Developing an Analytical Technique to Detect PFAS on Paper Food Packaging using SLE and NMR

A Major Qualifying Project Report

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by

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are a group of other 4,000 man-made chemicals widely used in industry and consumer products due to their water, grease, and heat resistant properties. These same properties are responsible for environmental contamination and human health problems. One application that PFAS have been used for is in the fast-food industry to make grease-resistant packaging. The goal of our project was to develop an analytical method to extract, detect, and identify PFAS intended for fast-food packaging. Solid-liquid extraction (SLE) using different solvents followed by ^{19}F-NMR analysis on select McDonald's® and Burger King® packaging allowed for the possible identification and quantification of three common PFAS: PFOA, GenX®, and 6:2 FTOH. Only some PFAS-spiked samples showed evidence of PFAS, suggesting inadequate SLE or the concentrations tested were too low. Some non-spiked samples showed evidence of PFAS which is an important finding even though concentrations could not be quantified. Organic solvents performed better overall over purified water. Further investigation into PFAS-contaminated fast-food packaging is needed to infer whether this pathway of exposure can cause human health issues. To further expand and improve on this project, it is recommended to increase surface area between the solvent and sample, test more organic solvents, replicate samples, increase spiking concentrations and its range, and limit the time between sample preparation and NMR analysis.

Executive Summary

The Issue

Per- and polyfluoroalkyl substances (PFAS) are widely used man-made chemicals. The strength and stability of the fluorinated chemicals as well as their hydrophobic and lipophobic qualities make them attractive for various consumer and industrial purposes and products. According to the Environmental Protection Agency (EPA), there are several hundred different chemicals that fall under the category of polyfluorinated alkylated substances (EPA, 2021b). Two of the most commonly used and studied PFAS are Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS), which are both considered long-chain PFAS (EPA, 2021b). However, since there has recently been abundant research and subsequent regulations on the use of long-chain molecules such as PFOS and PFOA, many manufacturers have switched to using short-chain PFAS alternatives, such as GenX and 6:2 FTOH. Short-chain PFAS molecules have weakened technical advantages as opposed to the long-chain molecules. Manufacturers, then, may use higher quantities of the material in production in order to create their desired chemical results (Mantripragada, 2021).

PFAS and their associated properties make them extremely persistent in the environment upon disposal; the carbon-fluorine bond is strong enough that PFAS compounds hardly break down under normal environmental conditions (Sunderland et al., 2019). As a result, the implications surrounding the accumulation of PFAS in the environment and communities is concerning. Furthermore, there are also direct means of exposure to the chemicals through ingestion of food in contact with certain packaging items. Coatings made from PFAS compounds are commonly found in food packaging in order to create water and grease-resistant wrappers for products such as fast-food, microwave popcorn, pizza boxes, or candy wrappers. (EPA, 2021b). Currently, there is no dedicated method for quickly and easily quantifying the amount of certain PFAS in fast-food packaging, and so consumers may be unaware of how much of the chemicals remain on the food they consume.

Objectives

Based on the limited information available surrounding the amount of PFAS on common food packaging items, our team outlined three objectives to guide our research and our approach to the issue at hand:

1. Develop an effective solid-liquid extraction (SLE) procedure to remove PFAS from food contact material.
2. Use NMR spectroscopy to quantify the amount of PFAS extracted from the food contact material.
3. Demonstrate if the extraction procedure is effective by spiking PFAS-free material using PFAS widely used in manufacturing.

These three objectives served as a guide for our team throughout the entirety of constructing and executing our project. At the project's conclusion, the objectives also served as a means of

assessing what we had learned and how methods developed during our project could be improved upon in the future.

Approach

To complete the objectives created at the beginning of the project, our team first designated the PFAS to be studied. Perfluorooctanoic acid (PFOA), 6:2 fluorotelomer alcohol (6:2 FTOH), and the ammonium salt of hexafluoropropylene oxide dimer acid (HFPO-DA, more commonly known under the Chemours trademarked name "GenX®") were selected and purchased from their respective distributors. Fast-food packaging samples were obtained from McDonald's and Burger King. The samples were submitted to the same SLE procedure, both with and without PFAS spiking. Spiking in the context of our project meant adding PFAS compounds in the form of aqueous solution to the packaging samples being tested to examine whether the extraction process was successful, even if no PFAS compounds existed on the packaging when first purchased. For all of the samples in our project, they may be thought of as either non-spiked, or spiked with a "low" or "high" concentration of spiking solution.

The extraction process used sonication and centrifugation in an attempt to relocate PFAS from the packaging to a liquid solvent. Solvents used included acetone, purified water, and methanol. From there, the centrifuged supernatant liquid was pipetted to specialized glassware and subsequently placed in the RapidVap nitrogen blowdown evaporation system for further concentration. After concentration in the RapidVap, the samples were transported via micropipette to glass tubes for use in the NMR spectrometer.

Findings

The spectra resulting from the completion of NMR spectrometry formed the basis for all of our findings. For PFASs, spectra are rather simple with two main areas of interest. An exemplary sample NMR spectrum is shown below in Figure 1.

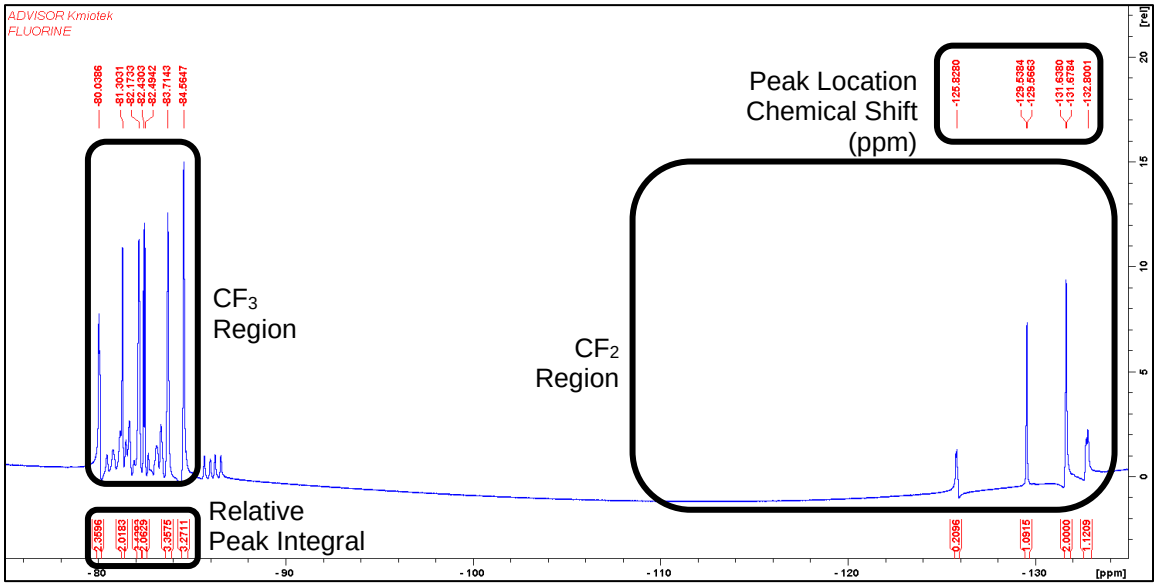
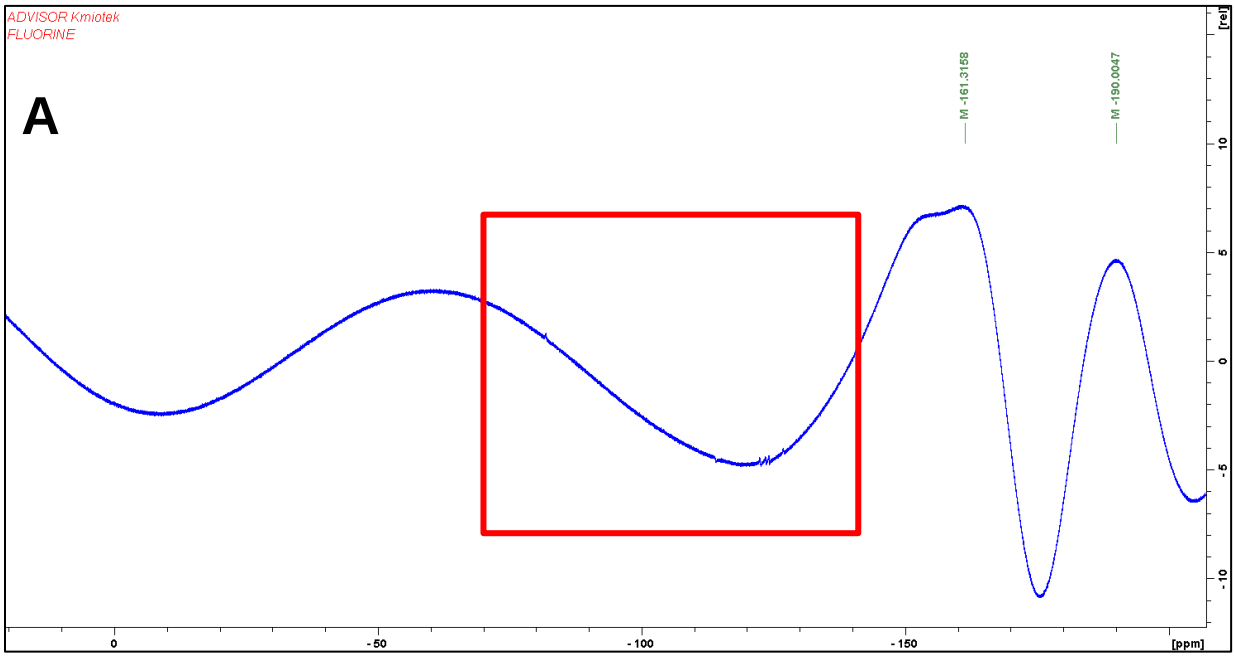


Figure 1. Model ^{19}F-NMR spectrum (specifically, 0.55 mM GenX standard solution).

As can be seen in Figure 11, the peaks on the left end of the spectrum, roughly located around -82 ppm, represent the CF_3 terminal group present for all PFASs. The particular spectrum contains more than one signal in this region since GenX has two CF_3 groups. The CF_3 peak stayed relatively constant for all samples analyzed. The multiple peaks on the right end of the spectrum, usually located between -110 and -130 ppm, represent the CF_2 groups on a PFAS molecule. The CF_2 peak locations vary from one PFAS to another since each molecule is structured slightly differently, leading to unique NMR signals. The ratio between the peak area of the CF_3 group to the CF_2 group is roughly 3:2 due to the number of fluorine molecules on each side. The peaks in Figure 1 are easily distinguishable due to the signal to noise ratio (S/N). Having a S/N ratio of 3:1 considers a peak to be true, in which proper identification of PFAS along with its concentration can be accomplished.

Almost all analyzed samples showed barely noticeable "peaks" in the -82 ppm region and between -110 ppm and -130 ppm, representative of the CF_3 and CF_2 groups on a PFAS molecule. Although they are small, the "peaks" within the spectra are still of importance. Although the S/N ratio of these peaks is below 3:1, the peaks were located in the two regions of interest for PFASs. Figure 2 below shows a NMR spectrum that is considered characteristic of most of our analyzed samples.



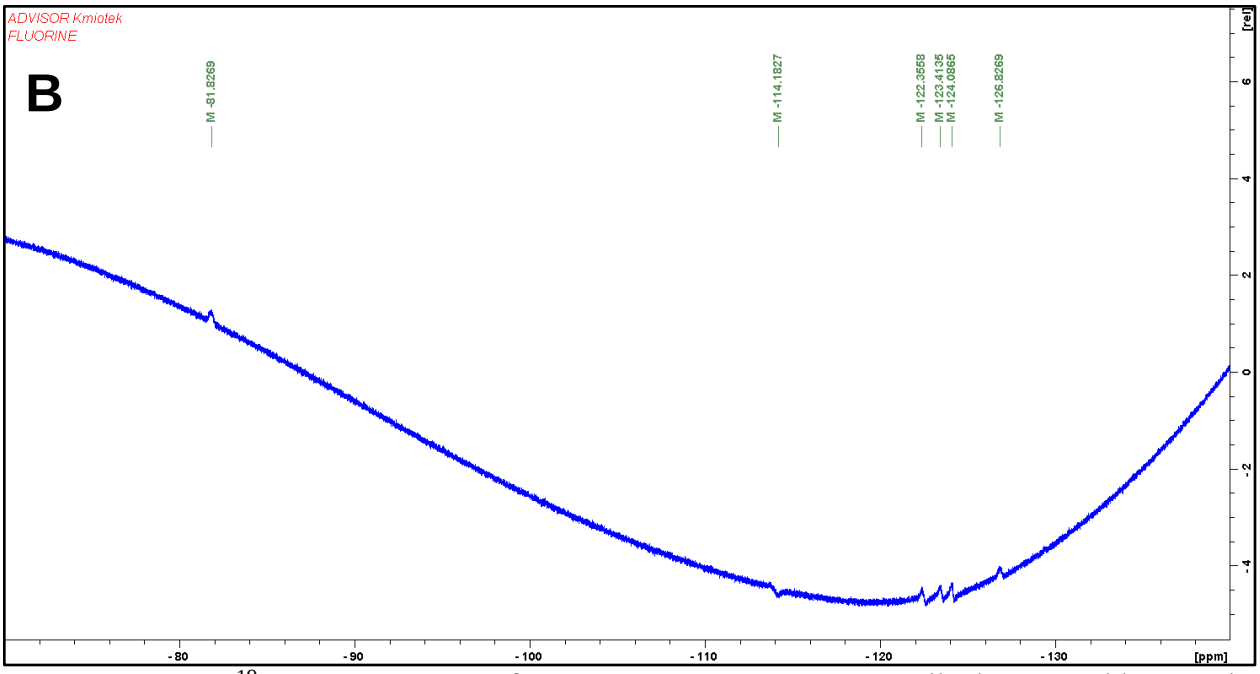


Figure 2. A) Full ^{19}F-NMR spectrum of the low concentration GenX-spiked McDonald's sample in acetone which was representative of most analyzed samples. Note the two broad peaks at -160 and -190 ppm. **B)** Region of interest within the full spectrum where signals due to PFAS are present. Notice the tiny peaks or "bumps" within the noise of the spectrum.

The difference in results between spiked and non-spiked samples was studied extensively in the concluding portion of our experiment. Not all spiked samples showed evidence of PFAS. There are several reasons why this may be, but most of them may be characterized as one of the following: either the PFAS within the spiking solution did not bind to the paper packaging samples, or the extraction method was not completely successful at removing the compounds before NMR analysis. Generally, acetone was a better solvent than water. This was most likely due to its increased solubility with fluorinated compounds. Samples spiked with solutions containing GenX produced the most peaks, which means that GenX was either able to bind better to the packaging during spiking or is just more soluble in acetone during extraction. Copy paper samples spiked with PFAS produced no noticeable peaks after NMR spectrometry was complete.

For those samples that were non-spiked, the samples that used purified water as a solvent did not produce any peaks. The acetone samples showed more evidence of PFAS, which is consistent with the results from the spiked samples (most likely due to higher solubility). The use of organic solvents appears to be the better choice for PFAS extraction rather than inorganic solvents. Both acetone and methanol samples produced stronger signals indicating CF_2 and CF_3 presence after NMR analysis than those samples utilizing water as a solvent. Finally, between McDonald's and Burger King, there was no clear distinction for which brand of packaging was higher in PFAS quantity.

