injector temperature and a 2 mL/min helium flow rate. The first dimension column was a Restek Rtx-DHA100 (100 m × 0.25 mm × 0.5 µm) (Bellefonte, PA) and the second dimension column was a Restek Rxi-17SilMS (1.0 m × 0.25 mm × 0.25 µm). The first dimension oven was programmed to maintain 50°C for 0.2 minutes after a 1µL 1:100 split injection and increase at 5°C/min to 315°C. The modulator temperature was offset

at 15°C and operated with a 2 second modulation period. The second dimension oven was offset by 5°C. The mass spectrometer operated with a 700 second acquisition delay, 250°C ion source temperature, and -70eV ionization energy. The collected mass range was 30-550 amu at 200 Hz. The sample was prepared for inorganic analysis by acid digestion in accordance to USEPA method 3005A.²⁹ Metal concentrations were determined using Thermo Scientific iCAP 6500 ICP-OES Duo (Waltham, MA).

Mussel tissue was prepared for organic analysis following Extraction of Biological Tissue or Trace Organic Analysis procedure outlined in the NOAA Mussel Watch program.²⁷ 2 mussels were pooled for analysis based on survival. Each treatment therefore had three samples: early mortality, mid mortality, and late mortality, with the exception of the control group that only had an early mortality and late mortality group. Approximately 3 g of homogenized fatty tissue was mixed with 10-15 g of sodium sulfate and tissuemized for 3 minutes with three aliquots of 25 mL of dichloromethane. The samples were concentrated to 1mL using a Kuderna-Danish apparatus and a sample cleanup was performed with 50 mg of PSA to remove the fatty acids native to mussel tissue. The samples were analyzed using GCxGC-TOFMS following the method described above with a 1:2 injector split.

Mussel tissues were dried in a speedvac until a constant weight was achieved. Thereafter, 0.5 grams of dried tissue was transferred to a 10 mL Teflon vial with 5 mL of 2N nitric acid, 20 μ L of hydrogen peroxide, and were heated at 60°C until dry (approximately 6-8 hours). This process was repeated 2 more times to degrade most of the organic matter in the tissue. Thereafter, the sample was refluxed in 5 mL of 2N nitric acid for 3 hours at 80°C. The refluxed samples were then diluted to 40 mL with 2N nitric acid and 4 mL of 12M HCl. The diluted digestants were transferred to a CEM Mars 6 microwave digested samples were diluted 100 times in 2% nitric acid before analysis on a Thermo Scientific iCAP 6000 inductively coupled plasma optical emission

spectrometer (ICP-OES; Na, Ca, Mg, Sr, K) or Thermo X-Series 2 mass spectrometer (ICP-MS; Cr, Mn, Fe, Co, Ni, Cu, Ba, Pb). All dilution factors were accounted for on a mass basis and elemental concentrations were normalized by mass of tissue digested.

5.3.4 Data Analysis

Statistical analysis was performed using MetaboAnalyst 4.0.³⁰ The GCxGC-TOFMS data were first subjected to basic data processing in the ChromaTOF software followed by peak alignment and peak wise comparison in the Statistical Compare feature of ChromaTOF. The alignment results were exported to Excel and manual quality control was applied to remove peaks corresponding to column bleed. The data were then subjected to normalization based on an internal standard, mean centering and log transformation in accordance to procedures outlined by Weggler et al.³¹ A non-parametric ANOVA (Kruskal-Wallis) was performed at p<005 to determine the analytes that contributed significantly. A principal component analysis (PCA) was performed on the significant analytes as a non-targeted data analysis tool to determine differences between treatment groups and the features which contribute to the differentiation.

5.4 Results and Discussion

Freshwater mussels were exposed to hydraulic fracturing produced water to simulate environmental contamination conditions and assessed for their longevity and bioaccumulation potential. The mussels were exposed to produced water concentrations comparable to those seen in previously reported surface waters affected by shale gas development, thus mimicking environmental conditions that might be encountered by freshwater mussels in watersheds where shale gas development is prevalent. The experiment was conduced over an 8-week period and 4 weeks after the beginning of the experiment the mussels were re-dosed. After each addition of produced water, the water in the tanks was orange-tinged and a visable organic layer on top of the water was observed. Water clarity returned 14 days after the addition of produced water, indicating that the mussels were actively filtering the water.