Recommendations

Our team has several recommendations for expanding upon the work performed during this project. One of the first improvements that could be made would be to use a more sophisticated piece of equipment than a pair of scissors for preparing the packaging samples for submersion and sonication. Using a device like a blender or grinding mill to turn the paper samples into smaller pieces would increase the surface area between the solvent and sample. Another way in which future work could be improved would be to increase the variety of organic solvents used throughout the entirety of the extraction process, starting with the initial sonication of the samples. Organic solvents should be used in favor of water and other inorganic solvents, based on the initial results from experimentation performed by our team. Other solvents also may be more favorable to the different PFAS structures, so it would be worth trying out several more solvents in an attempt to improve extraction. Another change to the methodology of the project that could yield better results includes additional repetition of sample-solvent combinations that show promising NMR spectra.

An additional aspect that could be further investigated and improved is the timeline of sample preparation to NMR analysis. Due to schedule restraints and instrumentation/lab access, many of the samples experienced a prolonged delay before analysis. The samples were often stored at different points of our procedure for various amounts of time, as long as a couple months. This opened the door for other factors to affect our samples that ultimately could have contributed to the extremely low signals observed after the completion of NMR spectrometry. The varied results in our spiked and non-spiked samples did not provide clear evidence on the success of our extraction procedure because there was no pattern to which samples had signals and which did not. Also, the samples that did have signals were all at a very low intensity, which could be indicative of a concentration near the border of the limit of detection of the NMR. Therefore, we recommend increasing the sample concentration to strengthen the signals that we did see and to potentially produce signals in the samples that we did not see any.

Acknowledgements

Through the competition of this project, we have been supported by several members of the WPI community who were all vital to the success of this project. We would like to recognize the Instrumentation Core Technician of the Life Sciences and Bioengineering Center, Daryl Johnson, for his assistance with the credential and training session required to use the Gateway Lab NMR Spectrometer. We would also like to thank the director of the Life Sciences & Bioengineering Center, Andrew Butler, for his assistance with the TopSpin software used to analyze our results. Their knowledge of NMR analysis was crucial for our project.

We would also like to extend gratitude to the 2018 MQP team of Ashley Choi, Dylan Muise, and Zachary Weiland for their advances with PFAS quantification using NMR. Their work greatly impacted the direction of our research and provided us with key understanding of NMR analysis. The 2020 MQP team of Jonathan Cain and Zachary Powers also provided us with solid information about PFAS quantification for us to build off of throughout the course of our research.

Finally, we are extremely grateful to our advisors John Bergendahl and Stephen Kmiotek for their constant support and positivity throughout the process of this project. This project would not have gotten to where it is today without their valuable advice and experience.

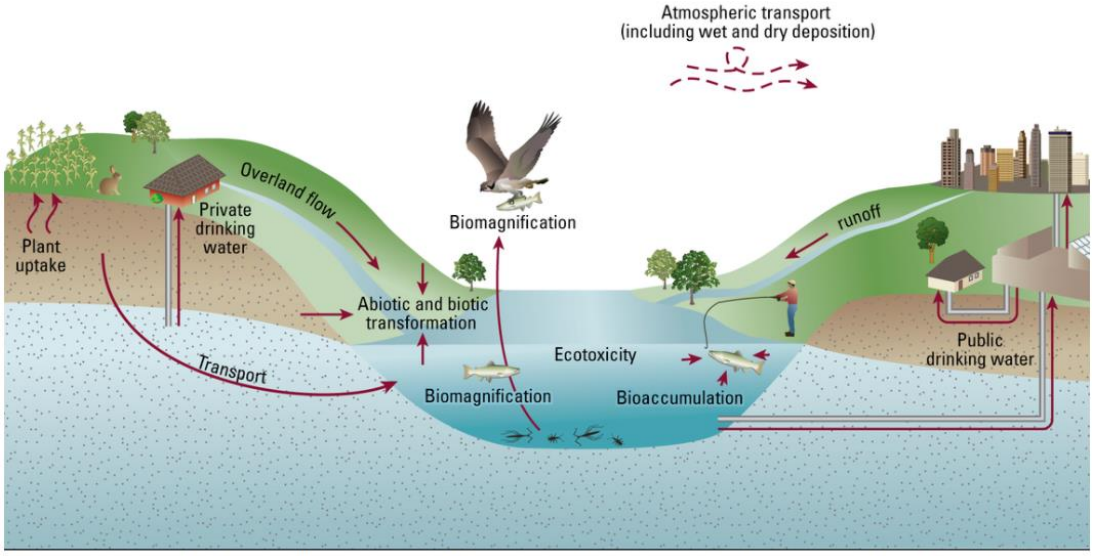
Table of Contents

Table of Figures

Table of Tables

1.0 Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a group of human manufactured chemicals. These are fluorinated compounds that are widely used due to their water, grease, and heat resistant properties. The strength and stability of the chemicals as well as their hydrophobic and lipophobic qualities make them attractive for various consumer and industrial purposes and products. However, these same properties create major environmental and health issues. The stability of PFAS compounds makes them extremely hard to break down, leaving them to accumulate in places such as drinking water sources, soil, and animal food products. Ultimately, the chemicals can end up accumulating within humans and cause health problems (EPA, 2021b). Figure 1 illustrates the movement of PFAS contamination through different aspects of life and the environment.



Mechanisms of per- and poly-fluoroalkyl substances (PfAS) fate, transport, and exposure in the environment (Credit: Jacqueline Olsen, USGS. Public domain.).

Figure 1. Mechanisms of PFAS fate, transport, and exposure in the environment. (USGS, 2021)

This study will focus on three types of PFAS molecules, PFOA, GenX®, and 6:2 FTOH. These chosen compounds are a combination of long- and short-chain molecules that have varying histories of being implemented into different products.

PFAS compounds have shown to be very useful in manufacturing water and grease-resistant fast-food packaging. There have been regulations put in place that resulted in common long-chain compounds, such as PFOS and PFOA, to be phased out. However, other PFAS alternatives such as GenX and 6:2 FTOH have been implemented in their place. A 2017 study by the Silent Spring Institute conducted a study involving data from 10,000 humans collected between 2003 and 2014 (Seltenrich, 2020). The study concluded that people who eat more food at home had lower levels of PFAS in their blood than those who ate out at fast-food restaurants.

The goal of our research was to develop an analytical method to extract and quantify PFAS in fast-food packaging from various popular fast-food chains. The current levels of PFAS compounds present in these wrappers are unknown. This study aimed to determine if there are any leachable PFAS compounds that are detectable in the fast-food packaging through nuclear magnetic resonance (NMR) spectroscopy and if a successful extraction method from solid packaging material could be developed. To accomplish this, it was necessary to develop and test a method to extract all or almost all PFAS from the solid food packaging material. Following a solid-liquid extraction (SLE) technique, the samples were analyzed with NMR to measure the PFAS compounds present in our samples. The NMR scans were analyzed and compared against each other in terms of the packaging material, PFAS compound, or solvent. Identification of specific PFAS was attempted by matching NMR spectra from standard solutions to those of the different types of packaging. In order to test the efficacy of the extraction method, spiked samples with known concentrations of PFAS compounds were subjected to the SLE procedure and their subsequent appearance on the NMR scans confirmed successful extraction.

2.0 Background

To analyze the presence of per- and polyfluoroalkyl substances in fast-food packaging, it is important to understand the unique properties, uses, and impacts of PFAS compounds in general as well as the specific molecules of interest in this study. This chapter will expand on information about PFAS and related topics for the experiment. There are numerous regulations relating to PFAS, which are necessary for understanding which compounds may be present in the packaging and estimating the concentration levels to expect. Also, in order to analyze the products and produce quantifiable results, it is essential to gather information on proper extraction and detection techniques for these compounds. The important information within this chapter is the basis for the rest of the study and was instrumental in achieving results.

2.1 Per- and Polyfluoroalkyl Substances

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) describe a group of thousands of human manufactured fluorinated chemicals with high stability, superior chemical bond strength, and hydrophobic and lipophobic qualities. Perfluorinated substances describe the category of compounds that are fully fluorinated, meaning the hydrogen substituents on the carbon atoms have all been replaced with fluorine atoms. Polyfluorinated substances have at least one carbon with all fluorine substituents (EFSA, 2008). The carbon and fluorine atoms form an incredibly strong bond and the compounds are very stable.

According to the Environmental Protection Agency (EPA), there are several hundreds of different chemicals that fall under the category of polyfluorinated alkylated substances, or PFAS (EPA, 2021b). These molecules contain a hydrophobic alkyl chain, R, with normally four to sixteen carbons and a hydrophilic end group, X. The hydrophobic chain may be partially or fully fluorinated. The general structure of fully fluorinated PFAS molecules is shown below in Figure 2.

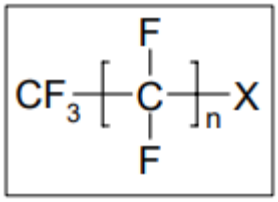


Figure 2. Basic structure of perfluorinated alkylated substances.

Two of the most commonly used and studied perfluorinated alkylated substances are Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS), which are both considered long-chain PFAS (EPA, 2021b). In this study, one of the compounds that is of focus is PFOA, which is a linear molecule with eight carbons and an acid group. The acid group is theorized to fully dissociate in water, while the perfluoroalkyl chain remains on the surface. This compound is used heavily as an emulsifying agent in manufacturing processes. The most common PFOA derivative is ammonium salt, APFO. The structure of PFOA is shown below in Figure 3 (EFSA, 2008).

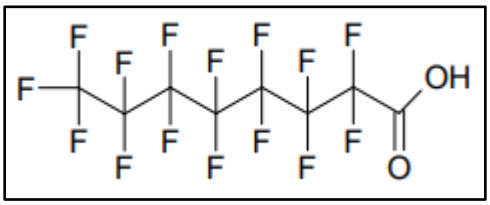


Figure 3. Molecular structure of Perfluorooctanoic Acid, PFOA.

PFOA will readily dissolve in water. Historically, there has been widespread evidence of PFOA in numerous industrial and consumer products. Because of these products and the manufacturing of these products, traces of PFAS are exposed to the environment and can build up throughout the food chain due to bioaccumulation (EFSA, 2008). PFOA is of particular interest due to its recorded presence in the environment and ability to bioaccumulate.

However, since there has been abundant recent research and subsequent regulations on the use of long-chain molecules such as PFOS and PFOA, many manufacturers have switched to using short-chain PFAS alternatives, such as GenX and 6:2 FTOH, which were examined in this study and shown in Figure 4 and 5, respectively. Short-chain PFAS molecules have weakened technical advantages as opposed to the long-chain molecules. As a result of this, manufacturers may use higher quantities of the material in production in order to create their desired chemical results (Mantripragada, 2021). However, these molecules have equal or greater ability to be hazardous to humans. GenX had previously been marketed as a sustainable alternative to PFOA. In an EPA assessment released in October of 2021, GenX was stated to be even more toxic and hazardous than PFOA (Hogue, 2021).

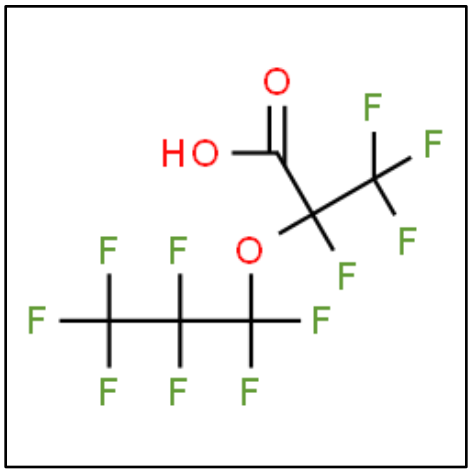


Figure 4. Molecular structure of Perfluoro-2-Propoxypropanoic Acid, GenX.

GenX is the name of the ammonium salt, perfluoro-2-propoxypropanoic acid. GenX presents a more difficult challenge for remediation of the molecule from water, once it has leached into drinking sources. It has a very high water solubility, which means the usual technique for PFAS compounds, adsorption and absorption, are not as effective. New research is being developed on better sorption techniques for this particularly stubborn molecule (Mantripragada, 2021).

FTOH products are a group of fluorotelomer alcohols, with varying lengths of carbon chains. This study focuses on 6:2 FTOH, shown below in Figure 5. 6:2 FTOH is known to be a starting chemical to produce fluoro monomers and polymers that are used in fast-food packaging (Yuan, 2016). The degradation of these products leads to the release of FTOH, which has shown toxic environmental impacts in soil, activated sludge, microbial groups, and small animals (Tseng, 2014). Humans can be exposed to FTOH from inhalation of dust on floor waxes or wood and stone sealants in the home, as well as the consumption of food that was wrapped in FTOH treated paper (Rice, 2020).

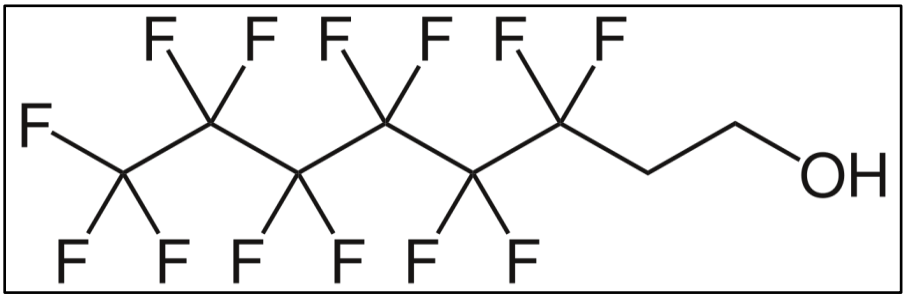


Figure 5. Molecular structure of Polyfluorinated 6:2 Fluorotelomer Alcohol, 6:2 FTOH.

2.2 Uses of Per- and Polyfluoroalkyl Substances

PFAS have been manufactured and used in various production systems since the 1940s. There are more than 4,000 substances used for many different applications and products (Glüge, 2020). The toxic chemicals are present in many aspects of human life, as they are very useful for a variety of applications. One major area that PFAS are used in is manufacturing and production facilities that either produce these compounds or use them in their processes. These products

range from electronics to textiles and paper. PFAS from these facilities can be introduced into the environment directly from the waste of the process or from the products themselves. Another common use of PFAS is in fire-extinguishing foams. While these products can be life-saving, there can be a great amount of pollution that comes from using these foams in training exercises as well as real emergencies (EPA, 2021b). PFAS are also present in various household products such as stain or water repellent for carpets, furniture, or clothing, as well as cleaning products, non-stick pots and pans, and some paints, or varnishes. They are also found in ammunition, climbing rope, guitar strings, and artificial turf (Glüge, 2020). Even specific types of shampoos, dental floss, or cosmetic products have had traces of PFAS. Coatings made from PFAS compounds are commonly found in food packaging in order to create water and grease-resistant wrappers for products such as fast-food, microwave popcorn, pizza boxes, or candy wrappers. (EPA, 2021b). The Silent Spring Institute led a study in 2017 that analyzed the use of PFAS in fast-food packaging. They found coatings that contained PFAS in 46% of food-contact papers and 20% of paperboard packages (Seltenrich, 2020).

The use of PFAS is very common and widespread, which has resulted in the unintended presence of PFAS in other areas of life. PFAS can now be found in drinking water systems and wells, or in soil and groundwater near hazardous waste locations such as Superfund sites. They also have been found in animal products that humans consume, such as fish or dairy products, as a result of the pollutant accumulating through the food chain (EPA, 2021b).

2.3 Health and Environmental Impacts

As stated previously, PFAS compounds are extremely stable and resistant to heat, water, and oil. These properties make them ideal for their industrial uses in cookware, firefighting foams, food packaging, and many more applications. However, these PFAS properties are what makes them so persistent in the environment; the carbon fluorine bond is strong enough that PFAS compounds hardly break down under normal environmental conditions (Sunderland et al., 2019). PFAS have been accumulating in the environment since the late 1940s and their widespread use has led to a large presence of PFAS in the environment. People can be directly exposed to PFAS by working in fields such as firefighting or chemical manufacturing, but the general population is typically exposed through food, drinking water, or inhaling dust or soil (EPA, 2021b). PFAS can migrate from treated food contact materials into food through food-simulants such as butter, vinegar, water, and water/ethanol mixtures (Sunderland et al., 2019). Contaminated water can also expose marine life, which impacts humans when they eat the contaminated seafood. All of these exposure pathways, combined with the stability of PFAS, has created a steadily contaminated environment with many potential risks to human health.

The buildup of PFAS in the body can create serious health effects even in very low concentrations. International concern for the health effects due to PFAS began in the early 2000s when PFOS was detected in the blood of polar bears and other wildlife in remote locations (Sunderland et al., 2019). Today, the CDC estimates that PFAS are detectable in the blood of 98% of Americans (Sunderland et al., 2019). However, it can be difficult to assess the impacts of PFAS to human health because the types and uses of PFAS have changed over time, and people can be exposed in a multitude of ways throughout their lives. Many studies only focus on a limited number of PFAS, when in reality there are over 4,000 PFAS that have various effects at

different toxicity levels. Additionally, PFAS can affect children and pregnant women in different ways than the general population. For example, children are more vulnerable to PFAS because they are going through vital stages of development (Sunderland et al., 2019). Therefore, it can be difficult to identify direct links between PFAS and human health issues, which is why there is a wide range of health issues that are associated with PFAS.

The Agency for Toxic Substances and Disease Registry (ATSDR) states that high levels of certain PFAS are linked to increased cholesterol levels, changes in liver enzymes, small decreases in infant birth weights, decreased vaccine response in children, increased risk of high blood pressure or pre-eclampsia in pregnant women, and increased risk of kidney or testicular cancer (ATSDR, 2020). In addition to those risks, the EPA estimates that PFAS exposure can also lead to decreased fertility, interference with the body's natural hormones, and risk of obesity (EPA, 2021b). Another study found probable links between PFOA exposure and thyroid disease, ulcerative colitis, renal function, and kidney cancer (Sunderland et al., 2019). In addition, a study found that drinking water contaminated with PFOA provided the strongest evidence for increased cancer risk (Sunderland et al., 2019). Altogether, PFAS have been found to create an impressive amount of serious human health problems even without a comprehensive evaluation of all types of PFAS at various toxicity levels. More research needs to be conducted to fully understand how PFAS can impact human health, but based on the current information available, it is clear that PFAS exposure should be a serious concern.

2.4 PFAS Regulation

As the impacts of PFAS have been uncovered, there has been governmental action to regulate the use of PFAS in industry in order to slow the levels of PFAS moving into the environment. These regulations have been mainly targeted at PFOA and PFOS due to their widespread use in consumer products. This has caused the use of PFOA and PFOS to decline. Many companies have even volunteered to phase out these types of PFAS in their products due to the negative health and environmental impacts from these compounds. However, new compounds with very similar properties have been introduced to get around these regulations and to allow companies to easily maintain the same qualities in their products. In the food packaging industry, the compounds 6:2 fluorotelomer alcohol (6:2 FTOH) and hexafluoropropylene oxide-dimer acid (HFPO-DA or "GenX", commonly) have increased in use in light of the PFOA and PFOS phase-out. There is less information and regulation surrounding these two compounds due to their recent introduction. This section details the introduction of PFAS regulations, specific regulations regarding 6:2 FTOH and GenX, and the EPA's plan for eventual PFAS regulations.

2.4.1 Regulation of 6:2 FTOH and GenX Chemicals

Two substances of interest for their past and currently declining use in fast-food packaging include the compounds 6:2 fluorotelomer alcohol (6:2 FTOH) and hexafluoropropylene oxide-dimer acid (HFPO-DA or "GenX", commonly). With regards to 6:2 FTOH, the Food and Drug Administration (FDA) reported in 2020 that a voluntary phase-out of the compound from grease-resistant food packaging had begun, with three manufacturers of food packaging products containing the compound agreeing to the phase-out. The announcement came upon a review of data that suggested biopersistence of the chemical in humans was possible. The phase-out

process is slated to take three years from the beginning of 2021, although it could take up to another 18 months for the compound-laced packaging to be completely removed from the market (FDA, 2020b). Although the current health effects of the accumulation of 6:2 FTOH and one of its metabolites, perfluorohexanoic acid, in the human body are not well understood, studies suggest that the effect on human health may be underestimated (Rice et al., 2020). Specifically, toxicology research completed by Rice et al. (2020) suggests that negative effects on liver and kidney health may occur with increased bioaccumulation of 6:2 FTOH in humans.

Alongside 6:2 FTOH, GenX has also been a compound at the center of discussion on the need to limit the amount of PFAS present in our environment. Broadly, GenX refers to a trade name for a process used to make many useful fluoropolymers without using perfluorooctanoic acid (PFOA), a chemical believed to have adverse effects in humans after bioaccumulation. Similarly to other PFAS, GenX has been found to be present in groundwater, drinking water, and air emissions as well. One use of GenX chemicals is as a grease resistant coating in food packaging materials to resist soiling from the time of packaging until consumption. Initial toxicology studies on the chemical suggest that oral exposure at high enough doses can eventually lead to liver and kidney damage, while also interfering with offspring development as well. While PFOA and perfluorooctane sulfonic acid (PFOS) have been specifically named and somewhat regulated through revisions to the Safe Drinking Water Act, the EPA is just beginning the process of regulating GenX chemicals as the necessary toxicology information comes forward (EPA, 2021a).

2.4.2 Previous U.S. Regulation

Despite the fact that PFAS have been linked to a wide variety of health problems afflicting the general population for quite many decades, there has previously been little regulation of the substances from agencies in the U.S. government, such as the EPA (Dean et al., 2020). Only recently has PFAS regulation come to the forefront in terms of goals for administrative groups like the EPA. For instance, until late 2019 the only regulatory action taken by the EPA with regards to PFAS was to create an advisory limit of 70 parts-per-trillion of PFOA and PFOS in drinking water. This advisory limit was criticized for addressing only two PFAS compounds (although over a thousand compounds have been characterized as PFAS) and for setting a concentration limit that is above levels known to be potentially harmful to humans (Dean et al., 2020). PFAS has been regulated by the EPA through the Toxic Substances Control Act of 1976 (TSCA). The TSCA consists of several components, all of which aim to limit the number of toxic substances entering the environment. For instance, the TSCA requires pre-manufacture notifications (PMNs) from companies before they begin using new chemicals in production cycles. Additionally, the TSCA calls for the issuing of "Significant New Use Rules" when a chemical is used for a novel application in order to test for potential sources of toxicity to humans or the environment (EPA, 2021c).

The TSCA serves some purpose in limiting the number of toxic substances that make it into human ecosystems through manufacturing. The law generally acts as a slow-moving tool and does aid in the following: " … to regulate chemical substances and mixtures which present an unreasonable risk of injury to health or the environment, and to take action with respect to chemical substances and mixtures which are imminent hazards…" (TSCA, 1976). That being

said, several drawbacks increase the need for a more aggressive set of regulations regarding harmful substances, especially PFAS. There is often no data regarding exposure limits or compound analysis when PMNs are submitted for PFAS (Richter et al., 2021). A severe lack of guidelines as to what toxicological data are required by the EPA during submission means that companies may begin using PFAS chemicals soon after submitting essentially no information to the agency. Additionally, companies often utilize low volume or exposure exemptions, or cite the proprietary nature of the chemical as a means of keeping information undisclosed, which is within the provision of the TSCA (Dean et al., 2020). As discussed by Richter et al. (2021), the TSCA also grandfathered-in some 60,000 chemicals after its enactment in 1976. Because PFAS have been used since the mid-20th century, it is extremely likely that PFAS compounds were used without pause despite the compounding negative effects they were having on the environment.

2.4.2 Proposed PFAS Regulation

Although regulation of PFAS compounds has not been a priority of the EPA since their creation, there are plans, at least at the time of this study, to begin remediating the damage that has already been caused and to limit the damage that could occur in the future. The EPA's "PFAS Strategic Roadmap" outlines goals that the agency has committed to striving for through the year 2024, divided into three main sections: "Research, Restrict, and Remediate" (EPA, 2021c). One commitment the EPA outlines in its PFAS roadmap is to generally deny low-volume exemption submissions from companies for PFAS chemicals based on the fact that there is relatively little knowledge of PFAS as a whole. The EPA also clarifies that it will strictly monitor any PFAS compounds that are no longer in use and enforce the "Significant New Use Rules" described previously to ensure their safety if a company desires to revive their utilization. The Office of Water has published a final toxicity assessment of the recently-developed GenX chemicals that have been found in increasing amounts in surface water, groundwater, and drinking water sources across the country.

While the EPA roadmap serves as an outline to *eventual* regulation of PFAS in the U.S., there is still a large gap in knowledge that could lead to things like quantitative limits of PFAS in water sources or in air emissions. The overwhelming lack of current knowledge is extremely evident from the document's goals and layout. Many environmental scientists and researchers have offered their opinion on the necessity of aggressive regulation in the face of ubiquitous environmental damage. For example, Ng et al. (2021) highlight the importance of transport modeling to understand how and where PFAS are moving through the environment. The authors also advocate for research into effective and cost-efficient ways in which to remediate PFAS contamination and for limiting emissions through careful monitoring of production processes. Dean et al. (2020) even call for the complete ban of non-essential PFAS by manufacturers. The EPA has established a plan to begin fighting PFAS contamination in the environment, but at the present time has not made the reform many believe necessary to seriously combat the problem.

2.5 Extraction Techniques

As a crucial part of this study, a technique for the extraction of PFAS from fast-food packaging was required for subsequent detection. Inefficient extraction may result in significant

underestimation of PFAS content or no detection at all (Camdzic et al., 2021). The extraction technique concerning this study was adapted from Dolman & Pelzing (2011), whose article was well-regarded by many other researchers, as well as research into effective PFAS extraction techniques. The technique followed involved a combination for solid-liquid extraction (SLE) using sonication, centrifugation, and nitrogen blowdown evaporation.

2.5.1 Solid-Liquid Extraction

Solid-liquid extraction is an overall method to remove solute from a solid material and disperse it into a solvent. It is assumed that the solute is spread throughout the solid matrix where an optimal solvent may extract all of the solute from the solid. The solid is then removed from the solute via filtration. In the case of the solute being PFAS, the compounds are added as a surface coating to food packaging and only partially seeps into the packaging itself. Therefore, the solvent has complete access to the solute for extraction. The solvent to use is a crucial decision which will affect the recovery of PFAS. Past researchers have used acetone, acetonitrile, and water (Dolman & Pelzing, 2011), ethanol (Begley et al., 2005; Curtzwiler et al., 2021), and acetonitrile and methanol (Zabaleta et al., 2017; Zabaleta et al., 2016). PFAS, the analyte, must be soluble in the solvent; known solubilities of the target analyte bases the decision of choice of solvent, along with the specific extraction techniques used (described below), and cost, safety, and environmental concerns (*Solid-Liquid Extraction*, n.d.).

2.5.2 Sonication

To aid in the extraction of PFAS from the packaging, sonication can be used in a series of techniques to achieve the maximum amount of PFAS compounds from the food contact material. Sonication uses sound waves to agitate and separate particles in a solution (*Sonication*, 2020). The sound energy is typically sent at ultrasonic frequencies of greater than 20 kHz. An electrical signal is converted into a physical vibration that breaks substances apart. The greater the frequency, the stronger the agitation. Thus, the ability to mix solutions increases and the dissolution of a solid into a liquid accelerates (*Sonication*, 2020).

Sonication can be applied to solutions in glassware via an ultrasonic bath. Ultrasonic frequencies produced by the tank create thousands of microscopic vacuum bubbles that collapse and implode violently on the glassware itself. This phenomenon is termed ultrasonic cavitation (Shchukin, 2011). The powerful vibrations release a large amount of energy which disrupts molecular interactions (*Sonication*, 2020). This energy travels through the glassware into the solution, driving the separation of solute from a solid and thoroughly mixing the solution in the appropriate solvent. Such great amounts of energy creates heat which increases the bath temperature about 10-15℃ over a one hour period. Such an occurrence is significant enough to be aware of if added heat may alter the amount of separation achieved or produce side reactions (Shchukin, 2011). The addition of sonication in SLE promotes higher solute recovery rates.

2.5.3 Centrifugation

Centrifugation is another technique to aid in extracting PFAS from food contact material. It can be thought of as a mechanical process that separates particles from a solution. A centrifuge is used for this process– a device that rotates tubes of samples around a fixed central axis.

Separation occurs according to the particle's size, shape, density, viscosity of the medium, and rotor speed (Masoodi, 2021). Centrifugal force is of relevance here which acts to move particles away from a central, rotating axis. If the centrifugal force exceeds the buoyant forces of the liquid media and the frictional force created by the particles, the particles will settle within the tubes (Masoodi, 2021). Particles denser than the solvent will sink in the direction of the applied centrifugal force.

There are two levels of centrifugation that can be performed: low and high speed (Masoodi, 2021). Low speed ranges from 4000 to 5000 rpm. This type is common for sedimentation of heavy particles. High speed centrifugation ranges from 15,000 to 20,000 rpm and is more pertinent for the case of the solid-liquid extraction of PFAS. This type is used in more sophisticated biochemical applications (e.g. PFAS extraction) in which speed and temperature can be controlled manually as required.

Upon the completion of PFAS extraction, the amount of material may be lower than expected. For example, researchers who strategized to determine PFOA levels in human plasma realized that the PFOA may bind to glass surfaces (Belisle & Hagen, 1980). They subsequently used polypropylene low-bind Eppendorf tubes and sample vials to improve recovery rates. The study that was used to shape our extraction methods examined the hydrophobic absorption of PFOA and PFOS to the Eppendorf tubes using standards and acetone as the solvent. Their results indicated no absorption of PFOA or PFOS on the tubes (Dolman & Pelzing, 2011). The characteristics of the specific types of PFAS used in experiment is imperative to avoid mistaking sample loss due to a piece of equipment or a certain technique instead of the glassware.