Mussel mortality was assessed daily and presented in the Kaplan–Meier survival curve in Figure 5-1. Each of the experimental groups experienced the mortality of one individual within the first 8 days of the experiments, with the high-dose mussel at day 6, followed by the low-dose mussel at day 7, and the control mussel at day 8. This initial mortality may be due to overpopulation in the experimental tanks or due to the stress of adapting to a new environment. At day 18, a mussel was deceased in the control tank (Figure 5-1). This occurred during a period of time where the mussels were not fed for 2 days and may have been due to a lack of resources. The high survival rates in the high-dose and low-dose tanks during this period may have been due to the availability of another organic food source from the produced water or more dormant behavior due to the stressed conditions.

Four weeks after the initial dose of produced water, the tanks were re-dosed with produced water. Prior to the re-dosing all mussel populations appeared to be stable and mussels were observed feeding regularly and moving within the tanks. After the re-dosing, large population crashes were observed in the low-dose and high-dose tanks (Figure 5-1). Between days 32-33, 6 mussels were deceased in the high-dose tank. A similar mortality was observed in the low-dose tank with 6 mussels dying between days 35-38. The high levels of mortality after the re-dose may be a result of high stress levels the mussels were subjected to and the inability to adapt to further stressors. Interestingly, with only one and two mussels in the low- and high-dose tanks, respectively, water clarity was restored after 2 weeks.



Figure 5-1: The Kaplan–Meier survival curves for mussels exposed to produced water from hydraulic fracturing operations (high-dose 1:50 dilution, low-dose 1:100 dilution). Note: n = 8 for control and low-dose groups, n=9 for high-dose group.

The fatty tissue of mussels was acid digested and subjected to ICP analysis. Overall, a slight uptake of divalent metals was observed in the mussels dosed with produced water (Figure 5-2). Large variability in the concentrations of metals was observed in mussels of the same experimental group, and is reflected in the standard deviations of the metal measurements. As such, the only metal that showed a statistically significant uptake in the dosed mussels was strontium. Strontium is often highly concentrated in produced waters from hydraulic fracturing, and the produced water used for the dosing experiments had a strontium concentration of 2698 mg/L.^{3,4} Strontium has been shown to be metabolically important in freshwater mussels in surface waters affected by shale gas activities.³¹ Geeza et al. reported elevated strontium incorporation into shells of mussels downstream from municipal wastewater treatment facilities that treated

shale gas wastewaters.³² The isotopic signature of the strontium in the shells matched that of shale gas wastewaters, indicating that the elevated strontium in the surface water from shale gas development was incorporated into the shell.³² The elevated uptake of strontium in the fatty tissue of the mussels indicates that the mussels exposed to the experimental conditions were metabolically active – filtering the water in the tanks and bioaccumulating a common pollutant of hydraulic fracturing wastewater that can be incorporated into the shells as the mussels grow.



Figure **5-2**: The metal uptake in fatty tissue of mussels exposed to produced water from hydraulic fracturing operations. All values are presented as molar calcium ratios. Error bars represent standard deviation.

The fatty tissue of mussels was also subjected to solvent extraction and analysis by GCxGC-TOFMS to evaluate the bioaccumulation of the organic compounds in produced water. Figure 5-3 shows the GCxGC-TOFMS chromatograms of the control, low-dose, and high-dose groups. While it is difficult to distinguish chromatographic differences between the three experimental groups, the mussels exposed to produced water did show a higher abundance and diversity of cyclic hydrocarbons such as cyclopentanes, cyclohexanes, and hydronaphthalenes, as highlighted in the white oval in Figure 5-3B and Figure 5-3C. Upon comparison of the GCxGC-TOFMS chromatograms of mussel fatty tissue (Figure 5-3) to the GCxGC-TOFMS