2.5.4 Nitrogen Blowdown Evaporation

After using several techniques for extracting and isolating PFAS from a solid substrate, it is desirable to concentrate the PFAS. The extraction techniques dealt with the use of a solvent. To concentrate the sample, the solvent may be eliminated by nitrogen blowdown evaporation.

Nitrogen blowdown evaporation directs a stream of nitrogen gas onto a sample. Doing so decreases the vapor pressure over the solvent and the constantly flowing gas pushes off the vapor-saturated layer of air (*Evaporators,* 2004). The vapor does not return to the solvent and speeds up the process of evaporation. A RapidVap N_2 Dry Evaporation System is one such system that can perform this method by using heat, nitrogen gas, and vortex motion. To evaporate efficiently, a bath temperature of 2-3℃ below the boiling point of the solvent should be set. If timed correctly with the correct parameters for temperature, gas pressure, and vortex speed, an extracted sample with excess solvent may be reduced to a concentration viable for subsequent PFAS detection.

2.6 Detection Techniques

PFAS compounds in the environment are typically found between the parts per trillion (ppt) to the parts per billion (ppb) range (USDA, 2021). Standards are typically focused on drinking water since PFAS are most likely to migrate into potable water sources. The EPA sets a recommended limit for PFAS in drinking water at 70 ng/L (EPA, 2016). Since this study is conducted in the state of Massachusetts, it is important to recognize that the Massachusetts

Department of Environmental Protection (MassDEP) set a stricter standard of 20 ng/L (MassDEP, 2021). The limits on drinking water are important to keep in mind, however, food contact material is of concern whose regulation is authorized by the Food and Drug Administration (FDA). FDA only allows PFAS that show no harm to humans with the available data and information (FDA, 2020a). Due to the ever-increasing spread and number of PFAS, scientific data may not keep up. As mentioned previously, there is known to be over 4,000 PFAS in existence. Manufacturers may take advantage of federal authorities having no knowledge of many different PFAS that could be used for food contact material. This concern emphasizes the need for a coupled extraction and detection method to identify PFAS in food packaging.

Once extracted from food packaging, the PFAS solution can be analyzed for multiple parameters including total fluorine content, concentration, and specific components. Pre-concentration during the extraction phase is necessary to relate the analysis to the minute amount of PFAS applied to food contact material. The type of analysis that is able to be conducted depends on the instrumentation used.

2.6.1 LC-MS & LC-MS/MS

A very common analytical technique to determine the composition and concentration is liquid chromatography-mass spectrometry (LC-MS). This technique separates compounds within a solution based on their interactions between two immiscible phases: mobile and stationary. The degree of separation of each compound depends on the compound's affinity for the mobile phase (*LC-MS*, n.d.). After chromatographic separation, compounds are ionized and the mass spectrometer identifies the structure of individual components based on their mass to charge ratio (m/z).

With over 4,000 PFAS currently known, only about 750 PFAS have been identified using LC-MS (Camdzic et al., 2021). There is limited identification with this technique due to poor ionization efficiency, lack of reference materials, variable recoveries during extraction, and the presence of unresolved isomers that do not have characteristic MS fragmentation patterns to facilitate identification (Camdzic et al., 2021). Liquid chromatography tandem mass spectrometry (LC-MS/MS) combines two mass analyzers in one mass spectrometer instrument. LC-MS/MS boasts increased sensitivity (i.e. reduces noise) and more structural information can be gained (*LC-MS/MS*, n.d.). This method differs from one MS by the first MS filters for the precursor ion followed by a fragmentation of the precursor ion into product ions. The second MS filters for and detects the product ions (*MS/MS*, n.d.). There are great hurdles to the use and access of LC-MS/MS including hundreds of thousands of dollars in capital and running costs as well as the need for skilled personnel.

Nuclear magnetic resonance (NMR) spectroscopy is advantageous over LC-MS. NMR is tolerant of matrix effects giving high reproducibility, inexpensive for analysis, simplified in terms of sample preparation, and quantifies PFAS without reference standards (Camdzic et al., 2021).

2.6.2 Nuclear Magnetic Resonance

Nuclear magnetic resonance spectroscopy was the technique of choice for this project. NMR is a non-destructive technique in which a liquid sample is placed in a strong magnetic field (*NMR*,

2014). The nuclei of each type of atom within the sample will resonate at their own specific frequencies. The nuclei of PFAS include an odd number of protons (^{19}F) which gives the required magnetic properties for NMR (*NMR*, 2013). The resonant frequencies of the nuclei are measured and converted into an NMR spectrum which are displayed as peaks on a graph. The height of each peak, or better known as the intensity of the signal, represents the number of nuclei that resonated at a specific frequency (*NMR*, 2013). The peaks are highly characteristic to individual compounds which is why NMR spectroscopy is a go-to technique to identify organic compounds.

As opposed to randomly oriented spins of nuclei in the absence of an external magnetic field, when the nuclei are placed in this field, the nuclear spins orient themselves into specific directions: either with or against the field. See Figure 6 below pictorially displaying the spin adjustment.

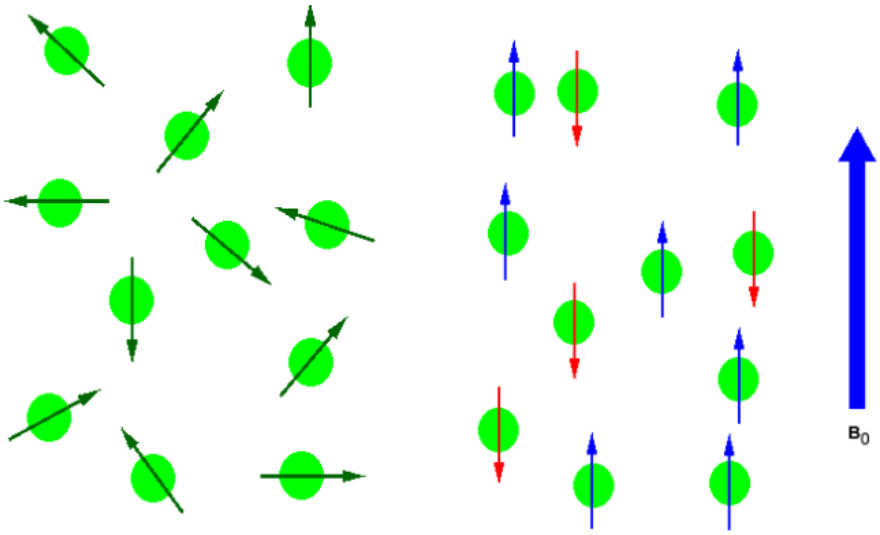


Figure 6. (Left) Random nuclear spin without an external magnetic field. (Right) Ordered nuclear spin in an external magnetic field. (*NMR*, 2014)

The nuclei are then exposed to electromagnetic radiation and those aligned with the field will absorb energy and "spin-flip" to align themselves against the field (*NMR*, 2014). The nuclei are then in a higher energy state and are in "resonance" with the field. See Figure 7 below showing the "spin flip" phenomena.

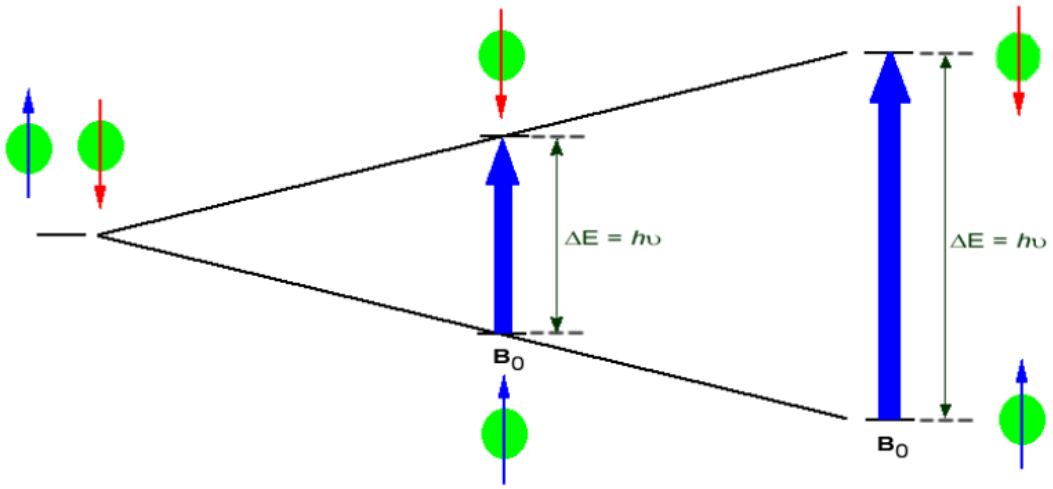


Figure 7. Change of energy states of nuclei as the strength of the magnetic field increases. The bigger the energy difference between two spin states, the higher the frequency needed to "spin-flip." (*NMR*, 2014)

The "spin-flip" is created within the spectrometer by a radio frequency generator pulsing a sample that is in between the poles of a strong magnet and thereby exciting the nuclei (*NMR*, 2014). A detector and amplifier comes before a computer processes and displays the spectrum. The spectrum is a plot of the applied radio frequency vs. absorption. The chemical shift is the position on the plot where the nuclei absorb and is typically calculated by the following equation:

$$Chemical\ shift = \frac{frequency\ of\ signal\ -\ frequency\ of\ standard}{spectrometer\ frequency}$$

This has an arbitrary value and therefore a standard reference point must be used. Tetramethylsilane (TMS) and deuterochloroform ($CDCl_3$) are the two most common standards. Higher chemical shifts have higher energy transitions and are therefore less electronically shielded (Clark, 2014). The opposite is true for lower chemical shifts. Fluorine typically has chemical shifts between -80 and -130 ppm which indicates high electron shielding (Clark, 2014). This may be due to fluorine being very electronegative with its electron clouds tightly held.

Fluorine (^{19}F) NMR spectroscopy was very practical for the project's experimental purposes due to carbon-fluorine bonds being much stronger than carbon-hydrogen bonds for hydrogen NMR. The chemical shift for hydrogen NMR is between 0 and 12 ppm which translates to low electron shielding with increased interference from other molecules. Fluorine NMR can differentiate PFAS from non-PFAS compounds and from F^- compounds (Camdzic et al., 2021). This ability is due to the distinguishable ^{19}F signal from the terminal trifluoromethyl moiety $-CF_3$ in PFAS alkyl chains (Camdzic et al., 2021). The CF_3 region occurs around -80 ppm on a NMR spectrum which corresponds to the CF_3 bond at the end of a PFAS chain. Moreover, there is a CF_2 region around -120 ppm which includes several peaks on a NMR spectrum that shows the chain structure of PFASs.

Researchers from the University of Buffalo report the first ever study of ^{19}F NMR signals to be useful in identifying and differentiating classes of PFASs within a sample (Camdzic et al., 2021). In addition, the intensity of the CF_3 signal can be used to determine the total PFAS content regardless of headgroup. The study further indicates that NMR spectra, such as the one in Figure 8, with ether linkages (e.g. GenX) show characteristic reference signals for both CF_2 and CF_3 signals which are useful for PFAS detection.

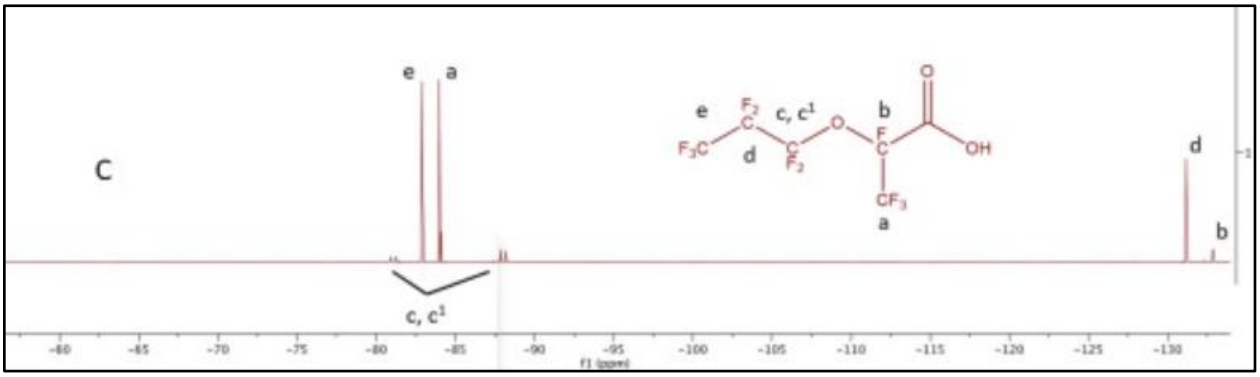


Figure 8. Representative NMR spectrum of GenX. Characteristic CF_2 signals around -130 ppm and CF_3 signals around -80 ppm.

Before using NMR spectroscopy, samples need to be prepared for proper quantitation and species identification. Since our compounds of interest are PFAS within food packaging, they need to be dissolved in a solvent. The solvent should not have any bonds that could produce and interfere with the NMR spectra. Deuterated solvents avoid this problem by having the deuterated atoms absorb at different frequencies and therefore not interfere with the spectra (*NMR*, 2013). This is more of an issue with hydrogen NMR which have chemical shifts between 0 and 12 ppm as opposed to fluorine NMR with chemical shifts between -80 to -120 ppm (Camdzic et al., 2021). Therefore, non-fluorinated molecules have little interference in fluorine NMR. Only a fraction of the amount of deuterated solvent used in hydrogen NMR needs to be used for fluorine NMR to result in a spectrum that is true to the compounds of interest.

3.0 Methodology

The goal of this MQP was to follow an appropriate procedure to extract, detect, and identify several PFAS intended for food packaging material found at fast-food establishments. To accomplish this goal, we created a unique procedure for sample extraction and followed the NMR detection and analysis techniques developed by Choi et al. (2019) and adjusted by Cain and Powers (2020). Our main objectives were as follows:

1. Develop an effective solid-liquid extraction procedure to remove PFAS from food contact material.
2. Use NMR spectroscopy to quantify the amount of PFAS extracted from the food contact material.
3. Demonstrate if the extraction procedure is effective by spiking PFAS-free material using PFAS widely used in manufacturing.

3.1 Materials

The PFAS compounds used in this study were perfluorooctanoic acid (PFOA), 6:2 fluorotelomer alcohol (6:2 FTOH), and the ammonium salt of hexafluoropropylene oxide dimer acid (HFPO-DA and more commonly known under the Chemours trademark name "GenX"). PFOA and 6:2 FTOH were purchased from Sigma Aldrich as a solid and liquid, respectively, and GenX was purchased from Matrix Scientific as a liquid. Acetone and methanol were purchased from VWR Chemicals and Fisher Chemical, respectively, and used as extraction solvents (along with purified water). Deuterated methanol (CD_3OD) was purchased as a liquid from Cambridge Isotope Laboratories and used as the deuterated solvent for NMR analysis. A Barnstead Labtower Reverse Osmosis water purifier from Thermo Fisher Scientific was used to produce purified water in the lab. The identifications of each chemical is displayed in Table 3.1 below.