chromatogram of the produced water utilized for dosing (Figure 5-4), it is possible to distinguish the effect of produced water exposure on the fatty tissue of mussels. The produced water is dominated by saturated hydrocarbons, including n-alkanes, branched alkanes, cyclohexanes, cyclopentanes, adamantanes, and hydronaphthalenes (Figure 5-4). While not every compound present in the produced water is also present in the mussels, it is likely that the higher abundance and diversity of cyclic hydrocarbons observed in the fatty tissues of mussels is a result of the produed water exposure.



Primary Retention Time (sec)





Figure **5-4:** The GCxGC-TOFMS total ion chromatograms of the produced water utilized for dosing. The produced water is dominated by saturated hydrocarbons, including n-alkanes, branched alkanes, and cyclic saturated hydrocarbons.

To determine if the observed increase in cyclic hydrocarbons in the fatty tissue of mussels exposed to produced water is statistically significant, principle component analysis (PCA) was performed and utilized as an exploratory data analysis tool. PCA has been previously applied to GCxGC-TOFMS data to differentiate particulate matter from the combustion of different wood sources.³¹ Similar procedures were applied to this study, and the results of the PCA are displayed as a score plot and shown in Figure 5-5. There is clear clustering within each experimental group and separation with presence of mussel tissue in PC1 and produced water exposure in PC2. Upon examining the loadings of the PCA, those features that clustered with the produced water dosed mussels were determined to contribute to the differentiation between dosed and undosed groups. Of the features that were attributed to produced water exposure, 74% were

cyclic hydrocarbons, a major class of compounds found in the produced water. The persistence of the cyclic hydrocarbons in the fatty tissue may be a potential fingerprinting mechanism of shale gas impacts on aquatic species.



Figure **5-5**: The principle component analysis score plot of GCxGC-TOFMS peak lists from fatty tissue of mussels exposed to produced water from hydraulic fracturing operations. Analytes were subjected to Kruskal-Wallis ANOVA and p<0.005 was required for PCA modeling, shading represents the 95% confidence interval.

Exposing freshwater mussels to hydraulic fracturing produced water demonstrated the toxicity and bioaccumulation of produced water constituents. Overall, prolonged exposure to produced water resulted in toxicity and increased strontium and cyclic hydrocarbon uptake in the mussels. While the study presented herein was designed to be a proof-of-concept in determining if mussels could be used as biomonitoring species of environmental impacts from unconventional gas development, more research is necessary to determine the full scale impacts of produced water exposure on freshwater mussels and greater-scale ecosystem impacts. Specifically, determining the metabolomics of produced water exposure would be beneficial in assessing the broader impacts on the mussels. Additionally, the elevated strontium uptake would allow for a comparison of strontium isotope ratios to those common in shale gas wastewaters as a further fingerprinting mechanism. While not comprehensive, this study demonstrated the potential of utilizing freshwater mussels as biomonitoring species for environmental impacts from unconventional oil and gas development.

5.5 References

(1) Howarth, R. W.; Ingraffea, A. Natural Gas: Should Fracking Stop? *Nature*. 2011, pp 271–273.

(2) Kahrilas, G. A.; Blotevogel, J.; Corrin, E. R.; Borch, T. Downhole Transformation of the Hydraulic Fracturing Fluid Biocide Glutaraldehyde: Implications for Flowback and Produced Water Quality. *Environ. Sci. Technol.* **2016**, *50* (20), 11414–11423.

(3) Haluszczak, L. O.; Rose, A. W.; Kump, L. R. Geochemical Evaluation of Flowback
Brine from Marcellus Gas Wells in Pennsylvania, USA. *Appl. Geochemistry* 2013, *28*, 55–61.
(4) Lester, Y.; Ferrer, I.; Thurman, E. M.; Sitterley, K. A.; Korak, J. A.; Aiken, G.; Linden,

K. G. Characterization of Hydraulic Fracturing Flowback Water in Colorado: Implications for Water Treatment. *Sci. Total Environ.* **2015**, *512–513*, 637–644.