Table 3.1: Chemical Compound Identification

Compound	Abbreviation	Formula	Purity (wt%)	CAS Number
Perfluorooctanoic Acid	PFOA	$C_8HF_{15}O_2$	96	335-67-1
6:2 Fluorotelomer Alcohol	6:2 FTOH	$C_8H_5F_{13}O$	97	647-42-7
Ammonium Salt of Hexafluoropropylene Oxide Dimer Acid	HFPO-DA ("GenX")	$C_6HF_{11}O_3$	97	13252-13-6
Methanol-d4	-	CD_3OD	99.8	811-98-3
Purified Water	-	H_2O	99	7732-18-5
Acetone	-	C_3H_6O	99.5	67-64-1
Methanol	-	CH_3OH	99.9	67-56-1

Glassware used included 150 mL and 250 mL Pyrex® Erlenmeyer flasks for sample preparation and storage. 100 mm x 15 mm Pyrex® Petri dishes were used for PFAS-spiked samples during extraction. Screw cap glass bottles were used to store stock PFAS solutions with known concentrations. 100 mL RapidVap tubes from Labconco were used to concentrate extracted samples. Nalgene™ Oak Ridge high-speed PPCO centrifuge tubes purchased from Thermo Scientific were used during sample extraction. Other materials included glass and plastic graduated cylinders, glass stir rods, school scissors, Pasteur pipettes, lab spatula, 100-1000 µL Fisherbrand Finnpipette, 10-50 µL Fisherbrand Finnpipette, and Fisherbrand Reference Pipet Tips were all used for sample preparation. Parafilm M sealing film was used to cover samples contained in Erlenmeyer flasks or RapidVap tubes that were not actively being worked with to prevent evaporation. After evaporating samples to their final volume, the samples were stored in 4 mL clear glass vials purchased from Chemglass Life Sciences.

Instruments used in this project included the Branson M1800 Ultrasonic Cleaner, Marathon 21000R Multi Purpose Centrifuge, RapidVap N2 Dry Evaporation System, and Bruker BioSpin 500 MHz Avance AV-III Digital NMR Spectrometer equipped with a ^{19}F-NMR cryoprobe. 5 mm NMR tubes from Fisher Scientific were used to insert samples into the spectrometer. The sonicator, centrifuge, and evaporation system were all located in WPI's Kaven Hall Laboratory. The spectrometer was located at WPI's Life Sciences and Bioengineering Center (LSBC).

3.2 Preparing and Cleaning Glassware

Throughout this experiment, we used the following glassware: Pyrex® beakers (100-500 mL), Pyrex® Erlenmeyer flasks (150 mL, 250 mL), Pyrex® graduated cylinders (10 mL, 100 mL, and 250 mL), Pyrex® petri dishes, glass Pasteur pipettes, and 5 mm glass NMR tubes.

The glass pasteur pipettes and NMR tubes were used directly out of the sterile packaging, so these materials were considered clean and there was no additional preparation before using those materials. However, all other glassware was provided in WPI's Kaven Hall Laboratory and was cleaned to ensure that contamination did not impact our results.

The glassware was cleaned using the following procedure:

1. Visual inspection of the item to look for damages that could lead to breakage or leaks (cracks, chips, etc.). Damaged items were not used and were discarded appropriately.
2. Rinse glassware with tap water.
3. Scrub with detergent solution made with Fisherbrand Sparkleen 1 and tap water.
4. Rinse with tap water.
5. Rinse with purified water.
6. 5-minute air dry on a paper towel with any remaining moisture wiped off with a Kimwipe.

3.3 Solid-Liquid Extraction

The application of PFAS on fast-food packaging is at very low concentrations and below the detection limit of the NMR. To be able to read concentrations from the NMR, samples needed to be concentrated. Concentrating samples involved the extraction of PFAS from solid food contact material with liquid solvents. The type of packaging used in this project was based on a previous study conducted by the Mind the Store campaign and Toxic-Free Future ("Packaged," 2020). They tested total fluorine content on numerous fast-food packaging samples from major chains. The fast-food packaging used was taken from select McDonald's® and Burger King® restaurants in the Worcester, MA area. Specifically, McDonald's small french fry bag and Burger King's small chicken nugget bag and Whopper wrapper composed our samples due to having the greatest fluorine content from the previous study. Sample pictures of the packaging are included in Appendix C. The sonicator, centrifuge, and evaporation system, as described in section 3.1, were used to help in this process.

Solid-liquid extraction was employed via the following procedure:

1. Obtain clean, unused pieces of fast-food packaging material (as described in the introduction to section 3.3). For the McDonald's small french fry bag, use 3 pieces. For the Burger King small chicken nugget bag, use 4 pieces. For the Burger King Whopper wrapper, use 1 piece.
2. Cut off adhesive and ink sections and then cut the remaining packaging into very small pieces of about 1 cm^2 using school scissors. **Note: Keep ink sections on the Whopper wrapper.**
3. Weigh the cut sample pieces using a mass balance.
4. Transfer cut sample pieces into a 150 mL, 200 mL, or 250 mL Pyrex® Erlenmeyer flask.
5. Fill Erlenmeyer flask with enough purified water to submerge the sample (approximately 50-100 mL).
6. Prepare a sonication bath in the Branson M1800 Ultrasonic Cleaner with tap water and utilize a clamp stand adjacent to the instrument to hold the Erlenmeyer flask.
7. Sonicate the sample for one hour.
8. Evenly transfer sample solution to polypropylene tubes designated for the Marathon 21000R Multi Purpose Centrifuge. As needed, use tap water in separate tubes to balance the centrifuge. **Note: Tubes must be balanced by mass. Damage could occur if the centrifuge is unbalanced.**
9. Insert tubes into centrifuge in an even spread and centrifuge sample at 3,600 rpm for 20 minutes.
10. Extract supernatant using a glass transfer pipette and place into a 100 mL RapidVap tube.
11. Fill a second 100 mL RapidVap tube with another sample or purified water to the same volume. **Note: Similarly to the centrifuge, proper balance needs to be achieved to maximize performance.**
12. Turn the RapidVap N2 Dry Evaporation System on. Place the exhaust pipe attachment into a fume hood.
13. Set the temperature to 90℃, rotational speed to 50, duration to 75 minutes, and select 2 samples on the control panel of the evaporation system.
14. Press the preheat button and let the evaporation system heat to 90℃.

15. Insert tubes into the designated spots in the evaporation system. Close the lid.
16. Open the cylinder valve on the nitrogen gas cylinder and adjust the regulator hand knob to raise delivery pressure to about 5 psi.
17. Open outlet valve on the regulator to establish gas flow to the evaporation system.
18. Press run on the control panel and allow the sample to evaporate for about 1.25 hours or until the volume in the tube is 2 mL. When sample volume is low, complete regular checks of RapidVap tubes to ensure the volume does not go below 2 mL.
19. Press stop on the control panel and close the outlet valve and cylinder valve on the nitrogen gas cylinder. Ensure pressure gauges read a value of 0 psi.
20. Remove RapidVap tube with 2 mL of sample and use a glass Pasteur pipette to transfer the sample into a 4 mL clear glass vial for temporary storage.
21. Use an automatic pipette to extract 540 μL out of the vial and add into a 5 mm NMR tube.
22. Use an automatic pipette to add 60 μL of deuterated methanol into the NMR tube. **Note: Following a 90% non-deuterated solvent, 10% deuterated solvent approach.**
23. Cap the NMR tube and label sample with marker on the cap or tube (do not use tape).

The above procedure was repeated for each sample and for each sample in a different solvent (acetone, methanol). The RapidVap N2 Dry Evaporation System was run at 5℃ below the boiling point of each solvent. The operating temperature for the acetone samples was 51℃ and 60℃ for the methanol samples. All samples were temporarily stored in NMR tubes before analysis.

3.4 Standard Curve Solutions

3.4.1 PFOA

Two standard PFOA solutions were made to validate the PFOA standard curve created by Choi et al. (2019). The concentrations were chosen to be within the range of the standard curve. Detailed calculations that determined these concentrations, along with the values for GenX and 6:2 FTOH, are shown in Appendix A.1. These two standard solutions were made from PFOA powder with purified water and were prepared using the following procedure:

1. Mass 4.56 mg of PFOA powder.
2. Use a 20 mL graduated cylinder to measure 20 mL of purified water and pour into a small beaker.
3. Dissolve the PFOA powder in purified water to make a 0.55 mM solution.
4. Measure 1 mL of the 0.55 mM solution and pour it into a new beaker.
5. Measure 9 mL of purified water and mix it into the new beaker with the 1 mL of 0.55 mM solution to make a 0.055 mM solution.
6. Prepare NMR tubes for both standard solutions following steps 21-23 in Section 3.3.

3.4.2 GenX and 6:2 FTOH

The solutions for the standard curves for GenX and 6:2 FTOH were made from liquid forms of those PFAS and were prepared using the following procedure:

1. Measure 0.1 mL of GenX solution from the bottle and mix with 100 mL of water to make a 5.59 mM stock solution.
2. Measure 2.95 mL of the 5.59 mM stock solution and mix with 27.05 mL of water to make a 0.55 mM solution. Label solution "GenX Solution 1."
3. Measure 2 mL of GenX Solution 1 and mix with 8 mL of water to make a 0.11 mM solution. Label solution "GenX Solution 2."
4. Measure 1 mL of GenX Solution 1 and mix with 9 mL of water to make a 0.055 mM solution. Label solution "GenX Solution 3."
5. Measure 1 mL of GenX Solution 2 and mix with 9 mL of water to make a 0.011 mM solution. Label solution "GenX Solution 4."
6. For the FTOH solution, measure 0.1 mL of FTOH solution from the bottle and mix with 50 mL of water to make a 9.07 mM stock solution.
7. Measure 1 mL of the 9.07 FTOH solution and mix with 16.5 mL of water to make a 0.55 mM FTOH solution. Label Solution "FTOH Solution 1."
8. Repeat steps 3-5 using FTOH Solution 1 to make the rest of the FTOH standard solutions of 0.11, 0.055, and 0.011 mM.
9. Prepare NMR tubes for all eight standard solutions following steps 21-23 in Section 3.3.

3.5 Spiked Sample Preparation

Since many PFAS compounds have been phased out or are in the process of being phased out of manufacturing, spiked samples were prepared to confirm that the extraction process was successful even if there were no PFAS in the packaging samples collected. For PFOA, GenX, and 6:2 FTOH, two spiked solutions were made of two different concentrations with the goal that the spiked samples would fall above a concentration of 0.08 mg/L, the minimum detectable concentration of PFAS via NMR analysis (Cain & Powers, 2020). These spiked solutions were stored in screw cap glass bottles. Spiked samples were extracted immediately and stored in Erlenmeyer flasks covered with parafilm before reaching the stage of NMR analysis. Sample calculations of these samples are found in Appendix A.2.

The spiked solutions for PFOA were made with PFOA powder and were prepared using the following procedure:

3.5.1 PFOA

1. Choose target concentrations for spiked solutions. For this experiment, the concentrations were 0.0232 mM, 0.00174 mM, and 0.00116 mM.
2. Weigh 2.4 mg of PFOA powder using a mass balance.
3. Use a 250 mL graduated cylinder to measure 250 mL of methanol and then pour into a large Pyrex® beaker.
4. Dissolve PFOA powder into the beaker of methanol to make a solution with a concentration of 0.0232 mM. Label solution "PFOA Solution A."
5. Use a 25 mL graduated cylinder to measure 10 mL of PFOA Solution A and pour into a new beaker.

6. Measure 190 mL of methanol in a graduated cylinder and pour into the beaker containing the 10 mL of PFOA Solution A. This makes a second solution with a concentration of 0.00174 mM. Label solution “PFOA Solution B.”
7. Measure 15 mL of PFOA Solution A in a graduated cylinder and pour into a new beaker.
8. Measure 185 mL of methanol in a graduated cylinder and pour into the beaker containing the 15 mL of PFOA Solution B. This makes a third solution with a concentration of 0.00116 mM. Label solution “PFOA Solution C.”
9. Store PFOA Solution A in a screw cap glass bottle for future dilution. Label bottle.
10. Use PFOA Solutions B and C for immediate use or store in separate screw cap glass bottles for future use. Label bottle.

The spiked solutions for GenX and 6:2 FTOH were made with the previously prepared standard solutions and were prepared using the following procedure:

3.5.2 GenX

1. Choose target concentrations for spiked solutions. For this experiment the target concentrations for GenX were 0.00224 mM, 0.00218 mM, and 0.00127 mM.
2. Use a 250 mL graduated cylinder to measure 250 mL of methanol and then pour into a large Pyrex® beaker.
3. Use an automatic pipette to measure 100 μL of the 5.59 mM GenX stock solution previously made in Section 3.4.
4. Mix into the beaker of methanol to make a solution with a concentration of 0.00224 mM. Label solution “GenX Solution A.”
5. Measure 243.3 mL of GenX Solution A and 6.7 mL of methanol and pour into a new Pyrex® beaker to make a solution with a concentration of 0.00218 mM. Label solution “GenX Solution B.”
6. Measure 52.4 mL of GenX Solution A and 197.6 mL of methanol and pour into a new Pyrex® beaker to make a solution with a concentration of 0.00127 mM. Label solution “GenX Solution C.”
7. Store GenX Solution A in a capped glass bottle for future dilution.
8. Use GenX Solutions B and C for immediate use or store in separate capped glass bottles for future use.

3.5.3 6:2 FTOH

1. Choose target concentrations for spiked solutions. For this experiment, the concentrations for 6:2 FTOH were 0.00363 mM, 0.00198 mM, 0.00115 mM.
2. Use a 250 mL graduated cylinder to measure 250 mL of methanol and then pour into a large Pyrex® beaker.
3. Use an automatic pipette to measure 100 μL of the 9.07 mM FTOH solution previously made in Section 3.4.
4. Mix into the beaker of methanol to make a solution with a concentration of 0.00363 mM. Label solution “FTOH Solution A.”

5. Measure 136.4 mL of FTOH Solution A and 113.5 mL of methanol and pour into a new Pyrex® beaker to make a solution with a concentration of 0.00198 mM. Label solution "FTOH Solution B."
6. Measure 79.4 mL of FTOH Solution A and 170.6 mL of methanol and pour into a new Pyrex® beaker to make a solution with a concentration of 0.00115 mM. Label solution "FTOH Solution C."
7. Store FTOH Solution A in a capped glass bottle for future dilution.
8. Use FTOH Solutions B and C for immediate use or store in separate capped glass bottles for future use.

The samples were then spiked using the following procedure:

3.5.4 PFAS Spiking

1. Repeat steps 1-4 from section 3.3.
2. Distribute sample pieces evenly into two Pyrex® Petri dishes.
3. Pour 10 mL of PFOA Solution B into each Petri dish and ensure all pieces are completely submerged in solution.
4. Place Petri dish covers on and store completely covered for at least 6 hours.
5. Move Petri dishes into a fume hood and partially remove the covers to allow liquid to evaporate.
6. Wait at least 24 hours for samples to completely dry before beginning the extraction procedure in Section 3.3.
7. Repeat steps 1-7 using the following spiked solutions: PFOA Solution C, GenX Solutions B and C, and FTOH Solutions B and C.

3.6 NMR

Once all samples were prepared, they were transported to the LSBC to be analyzed by the Bruker BioSpin 500 MHz Avance AV-III Digital NMR Spectrometer equipped with a ^{19}F-NMR cryoprobe. Under the direction of Daryl Johnson, Instrumentation Core Technician of the Life Sciences and Bioengineering Center, a training session was performed and a user profile created to safely operate the instrument. The NMR spectrometer was operated according to the following procedure:

Figure 9. NMR leveling jig used for proper sample preparation for NMR analysis.