(5) Thurman, E. M.; Ferrer, I.; Blotevogel, J.; Borch, T. Analysis of Hydraulic Fracturing Flowback and Produced Waters Using Accurate Mass: Identification of Ethoxylated Surfactants. *Anal. Chem.* **2014**, *86* (19), 9653–9661.

(6) Hoelzer, K.; Sumner, A. J.; Karatum, O.; Nelson, R. K.; Drollette, B. D.; O'Connor, M. P.; D'Ambro, E. L.; Getzinger, G. J.; Ferguson, P. L.; Reddy, C. M.; et al. Indications of Transformation Products from Hydraulic Fracturing Additives in Shale-Gas Wastewater. *Environ. Sci. Technol.* 2016, *50* (15), 8036–8048.

(7) Piotrowski, P. K.; Weggler, B. A.; Barth-Naftilan, E.; Kelly, C. N.; Zimmermann, R.; Saiers, J. E.; Dorman, F. L. Non-Targeted Chemical Characterization of a Marcellus Shale Gas Well through GC \times GC with Scripting Algorithms and High-Resolution Time-of-Flight Mass Spectrometry. *Fuel* **2018**, *215*, 363–369.

(8) Piotrowski, P. K.; Weggler, B. A.; Yoxtheimer, D. A.; Kelly, C. N.; Barth-Naftilan, E.; Saiers, J. E.; Dorman, F. L. Elucidating Environmental Fingerprinting Mechanisms of Unconventional Gas Development Through Hydrocarbon Analysis. *Anal. Chem.* **2018**, acs.analchem.8b00822.

(9) He, Y.; Flynn, S. L.; Folkerts, E. J.; Zhang, Y.; Ruan, D.; Alessi, D. S.; Martin, J. W.; Goss, G. G. Chemical and Toxicological Characterizations of Hydraulic Fracturing Flowback and Produced Water. *Water Res.* **2017**, *114*, 78–87.

(10) Llewellyn, G. T.; Dorman, F.; Westland, J. L.; Yoxtheimer, D.; Grieve, P.; Sowers, T.; Humston-Fulmer, E.; Brantley, S. L. Evaluating a Groundwater Supply Contamination Incident Attributed to Marcellus Shale Gas Development. *Proc. Natl. Acad. Sci.* **2015**, *112* (20), 6325–6330.

(11) Drollette, B. D.; Hoelzer, K.; Warner, N. R.; Darrah, T. H.; Karatum, O.; O'Connor, M. P.; Nelson, R. K.; Fernandez, L. A.; Reddy, C. M.; Vengosh, A.; et al. Elevated Levels of Diesel Range Organic Compounds in Groundwater near Marcellus Gas Operations Are Derived from Surface Activities. *Proc. Natl. Acad. Sci.* **2015**, *112* (43), 13184–13189.

(12) Cozzarelli, I. M.; Skalak, K. J.; Kent, D. B.; Engle, M. A.; Benthem, A.; Mumford, A. C.; Haase, K.; Farag, A.; Harper, D.; Nagel, S. C.; et al. Environmental Signatures and Effects of an Oil and Gas Wastewater Spill in the Williston Basin, North Dakota. *Sci. Total Environ.* **2017**, *579*, 1781–1793.

(13) Digiulio, D. C.; Jackson, R. B. Impact to Underground Sources of Drinking Water and Domestic Wells from Production Well Stimulation and Completion Practices in the Pavillion, Wyoming, Field. *Environ. Sci. Technol.* **2016**, *50* (8), 4524–4536.

(14) Gross, S. A.; Avens, H. J.; Banducci, A. M.; Sahmel, J.; Panko, J. M.; Tvermoes, B. E. Analysis of BTEX Groundwater Concentrations from Surface Spills Associated with Hydraulic Fracturing Operations. *J. Air Waste Manag. Assoc.* **2013**, *63* (4), 424–432.

(15) Hildenbrand, Z. L.; Carlton, D. D.; Fontenot, B. E.; Meik, J. M.; Walton, J. L.; Thacker, J. B.; Korlie, S.; Shelor, C. P.; Kadjo, A. F.; Clark, A.; et al. Temporal Variation in Groundwater Quality in the Permian Basin of Texas, a Region of Increasing Unconventional Oil and Gas Development. *Sci. Total Environ.* **2016**, *562*, 906–913.

(16) Kassotis, C. D.; Iwanowicz, L. R.; Akob, D. M.; Cozzarelli, I. M.; Mumford, A. C.; Orem, W. H.; Nagel, S. C. Endocrine Disrupting Activities of Surface Water Associated with a West Virginia Oil and Gas Industry Wastewater Disposal Site. *Sci. Total Environ.* **2016**, *557–558*, 901–910.

(17) Burgos, W. D.; Castillo-Meza, L.; Tasker, T. L.; Geeza, T. J.; Drohan, P. J.; Liu, X.; Landis, J. D.; Blotevogel, J.; McLaughlin, M.; Borch, T.; et al. Watershed-Scale Impacts from Surface Water Disposal of Oil and Gas Wastewater in Western Pennsylvania. *Environ. Sci. Technol.* **2017**, *51* (15), 8851–8860.

(18) Getzinger, G. J.; O'Connor, M. P.; Hoelzer, K.; Drollette, B. D.; Karatum, O.; Deshusses, M. A.; Ferguson, P. L.; Elsner, M.; Plata, D. L. Natural Gas Residual Fluids: Sources, Endpoints, and Organic Chemical Composition after Centralized Waste Treatment in Pennsylvania. *Environ. Sci. Technol.* **2015**, *49* (14), 8347–8355.

(19) Blewett, T. A.; Delompré, P. L. M.; He, Y.; Folkerts, E. J.; Flynn, S. L.; Alessi, D. S.; Goss, G. G. Sublethal and Reproductive Effects of Acute and Chronic Exposure to Flowback and Produced Water from Hydraulic Fracturing on the Water Flea Daphnia Magna. *Environ. Sci. Technol.* **2017**, *51* (5), 3032–3039.

(20) Blewett, T. A.; Delompre, P. L. M.; Glover, C. N.; Goss, G. G. Physical immobility as a sensitive indicator of hydraulic fracturing fluid toxicity towards Daphnia magna. *Sci. Total Environ.* **2018**, *635*, 639–643.

(21) He, Y.; Sun, C.; Zhang, Y.; Folkerts, E. J.; Martin, J. W.; Goss, G. G. Developmental Toxicity of the Organic Fraction from Hydraulic Fracturing Flowback and Produced Waters to Early Life Stages of Zebrafish (Danio Rerio). *Environ. Sci. Technol.* 2018, *52* (6), 3820–3830.
(22) He, Y.; Folkerts, E. J.; Zhang, Y.; Martin, J. W.; Alessi, D. S.; Goss, G. G. Effects on Biotransformation, Oxidative Stress, and Endocrine Disruption in Rainbow Trout (Oncorhynchus Mykiss) Exposed to Hydraulic Fracturing Flowback and Produced Water. *Environ. Sci. Technol.* 2017, *51* (2), 940–947.

(23) Blewett, T. A.; Weinrauch, A. M.; Delompré, P. L. M.; Goss, G. G. The Effect of Hydraulic Flowback and Produced Water on Gill Morphology, Oxidative Stress and Antioxidant Response in Rainbow Trout (Oncorhynchus Mykiss). *Sci. Rep.* **2017**, *7*, 46582.

(24) De los Ríos, A.; Echavarri-Erasun, B.; Dévier, M. H.; Le Menach, K.; Budzinski, H.; Ortiz-Zarragoitia, M.; Orbea, A.; Juanes, J. A.; Cajaraville, M. P. Assessment of the Effects of Discontinuous Sources of Contamination through Biomarker Analyses on Caged Mussels. *Sci. Total Environ.* **2018**, *634*, 116–126.