1. Insert the prepared NMR tube into a NMR tube holder to the correct depth using the level jig shown in Figure 9.
2. Remove the NMR tube from the level jig and thoroughly wipe it down with a Kimwipe.
3. Select an available spot on the NMR track and place the sample tube into the open slot.

Figure 10. Diagram of F-NMR sample input.

4. Select the number enclosed by box A in Figure X that corresponds to the slot that the sample was just placed in and then click ‘Add’ at the bottom of the screen.
5. Label the sample in the Name section enclosed by box B.
6. Choose the appropriate deuterated solvent used in the sample in the Solvent section enclosed by box C (i.e. methanol D-4).
7. In the Experiment section enclosed by box D, select F_{19} - NMR.
8. Select the time (day or night) for the experiment to run by clicking the icon in box E.
9. Set the number of cycles for analysis to 1024 by clicking on the icon in box F.
10. Click the number in box A again and then click ‘Submit’ at the bottom of the screen to add the sample to the queue.

4.0 Results and Discussion

4.1 NMR Spectra of PFAS

The NMR spectra of PFASs can be easily distinguished from other chemicals. The spectra can also be considered simple with only two regions of interest. Figure 11 below shows an exemplary NMR spectrum that resulted from one of our samples.

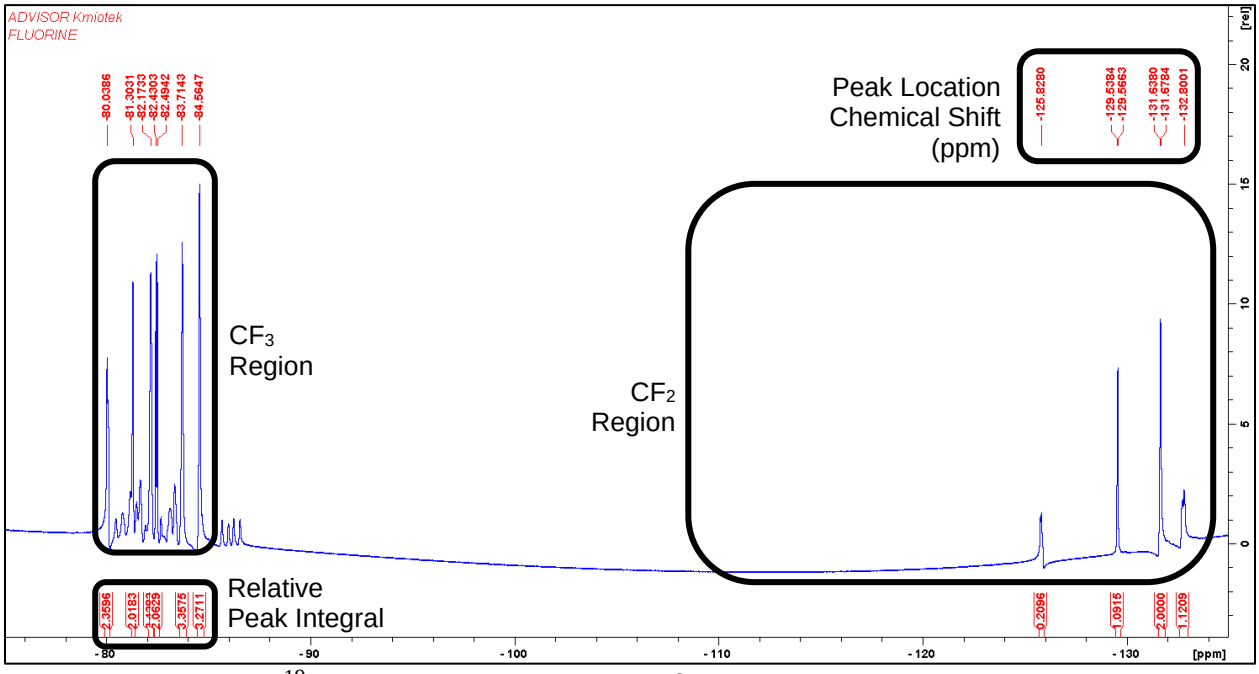


Figure 11. Model ^{19}F-NMR spectrum. The specific sample is the 0.55 mM GenX standard solution.

The peaks on the left end of the spectrum, roughly located around -82 ppm, represent the CF_3 terminal group present for all PFASs. The particular spectrum contains more than one signal in this region since GenX has two CF_3 groups, making it unique. The CF_3 peak stayed relatively constant for all samples analyzed. The multiple peaks on the right end of the spectrum, usually located between -110 and -130 ppm, represent the CF_2 groups on a PFAS molecule. The CF_2 peak locations vary from one PFAS to another since each molecule is structured slightly differently, leading to unique NMR signals. The ratio between the peak area of the CF_3 group to the CF_2 group is roughly 3:2 due to the number of fluorine molecules on each side. The peaks in Figure 11 are easily distinguishable due to the signal to noise ratio (S/N). Having a S/N ratio of 3:1 considers a peak to be true, in which proper identification of PFAS along with its concentration can be accomplished. The number of scans can clarify NMR spectra in terms of detection. Increasing the number of scans increases the S/N ratio and thereby the limit of detection. In the above figure, 64 scans were completed which resulted in a S/N ratio above 3:1. Other samples had 1024 scans to try to bring the S/N ratio to an acceptable value due to its particular composition.

All of our samples did not depict such a clear spectrum. Figure 12 below shows a NMR spectrum that is considered characteristic of most of our analyzed samples. See Appendix D for all NMR spectra that produced signals. In addition, a link to a OneDrive folder is provided in Appendix D which holds the files of all NMR samples that were run, regardless if it produced signals or not.

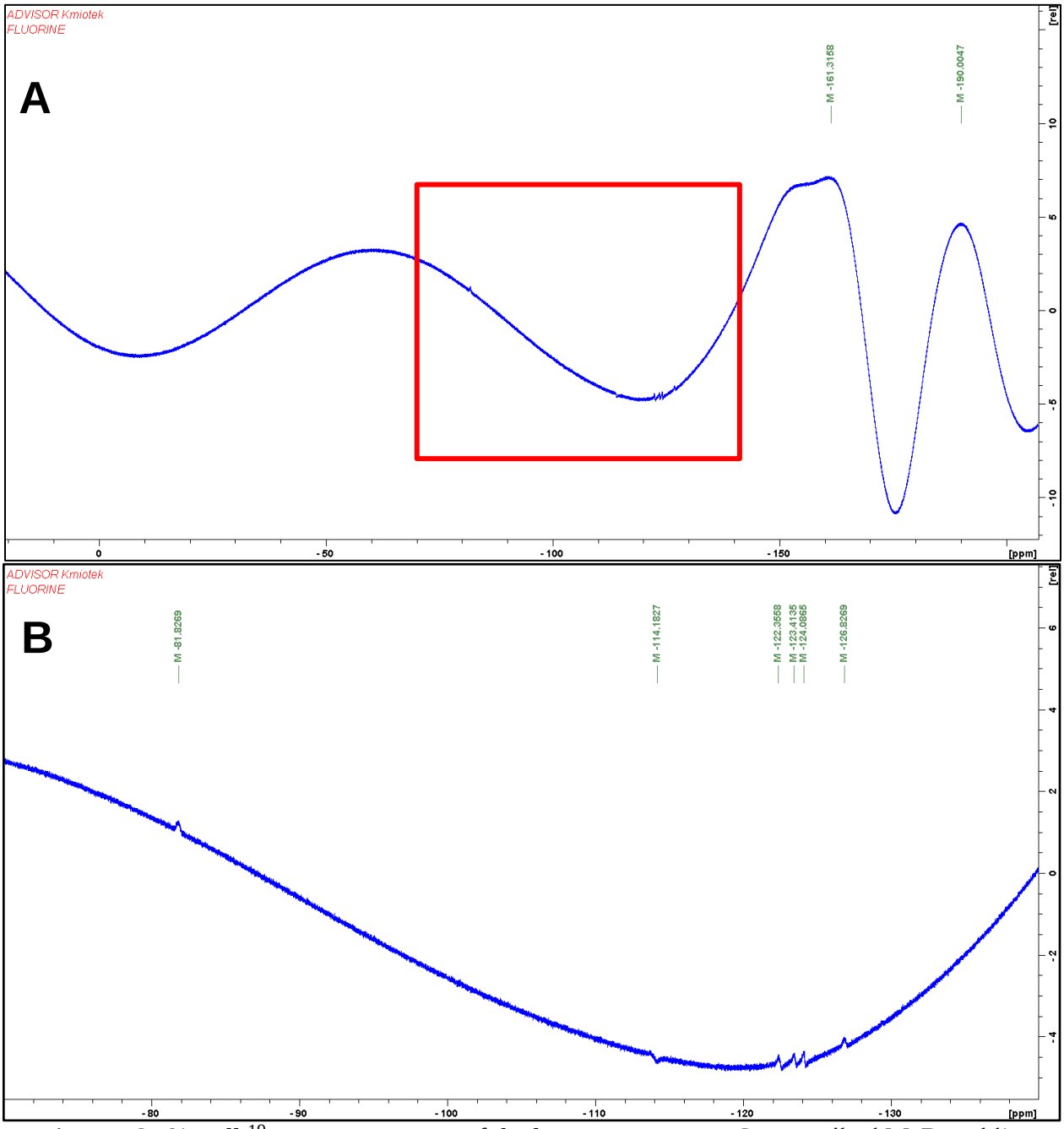


Figure 12. A) Full ^{19}F-NMR spectrum of the low concentration GenX-spiked McDonald's sample in acetone which was representative of most analyzed samples. Note the two broad peaks at -160 and -190 ppm. **B)** Region of interest within the full spectrum where signals due to PFAS are present. Notice the tiny peaks or "bumps" within the noise of the spectrum.

Almost all analyzed samples showed barely noticeable “peaks” in the -82 ppm region and between -110 ppm and -130 ppm. The chemical composition of the ^{19}F-NMR cryoprobe contains traces of PTFE (i.e. Teflon), a synthetic fluoropolymer, from its fabrication and was responsible for a large broad double peak at about -160 and -190 ppm. This peak was responsible for the curved baseline shown in Figure 12, after manipulation of the spectrum. In a typical NMR spectrum, this “large” peak would be considerably smaller than the peaks for the analyte in solution. However, our analyte concentrations were not sufficient enough to dwarf the background interference from the cryoprobe. The samples were initially calculated to be within the detection range of the NMR, however the percent recovery could have been a considerable factor that diluted the samples past the limit of detection. For the spiked samples, the final concentration was theoretically higher than the original spiking solutions, however, it is assumed that the percent recovery of PFAS was low enough to not be appreciably above the concentration of the original spiking solutions. Some sample concentrations were not high enough to produce a strong enough signal to be distinguished from the baseline noise, regardless if the samples were analyzed at 64 or 1024 scans. These issues are further explained in Section 4.2 and 4.3.

The small “peaks” within the spectra are still of importance. Although the S/N ratio of these peaks is below 3:1, the peaks were located in the two regions of interest for PFASs. Some samples were spiked with a particular PFAS which would ideally result in signals distinguishable from the noise at the baseline. However, as discussed in subsequent sections, spiking samples with a higher and lower concentration of PFAS did not return the expected NMR spectra as results varied widely. Due to the NMR signals being too small to integrate and calculate quantifiable concentrations, the standard curve solutions of varying concentration could not be used to produce standard curves for each PFAS. The standard curves would have been used to find the concentration of PFAS in non-spiked and spiked samples as well as match peaks to a PFAS compound. Detailed calculations for standard solution concentrations are found in Appendix A.1. A few NMR spectra for these samples are presented in Appendix D. For the samples that did not undergo spiking, the small peaks were still of immense importance due to the fact that the peaks appeared in the locations where PFAS signals are common, especially in the CF_3 region. Even though quantitative concentrations could not be calculated from the analyzed spectra, the presence of the low intensity peaks (or rather disturbances) suggest that the SLE technique was capable of returning qualitative findings.

4.2 NMR Analysis for Samples Spiked with Different PFAS

There were 24 packaging samples that were spiked with a known concentration of various PFAS. It was expected that NMR spectra of the spiked samples would show the presence of the specific compound that it was soaked in. However, not all spiked samples showed evidence of PFAS. There are several possible reasons for this. The first is that the method for spiking the paper, by letting it soak in solution until dry, did not allow for the PFAS molecules to bind to the paper and instead binded to the petri dishes. Another issue would be an inadequate extraction technique to leach the PFAS back off the paper after spiking. Either of these methods failing or a combination of the two would result in a lack of PFAS presence in the samples sent to NMR. It is also likely that the amount of PFAS in the NMR sample was not at a high enough concentration to get a reading. There was also some concern with the state of our NMR samples as they were entered into the machine. When the NMR tubes were prepared with our sample solutions, there were

some precipitates that came out of solution when the deuterated methanol was added. This could be a solubility issue, where the analytes in our sample were not as soluble in the methanol. Having solid particles in the NMR tubes can cause disturbances in the reading.

As mentioned and shown in Figure 12, the NMR spectra for these spiked samples showed small "peaks" that are not prominent enough to pass a 3:1 S/N ratio test. However, for the purposes of this experiment, these peaks will be noted as important because when there were small peaks on the spectra, they consistently appeared in the CF_3 and CF_2 regions. This indicates that there are traces of PFAS compounds within the sample, but not at high enough concentration to generate significant signals beyond the noise. Since the samples were spiked with a known concentration of PFAS, either higher concentrations must be used or the extraction method must be greatly improved.

Some trends appeared between the different PFAS compounds and the solvents used. Table 4.1 shown below illustrates the NMR results for the packaging spiked with PFOA, GenX, and 6:2 FTOH. Red indicates that there were no peaks observed in the desired locations. Green indicates that there were small peaks observed. The samples are also labeled "High" or "Low." This is indicative of which concentration of the spiking solution was used. For PFOA, the high and low concentration was 0.00174 ± 0.000063 mM and 0.00116 ± 0.0000066 mM, respectively. For GenX, the high and low concentrations were 0.00218 ± 0.000068 mM and 0.00127 ± 0.000049 mM, respectively. The 6:2 FTOH high and low concentrations were 0.00198 ± 0.000075 mM and 0.00115 ± 0.000046 mM, respectively. The error values for these concentrations are shown in detail in Appendix B.

Table 4.1. Qualitative analysis of ^{19}F-NMR spectra from 24 spiked packaging samples and the presence of small "peaks" at designated locations indicating CF_3 and CF_2 groups.