(25) Pilote, M.; André, C.; Turcotte, P.; Gagné, F.; Gagnon, C. Metal Bioaccumulation and Biomarkers of Effects in Caged Mussels Exposed in the Athabasca Oil Sands Area. *Sci. Total Environ.* **2018**, *610–611*, 377–390.

(26) Bonnefille, B.; Arpin-Pont, L.; Gomez, E.; Fenet, H.; Courant, F. Metabolic Profiling Identification of Metabolites Formed in Mediterranean Mussels (Mytilus Galloprovincialis) after Diclofenac Exposure. *Sci. Total*

(27) National Oceanic and Atmospheric Association. Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects **1984-1992**.

(28) United States Environmental Protection Agency. Method 3510C (SW-846): Separatory Funnel Liquid-Liquid Extraction, **1996**, Revision 3.

(29) United States Environmental Protection Agency. Method 3005A (SW-846): Acid Digestion of Waters for Total Recoverable or Dissolved Metals for Analysis by FLAA or ICP Spectroscopy, **1992**, Revision 3.

(30) Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., Wishart, D.S. and Xia, J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucl. Acids Res.* **2018.** doi:10.1093/nar/gky310

(31) Weggler, B. A.; Ly-Verdu, S.; Jennerwein, M.; Sippula, O.; Reda, A. A.; Orasche, J.; Gröger, T.; Jokiniemi, J.; Zimmermann, R. Untargeted Identification of Wood Type-Specific Markers in Particulate Matter from Wood Combustion. *Environ. Sci. Technol.* **2016**, *50* (18), 10073–10081.

(32) Geeza, T., Warner, N. and Gillikin, D., 2017, August. Trace and major element ratios in Elliptio dilatatus shells as indicators of surface water quality in Western Pennsylvania.
 In ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY (Vol. 254). 1155
 16TH ST, NW, WASHINGTON, DC 20036 USA: AMER CHEMICAL SOC.

Chapter 6

Summary and Outlook

The overarching goal of this dissertation was to better understand the chemistry of shale gas extraction practices. The research presented herein largely focused on developing analytical methodologies for the detailed analysis of organic compounds found in wastewaters from shale gas development, shale rocks, and biota that could be affected by environmental contamination from shale gas operations. This work expanded the understanding of the hydrocarbons in shale gas wastewaters through systematic studies of field samples and rock cores. Additionally, this research showed the potential of assessing environmental contamination from shale gas exploitation by hydrocarbon fingerprinting.

In Chapter 2, GCxGC-TOFMS was used to investigate the hydraulic fracturing fluids and subsequent flowback fluids from single well. This study was the first report of the characterization of a single well, which enabled the direct comparison of chemical signatures in the fluids before and after hydraulic fracturing. Characterization by comprehensive two-dimensional gas chromatography coupled time-of-flight mass spectrometry provided a complex data set. As such, data reduction strategies were undertaken though the use of mass spectral scripting algorithms, which allowed for a rapid, yet detailed, non-targeted analysis of hydrocarbon signatures. The impact of fluid handling and selective additive use was demonstrated in the fracturing fluids. The analysis of the flowback fluids also showed some unprecedented results. Namely, the hydrocarbon composition of the flowback remained relatively constant with time even though the concentration increased throughout the flowback period. This

consistent hydrocarbon chemistry, likely representative of that of the shale, may indicate a potential fingerprinting mechanism for environmental contamination events from unconventional gas development.

Chapter 3 aimed to elucidate environmental fingerprinting mechanisms of unconventional gas development through hydrocarbon analysis. Characterization of wastewaters from four Marcellus shale gas wells in geographic proximity by comprehensive two-dimensional gas chromatography (GCxGC) coupled with high-resolution time-of-flight detection allowed for petroleomics hydrocarbon fingerprinting. While Kendrick mass defect plots and GCxGC separation revealed differences in the four samples in heteroatomic substituted hydrocarbons and polycyclic aromatic hydrocarbons, respectively. A novel visualization strategy was developed that illustrates Kendrick mass defect classes as a function of retention time in both chromatographic dimensions, which served to further differentiate the hydrocarbon speciation in each of the four samples and could be easily applied as an aid in environmental forensic investigations.

Chapter 4 presented a novel analytical method that utilized thermal desorption as a sample introduction for GCxGC-TOFMS to investigate the source shale rock that is exploited during hydraulic fracturing. The methodology was compared to solvent extraction to verify validity and subjected to repeatability and efficiency assessments. This study was the first report of detailed shale hydrocarbon chemistry of formations that may undergo hydraulic fracturing. A comparison of Marcellus Shale and Utica Shale hydrocarbon signatures showed many differentiating features, especially when comparing the aromatic hydrocarbons. These differences may be a result of the kerogen that was deposited during the formation of the shales. This difference, while merely a proof of concept at this point, may play an important role in point-source identification of environmental contamination or as an aid for determining locations within the well to target by hydraulic fracturing.
Lastly in Chapter 5, the bioaccumulation potential of produced water from hydraulic fracturing was assessed in the freshwater mussel, *Ellipto complanata*. Mussels were grown in a controlled laboratory environment and dosed with produced water from a hydraulically fractured well in the Utica Shale play. The fatty tissue of the mussels was analyzed for both organic and inorganic components that could be indicators of bioaccumulation of the produced water. Principle Component Analysis was used as an exploratory data analysis/reduction method and demonstrated the uptake of cyclic hydrocarbons and strontium. This study demonstrated the potential of biogeochemical fingerprinting of environmental contamination form shale gas operations.

To this day, the interactions of hydraulic fracturing fluids with shale are poorly understood. It is unknown to what extent hydrocarbon extraction occurs in the subsurface, therefore it cannot be concluded that all hydrocarbon content is extracted from the shale during hydraulic fracturing. In order to assess if hydrocarbon differences may be utilized towards pointsource identification methodologies, a better understanding of the relationship between native shale hydrocarbons and those present in the flowback fluids must be established. However, performing hydrocarbon extraction experiments on the unconventional well scale is not feasible, therefore, a bench-scale model must be developed. While the thermal desorption methods described in Chapter 4 can be applied to studying shale chemistry, they cannot provide an accurate representation of they hydrocarbons that will be present in the wastewaters after hydraulic fracturing occurs.

High pressure and temperature (HPT) experiments can be utilized to mimic hydraulic fracturing conditions. By utilizing HPT experiments with core shale samples that were characterized by thermal desorption, it will be possible to reproduce hydraulic fracturing conditions, thus modeling flowback fluid composition. This will allow for a comparison between the native shale hydrocarbons and those that are extracted into the aqueous phase during hydraulic fracturing and would subsequently return to the surface as flowback. These model experiments also enable the study of fluid-rock interactions though the controlled addition of hydraulic fracturing additives into HPT experiments, which can provide insight into additives that may be enhancing or suppressing hydrocarbon extraction or inducing degradation or transformation. Studying these key fluid-rock interactions will enable a better understanding of flowback fluids hydrocarbon chemistries and differences that can serve as groundwork for pointsource identification methodologies.

Appendix

Portions of this dissertation, including text and figures were from the following peer reviewed

publications:

Paulina K. Piotrowski, Benedikt A. Weggler, Erica Barth-Naftilan, Christina N. Kelly, Ralf Zimmermann, James E. Saiers, Frank L. Dorman. Non-Targeted Chemical Characterization of a Marcellus Shale Gas Well Through GCxGC with Scripting Algorithms and High-Resolution Time-of-Flight Mass Spectrometry. *Fuel.* **2018**, *215*, 363-369. doi.org/10.1016/j.fuel.2017.11.026.

Paulina K. Piotrowski, Christina N. Kelly, Erica Barth-Naftilan, James E. Saiers, Frank L. Dorman. Elucidating Environmental Fingerprinting Mechanisms of Unconventional Gas Impacts Through Hydrocarbon Analysis. *Analytical Chemistry.* **2018**, *90* (8), 5466–5473. DOI: 10.1021/acs.analchem.8b00822.