PFOA				
	CF_3 Presence	CF_2 Presence	CF_3 Presence	CF_2 Presence
Solvent:	Water		Acetone	
Low McDonald's				
High McDonald's				
Low Burger King				
High Burger King				
GenX				
	CF_3 Presence	CF_2 Presence	CF_3 Presence	CF_2 Presence
Solvent:	Water		Acetone	
Low McDonald's				
High McDonald's				
Low Burger King				
High Burger King				
6:2 FTOH				
	CF_3 Presence	CF_2 Presence	CF_3 Presence	CF_2 Presence
Solvent:	Water		Acetone	
Low McDonald's				
High McDonald's				
Low Burger King				
High Burger King				

Out of all three compounds, the samples spiked with GenX resulted in the most peaks present on the NMR spectra. This could indicate that GenX either binded with the paper packaging more readily than the other compounds or was more readily extracted through sonication and centrifugation. The spiking solutions were prepared at a high and low concentration. The results did not show any clear distinction between the different concentrations. The low concentration was about half the molarity of the high concentration. The results support that this disparity

between the two concentrations is not large enough to show changes in the results. For all three compounds, the samples in acetone showed evidence of PFAS more often. Acetone is a better extraction solvent for these PFAS compounds because it is slightly more polar than water and therefore has a higher solubility. Fluorinated compounds are hydrophobic and therefore water does not make a successful extractant. GenX showed the most extracted PFAS from the acetone solvent. Since acetone is so polar, it is likely that it was able to dissolve the carboxylic acid group in the GenX molecule (see Figure 4) and extract the compounds better. The varying structures of each PFAS molecule have their own specific solubilities in every solvent, resulting in the changing results between each compound. The effectiveness of each solvent is further discussed in Section 4.4. Each of the spiked solutions were also used on plain copy paper as shown in Table 4.2.

Table 4.2. Qualitative analysis of ^{19}F-NMR spectra from 12 spiked batches of copy paper and the presence of small "peaks" at designated locations indicating CF_3 and CF_2 groups.

Spiked Copy Paper					
	CF_3 Presence	CF_2 Presence		CF_3 Presence	CF_2 Presence
Solvent:	Water			Acetone	
Low PFOA					
High PFOA					
Low GenX					
High GenX					
Low FTOH					
High FTOH					

Interestingly, none of these samples produced any peaks on the NMR results. This may indicate that the PFAS compounds do not easily bind to the copy paper. Perhaps the compounds more readily bind to the other packaging samples since that paper had been previously treated with a PFAS coating. Another possible explanation for this could be that the concentration of the spiking solutions was too low to recover enough PFAS to produce an NMR signal. It is possible that some PFAS did bind to the copy paper but once the samples went through the extraction process, the concentration of PFAS in the sample may have been below the limit of detection of the NMR. These possibilities and inconsistent spiked sample results make it difficult to conclude about the success of the extraction technique.

4.3 NMR Analysis of Food Packaging Samples

As stated prior, the peaks observed did not have a 3:1 S/N ratio, but given the expected low concentrations, they still offered meaningful information. The most important takeaway from these results is that NMR picked up signals of PFAS within common food packaging that consumers eat from every day. Figure 13 shows an example of the peaks found for the samples with no spiking. This spectrum shows both CF_3 and CF_2 presence in their respective regions.

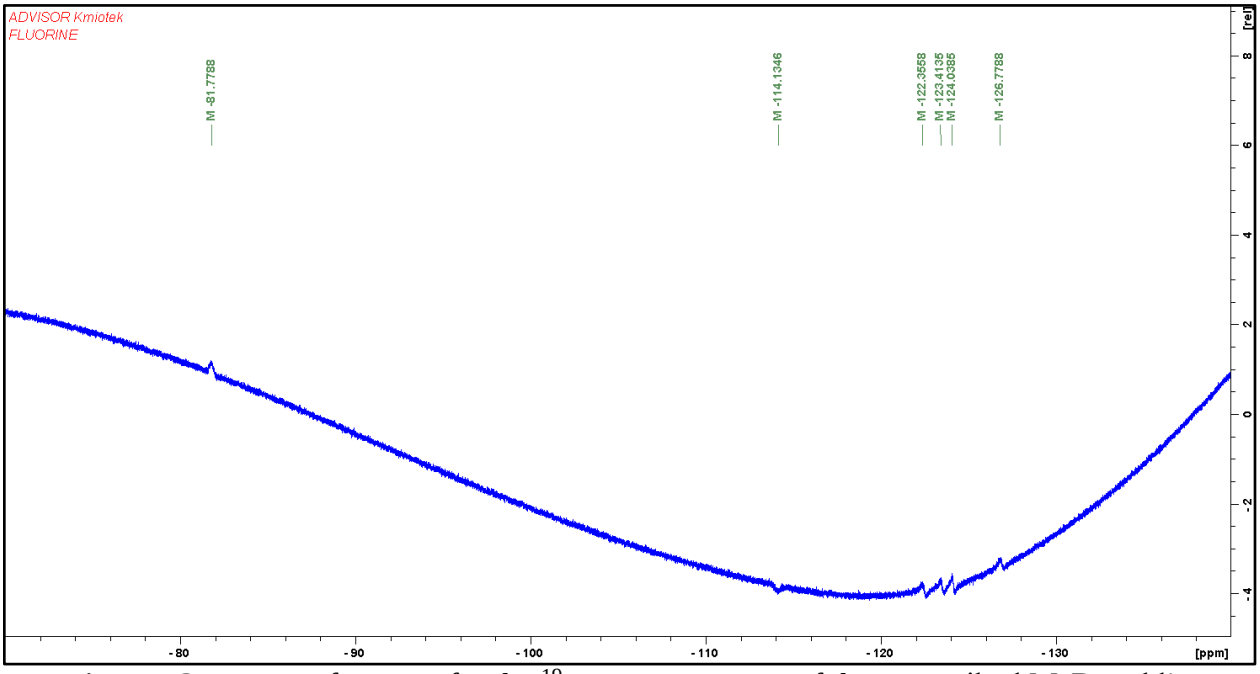


Figure 13. Region of interest for the ^{19}F-NMR spectrum of the non-spiked McDonald's wrappers in acetone solvent.

The results for all the McDonald's and Burger King packaging with no spiking are illustrated in Table 4.3. It is important to note that the "McD" and "BK" samples used three pieces of packaging, while the "BK Whop" samples used one piece. Red indicates no peaks observed in the regions of interest and green indicates there were peaks observed as evidence of PFAS presence. Repeats for each packaging material were prepared and analyzed and are labeled replicate number 0, 1, and 2.

Table 4.3. Qualitative analysis of ^{19}F-NMR spectra from 18 packaging samples and the presence of small "peaks" at designated locations indicating CF_3 and CF_2 groups.

Food Packaging Comparison						
	CF_3 Presence	CF_2 Presence	CF_3 Presence	CF_2 Presence	CF_3 Presence	CF_2 Presence
Solvent:	Water		Acetone		Methanol	
McD [0]					Not Available	Not Available
McD [1]					Not Available	Not Available
McD [2]						
BK [0]						
BK [1]						
BK [2]						
BK Whop[0]	Not Available	Not Available	Not Available	Not Available		
BK Whop[1]	Not Available	Not Available	Not Available	Not Available		

Some data were not retrievable from the NMR due to its failure to run, and time constraints did not allow for new samples to be prepared and run again. Those samples unfortunately leave gaps in the data that otherwise would have been useful for comparison. The samples that used purified water as a solvent did not produce any peaks. The acetone samples showed more evidence of PFAS. This is consistent with the results from the spiked samples due to higher solubility. As far as comparing acetone and methanol, it is difficult to compare their effectiveness as solvents due to the missing data. The "BK Whop" wrappers are a different type of packaging from Burger King, so the signals observed could be because the Whopper wrappers may have a higher presence of PFAS than the other wrappers. However, due to the lack of Whopper data for the other solvents, it is unclear if the Whopper wrappers actually contain a higher amount of PFAS. Another possibility for the signals in the Whopper samples could be that methanol was a better solvent for extracting PFAS. But without any results from the McD methanol samples, there is not enough evidence to make any solid conclusions about the success of acetone and methanol as extractants. Based on the obtained results, the Whopper results could be due to a higher PFAS presence in the Whopper wrappers, methanol being a better solvent, or a combination of both. Sample repetition could provide more clarity about the different types of packaging and could help differentiate the success of each solvent.

Between McDonald's and Burger King, there was no clear distinction for a higher presence of PFAS. By increasing the yield of the extraction process and finding the optimal solvent, the concentration of each sample would increase and possibly show significant enough peaks to integrate and calculate the concentration of PFAS within the packaging. This level of analysis could better distinguish between the levels of PFAS in McDonald's or Burger King packaging.

4.4 Solvent Effects on Sample Solubility

Throughout the duration of the project, the final concentrated solution for each of the samples displayed varying amounts of a blue tinge depending on the solvent or the material. Figure 14 below compares different sets of sample vials in terms of a particular solvent or material.

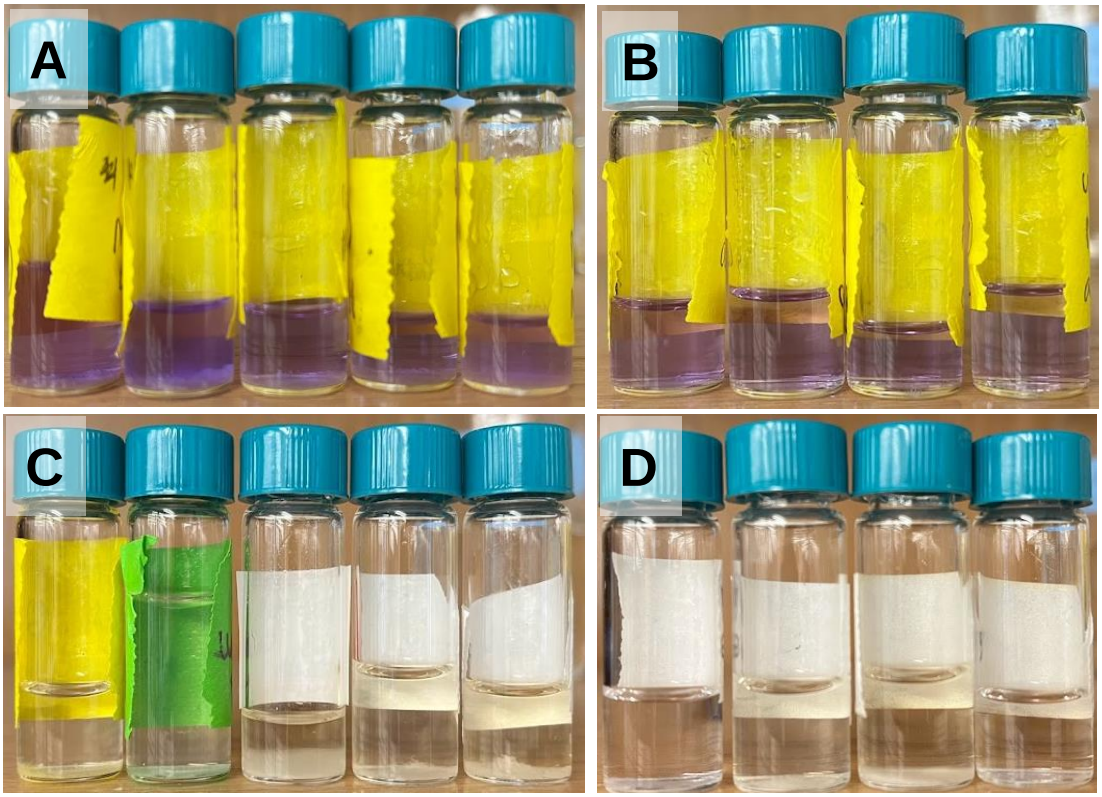


Figure 14. A) Non-spiked McDonald's and Burger King samples in methanol. **B)** Non-spiked McDonald's and Burger King samples in acetone. **C)** Non-spiked and PFAS-spiked samples in purified water for all material. **D)** PFAS-spiked plain copy paper in purified water and acetone.

The first two pictures (A and B) show a few sample vials of non-spiked food packaging material dissolved in methanol and acetone. These samples were observed to have a distinct blue tinge. Those dissolved in methanol appeared to have a slightly darker blue tinge than those dissolved in acetone. Both methanol and acetone are polar solvents and thus have a higher solubility for polar solutes. All the sample vials in picture C contain purified water which is also polar. However, PFAS compounds are hydrophobic as hydrocarbons and fluorinated compounds will repel water molecules. The powerful hydrophobic nature of the compounds is what makes them useful compounds, however it also means that water will not be as successful as an extractant solvent. Picture D shows that the sample vials with plain copy paper do not contain any blue tinge regardless of the solvent of choice. Furthermore, as seen in the tables in sections 4.2 and 4.3, samples dissolved in purified water consistently resulted in NMR spectra with little to no signals. PFAS are known to dissolve in water, however the data suggests that it is unsuccessful at extracting off a solid (EPA, 2016). NMR spectra of the samples dissolved in acetone and methanol varied in the presence and intensity of signals but showed a meaningful difference

from samples in purified water. For the plain copy paper samples, the inherent chemical properties between the solvent and the surface of the paper did not allow for the various PFAS to attach to the material, resulting in no color change or signals on the NMR spectra.

The use of organic solvents appears to be the better choice for PFAS extraction rather than inorganic solvents. However, the differences between organic solvents themselves were not clear from this project. Both methanol and acetone resulted in some number of signals from NMR, but their intensity, along with which samples showed the signals, may be due to analyte-solvent molecular interactions. Every PFAS has its own unique structure, and therefore the optimal solvent may be different for each separate compound. These possibilities are further explored in the chapter discussing recommendations for further experimentation.

5.0 Conclusions and Recommendations

Our team was able to compare the results of our project to the objectives outlined at the beginning to assess our work. With regards to the first objective, our team found that our solid-liquid extraction process had potential for removing PFAS from food contact material but requires work to become more effective. The second objective pertained to quantifying the amount of PFAS extracted from the food packaging samples. This is currently out of the scope of our work until serious improvements to the timeline and execution of our procedure are finished. That being said, quantification could become a possibility with procedure optimization. The third objective, which aimed to demonstrate if the SLE procedure was effective by spiking PFAS-free material using PFAS widely used in manufacturing, was completed partially with several of the spiked samples demonstrating weak NMR signals. Again, with streamlining the procedure and more testing of spiked packaging samples, it is possible that our generated method would in the future show continuous success at extracting spiked compounds from samples being analyzed. Recommendations to expand upon our work in the future are discussed in the next section.

5.1 Recommendations

After the completion of work in the laboratory and the analysis of the resulting data, our team has several recommendations for those wishing to expand upon the work completed in this project or those beginning to work with PFAS on food packaging materials. One of the first improvements to be conducted is to use a more sophisticated piece of equipment than a pair of scissors for preparing the packaging samples for submersion and sonication. Using a device like a blender or grinding mill to turn the paper samples into smaller pieces would increase the surface area between the solvent and sample. Some kind of solid filtration could be applied if necessary to remove any powders or small solid particles from the sample to ensure effective NMR analysis. One of the issues with utilizing a grinding mill is the cost of the device, and so it could be desirable to begin with a blender and see what results are presented at the end of solid-liquid extraction. This would give insight into whether increasing the surface area of the sample within the solvent increased the amount of PFAS extracted. Another way in which future work could be improved would be to increase the variety of organic solvents used throughout the entirety of the extraction process, starting with the initial sonication of the samples. Organic solvents should be used in favor of water and other inorganic solvents, based on the initial results from experimentation. Other solvents also may be more favorable to the different PFAS structures, so it would be worth trying out several more solvents in an attempt to improve extraction. More nonpolar options should be utilized as well, with regards to organic solvents, such as hexane and toluene, to test for their usefulness opposed to polar solvents.