Portions of this dissertation, including text and figures were from the following manuscripts

submitted or to-be submitted to peer reviewed journals for publication:

Paulina K. Piotrowski, Travis L, Tasker, William D. Burgos, Frank L. Dorman. Applications of Thermal Desorption coupled to GCxGC-TOFMS for Hydrocarbon Fingerprinting of Unconventionally Exploited Shale Rocks. Submitted for Publication in *Journal of Chromatography A*.

Paulina K. Piotrowski, Travis L, Tasker, Thomas J. Geeza, Nathaniel R. Warner, Frank L. Dorman. Determination of Bioaccumulation Potential of Hydraulic Fracturing Produced Water in the Freshwater Mussel, *Ellipto complanata*. Manuscript in Preparation.

VITA

Paulina K. Piotrowski

EDUCATION

Ph.D. (2018) Chemistry and Biogeochemistry, The Pennsylvania State University B.S. (2014) Chemistry, Union College

PUBLICATIONS

8. **Paulina K. Piotrowski,** Travis L, Tasker, Thomas J. Geeza, Nathaniel R. Warner, Frank L. Dorman. Determination of Bioaccumulation Potential of Hydraulic Fracturing Produced Water in the Freshwater Mussel, *Ellipto complanata*. Manuscript in Preparation.

7. **Paulina K. Piotrowski,** Travis L, Tasker, William D. Burgos, Frank L. Dorman. Applications of Thermal Desorption coupled to GCxGC-TOFMS for Hydrocarbon Fingerprinting of Unconventionally Exploited Shale Rocks. Submitted for Publication in *Journal of Chromatography A*.

6. Luis Castillo Meza, **Paulina K. Piotrowski**, Travis L. Tasker, James Farnan, Boya Xiong, Frank Dorman, Jack Vanden Heuvel, Colin Lennox, William D. Burgos. Biogenic Manganese Oxides Capable of Treating Hospital Wastewater. Submitted for Publication in *Environmental Science and Technology*.

5. Beate Gruber, Benedikt Weggler, Roman Jaramillo, Kyra Murrell, **Paulina K. Piotrowski**, Frank Dorman. Comprehensive two-dimensional gas chromatography in forensic science: A critical review of recent trends. Accepted for Publication in *Trends in Analytical Chemistry*.

4. Travis L. Tasker, William D. Burgos, **Paulina K. Piotrowski**, Luis E. Castillo, Tamzin Blewett, Kyle Ganow, Perrine Delompre, Greg Goss, Lara Fowler, John Vanden Heuvel, Frank L. Dorman, Nathaniel R. Warner. Environmental and human health impacts of spreading oil and gas wastewater on roads. Accepted for Publication in *Environmental Science and Technology*.

3. **Paulina K. Piotrowski**, Christina N. Kelly, Erica Barth-Naftilan, James E. Saiers, Frank L. Dorman. Elucidating Environmental Fingerprinting Mechanisms of Unconventional Gas Impacts Through Hydrocarbon Analysis. *Analytical Chemistry*. **2018**, *90* (8), 5466–5473.

2. **Paulina K. Piotrowski**, Benedikt A. Weggler, Erica Barth-Naftilan, Christina N. Kelly, Ralf Zimmermann, James E. Saiers, Frank L. Dorman. Non-Targeted Chemical Characterization of a Marcellus Shale Gas Well Through GCxGC with Scripting Algorithms and High-Resolution Time-of-Flight Mass Spectrometry. *Fuel.* **2018**, *215*, 363-369.

1. Travis L. Tasker, **Paulina K. Piotrowski**, Frank L. Dorman, William D. Burgos. Inorganic mobilization and organic fate by reactions between Marcellus Shale and additives in synthetic hydraulic fracturing fluids. *Environmental Engineering Science*. **2016**, *33*(10), 753-765.