In addition to those listed above, another change to the methodology that could yield better results includes additional repetition of sample-solvent combinations that show promising NMR spectra. Had the timeline of our project been slightly more accelerated regarding access to the Kaven laboratory and NMR machinery, it may have been in our team's best interest to repeat the process on samples that showed any sort of prominent NMR spike to see if the spike persisted between runs. Generally, it would have been beneficial as well for our team to repeat all the samples we could to test the reproducibility of the results using our SLE procedure.

The varied results in the spiked and non-spiked samples did not provide clear evidence on the success of the SLE procedure because there was no pattern to which samples had signals and which did not. The samples that did have signals were all at a very low intensity, which could be indicative of a concentration near the border of the limit of detection of the NMR. Therefore, we recommend increasing the sample concentration to strengthen the signals that we did see and to potentially produce signals in the samples that we did not see any. For the non-spiked samples, this could be done by using more packaging per sample. For the spiked samples, we recommend increasing the concentration of the spiking solutions with the hope that more PFAS would end up in the extracted sample. The concentrations of the spiking solutions were known, but it was unclear how much PFAS actually bound to the packaging or how much was actually extracted and ended up in the NMR tube (i.e. percent recovery). Increasing the concentration of the samples to be above the limit of detection of the NMR could show a clearer pattern in the results and provide more clarity on the success of our extraction procedure. A higher concentration would also provide more evidence of how much PFAS from the spiking solutions actually made it into the NMR tube.

Another aspect that could be further investigated and improved is the timeline of sample preparation to NMR analysis. Due to schedule restraints and instrumentation/lab access, many of the samples experienced a prolonged delay before analysis. The samples were often stored at different points of our procedure for various amounts of time, as long as a couple months. This opened the door for other factors to affect our samples that ultimately could have contributed to the extremely low signals observed after the completion of NMR spectrometry. One issue was that the longer the packaging was immersed in a solvent, (i.e. before using the RapidVap evaporation system) the more the packaging broke down in the solvent and thereby more solid particles suspended in solution, which did not resemble ideal conditions for NMR analysis. In a perfect world, our samples would be completely in the liquid phase and should not contain any solid matter that could potentially interfere with the signal. Another problem with the ample time between sample making and NMR testing was that there was more time for the PFAS to bind to the glass containers that they were stored in. Cain and Powers (2020) found that PFAS solutions stored in glass beakers for a period of 50 days showed an average decrease of 6.6% in concentration compared to the samples that were tested the day they were made. Other sources of literature describe the possibility of the absorption of PFASs onto glass or plastic containers without a clear answer as to the best solution for containment (Belisle & Hagen, 1980; Dolman & Pelzing, 2011). Such a period of time can make a difference especially when the samples were already in very low concentrations and producing very weak signals. Therefore, we recommend shortening the time between completing the extraction procedure and performing the NMR test to retain the original sample concentration and improve the intensity of the signal.

In terms of preventing or decreasing the number of solid particles in the NMR tubes, we recommend experimenting with other deuterated solvents to increase sample solubility. As stated previously, the extended timeline between extraction and NMR testing contributed to more solid particles suspended in solution. However, there were several occasions where the sample appeared clear in the NMR tube and then became cloudy upon adding the deuterated solvent. Using a different deuterated solvent in combination with a shortened window between extraction and testing could help improve sample quality in the NMR tube and result in better signals.

References

Begley, T. H., White, K., Honigfort, P., Twaroski, M. L., Neches, R., & Walker, R. A. (2005). Perfluorochemicals: Potential sources of and migration from food packaging. *Food Additives & Contaminants*, *22*(10), 1023–1031. https://doi.org/10.1080/02652030500183474

Belisle, J., & Hagen, D. F. (1980). A method for the determination of perfluorooctanoic acid in blood and other biological samples. *Analytical Biochemistry*, *101*(2), 369–376. https://doi.org/10.1016/0003-2697(80)90202-X

Cain, J. & Powers, Z. (2020). *Developing an analytical technique for PFAS in water using SPE and NMR* (Undergraduate Major Qualifying Project No. E-project-040720-202541). Retrieved from Worcester Polytechnic Institute Electronic Projects Collection: https://digital.wpi.edu/concern/student_works/xw42nb43j?locale=en

Camdzic, D., Dickman, R. A., & Aga, D. S. (2021). Total and class-specific analysis of per- and polyfluoroalkyl substances in environmental samples using nuclear magnetic resonance spectroscopy. *Journal of Hazardous Materials Letters*, *2*, 100023. https://doi.org/10.1016/j.hazl.2021.100023

Choi, A., Muise, D., & Weiland, Z. (2019). *Designing a total organic fluorine test: PFAS characterization & quantitation utilizing NMR analysis* (Unpublished Undergraduate Major Qualifying Project). Worcester Polytechnic Institute.

Clark, J. (2014). What is nuclear magnetic resonance (NMR)? *Chemguide*. https://www.chemguide.co.uk/analysis/nmr/background.html

Curtzwiler, G.W., Silva, P., Hall, A., Ivey, A. and Vorst, K. (2021). Significance of perfluoroalkyl substances (PFAS) in food packaging. *Integr Environ Assess Manag*, *17*, 7-12. https://doi.org/10.1002/ieam.4346

Dean, W. S., Adejumo, H. A., Caiati, A., Garay, P. M., Harmata, A. S., Li, L., Rodriguez, E. E., & Sundar, S. (2020). A Framework for Regulation of New and Existing PFAS by EPA. *POLICY ANALYSIS*, *16*(1), 14.

Dolman, S., & Pelzing, M. (2011). An optimized method for the determination of perfluorooctanoic acid, perfluorooctane sulfonate and other perfluorochemicals in different matrices using liquid chromatography/ion-trap mass spectrometry. *Journal of Chromatography B*, *879*(22), 2043–2050. https://doi.org/10.1016/j.jchromb.2011.05.032

Environmental Health Program. (2021). *Per-and Polyfluoroalkyl Substances movement through the environment.* U.S. Geological Survey. https://www.usgs.gov/media/images/and-polyfluoroalkyl-substances-movement-through-environment

Evaporators. (2004). American Chemical Society. *Today's Chemist at Work*, 47-50. https://pubsapp.acs.org/subscribe/archive/tcaw/13/i05/pdf/504prodprofile.pdf

Farm Production and Conservation (2021). *Per- and polyfluoroalkyl substances (PFAS)* [Fact sheet]. USDA. https://www.fsa.usda.gov/Assets/USDA-FSA-Public/usdafiles/FactSheets/fpac-pfas-fact-sheet-july-2021.pdf

Glüge, J., Scheringer, M., T. Cousins, I., C. DeWitt, J., Goldenman, G., Herzke, D., Lohmann, R., A. Ng, C., Trier, X., & Wang, Z. (2020). An overview of the uses of per- and polyfluoroalkyl substances (PFAS). *Environmental Science: Processes & Impacts*, 22(12), 2345–2373. https://doi.org/10.1039/D0EM00291G

Hogue, S. (2018). EPA toxicity analysis of GenX-related compounds coming soon. (2018). *C&EN*

Global Enterprise, *96*(37), 18–18. https://doi.org/10.1021/cen-09637-polcon1

Hogue, C. (2021). US EPA deems two GenX PFAS chemicals more toxic than PFOA | *C&EN*

Global Enterprise. (n.d.). https://pubs.acs.org/doi/10.1021/cen-09940-polcon2

Liquid Chromatography Mass Spectrometry (LC-MS) Information. (n.d.). ThermoFisher Scientific. //www.thermofisher.com/us/en/home/industrial/mass-spectrometry/mass-spectrometry-learning-center/liquid-chromatography-mass-spectrometry-lc-ms-information.html

Mantripragada, S., Deng, D., & Zhang, L. (2021). Remediation of GenX from water by amidoxime surface-functionalized electrospun polyacrylonitrile nanofibrous adsorbent. *Chemosphere*, *283*, 131235. https://doi.org/10.1016/j.chemosphere.2021.131235

Masoodi, K. Z., Lone, S. M., & Rasool, R. S. (2021). Chapter 23—Study of principle of centrifugation. In K. Z. Masoodi, S. M. Lone, & R. S. Rasool (Eds.), *Advanced Methods in Molecular Biology and Biotechnology* (pp. 133–137). Academic Press. https://doi.org/10.1016/B978-0-12-824449-4.00023-2

MassDEP. (2021). *Per- and polyfluoroalkyl substances (PFAS) in drinking water: Questions and answers for consumers*. [Fact sheet]. https://www.mass.gov/doc/massdep-fact-sheet-pfas-in-drinking-water-questions-and-answers-for-consumers/download

Ng, C., Cousins, I. T., DeWitt, J. C., Glüge, J., Goldenman, G., Herzke, D., Lohmann, R., Miller, M., Patton, S., Scheringer, M., Trier, X., & Wang, Z. (2021). Addressing Urgent Questions for PFAS in the 21st Century. *Environmental Science & Technology*, acs.est.1c03386. https://doi.org/10.1021/acs.est.1c03386

Nuclear Magnetic Resonance Spectroscopy. (2013). https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/nmr/nmr1.htm

Nuclear Magnetic Resonance (NMR) Spectroscopy. (2014). Chemistry LibreTexts. https://chem.libretexts.org/Courses/Purdue/Purdue%3A_Chem_26505%3A_Organic_Chemistry_I_(Lipton)/Chapter_5._Spectroscopy/5.3_Nuclear_Magnetic_Resonance_(NMR)_Spectroscopy

Nutrition, C. for F. S. and A. (2020a). Authorized uses of PFAS in food contact applications. *FDA*. https://www.fda.gov/food/chemical-contaminants-food/authorized-uses-pfas-food-contact-applications

Nutrition, C. for F. S. and A. (2020b). FDA announces the voluntary phase-out by industry of certain PFAS used in food packaging. *FDA*. https://www.fda.gov/food/cfsan-constituent-updates/fda-announces-voluntary-phase-out-industry-certain-pfas-used-food-packaging

Packaged in Pollution: Are food chains using PFAS in packaging? (2020). Toxic-Free Future. https://toxicfreefuture.org/packaged-in-pollution/

Potential health effects of PFAS chemicals | ATSDR. (2020, June 24). https://www.atsdr.cdc.gov/pfas/health-effects/index.html

Rice, P. A., Aungst, J., Cooper, J., Bandele, O., & Kabadi, S. V. (2020). Comparative analysis of the toxicological databases for 6:2 fluorotelomer alcohol (6:2 FTOH) and perfluorohexanoic acid (PFHxA). *Food and Chemical Toxicology*, *138*, 111210. https://doi.org/10.1016/j.fct.2020.111210

Richter, L., Cordner, A., & Brown, P. (2021). Producing Ignorance Through Regulatory Structure: The Case of Per- and Polyfluoroalkyl Substances (PFAS). *Sociological Perspectives*, *64*(4), 631–656. https://doi.org/10.1177/0731121420964827

Schaider, L. A., Balan, S. A., Blum, A., Andrews, D. Q., Strynar, M. J., Dickinson, M. E., Lunderberg, D. M., Lang, J. R., & Peaslee, G. F. (2017). Fluorinated Compounds in U.S. Fast Food Packaging. *Environmental Science & Technology Letters*, *4*(3), 105–111. https://doi.org/10.1021/acs.estlett.6b00435

Seltenrich, N. (2020). PFAS in Food Packaging: A Hot, Greasy Exposure | *Environmental Health Perspectives | Vol. 128, No. 5*. (n.d.). https://doi.org/10.1289/EHP6335

Shchukin, D. G., Skorb, E., Belova, V., & Möhwald, H. (2011). Ultrasonic Cavitation at Solid Surfaces. *Advanced Materials*, *23*(17), 1922–1934. https://doi.org/10.1002/adma.201004494

Solid-Liquid Extraction. (n.d.). JoVE. https://www.jove.com/v/5538/solid-liquid-extraction

Sunderland, E.M., Hu, X.C., Dassuncao, C. et al. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J Expo Sci Environ Epidemiol 29, 131–147 (2019). https://doi.org/10.1038/s41370-018-0094-1

Tandem Mass Spectrometry (MS/MS). (n.d.). National High Magnetic Field Laboratory. https://nationalmaglab.org/user-facilities/icr/techniques/tandem-ms

Toxic Substances Control Act, 15 U.S.C. § 53 (1976). https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim

Tseng, N., Wang, N., Szostek, B., Mahendra, S. (2014). *Biotransformation of 6:2 Fluorotelomer Alcohol (6:2 FTOH) by a Wood-Rotting Fungus | Environmental Science & Technology*. (n.d.)., University of California, DuPont Haskell Global Centers for Health and Environmental Sciences. https://pubs.acs.org/doi/10.1021/es4057483

US EPA, O. (2016). *Drinking Water Health Advisories for PFOA and PFOS*. [Overviews and Factsheets]. https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos

US EPA, O. (2021a). *Human Health Toxicity Assessments for GenX Chemicals* [Reports and Assessments]. https://www.epa.gov/chemical-research/human-health-toxicity-assessments-genx-chemical

US EPA, O. (2021b). *Our Current Understanding of the Human Health and Environmental Risks of PFAS* [Overviews and Factsheets]. https://www.epa.gov/pfas/our-current-understanding-human-health-and-environmental-risks-pfas

US EPA, O. (2021c). *PFAS Strategic Roadmap: EPA's Commitments to Action 2021-2024* [Overviews and Factsheets]. https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-action-2021-2024

US EPA, O. (2013). *Summary of the Toxic Substances Control Act* [Overviews and Factsheets]. https://www.epa.gov/laws-regulations/summary-toxic-substances-control-ac

What are the differences between LC/MS and LC/MS/MS ? (n.d.). ResearchGate. https://www.researchgate.net/post/What-are-the-differences-between-LC-MS-and-LC-MS-MS

What is Sonication and How Does it Work? (2020). Toppr-Guides. https://www.toppr.com/guides/physics/waves/what-is-sonication/

Yuan, G., Peng, H., Huang, C. (2016). *Ubiquitous Occurrence of Fluorotelomer Alcohols in Eco-Friendly Paper-Made Food-Contact Materials and Their Implication for Human Exposure | Environmental Science & Technology*. (n.d.). Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China. https://pubs.acs.org/doi/10.1021/acs.est.5b03806

Zabaleta, I., Bizkarguenaga, E., Bilbao, D., Etxebarria, N., Prieto, A., & Zuloaga, O. (2016). Fast and simple determination of perfluorinated compounds and their potential precursors in different packaging materials. *Talanta*, *152*, 353–363. https://doi.org/10.1016/j.talanta.2016.02.022

Zabaleta, I., Negreira, N., Bizkarguenaga, E., Prieto, A., Covaci, A., & Zuloaga, O. (2017). Screening and identification of per- and polyfluoroalkyl substances in microwave popcorn bags. *Food Chemistry*, *230*, 497–506. https://doi.org/10.1016/j.foodchem.2017.03.074

Appendix

Appendix A: Sample Calculations

Appendix A.1: Standard Solution Concentrations

PFOA

Molar mass of PFOA = 414.1 g/mol

Need concentration 4-250 mg/L → two solutions at 0.55 mM and 0.055 mM

Volume of solution = 20 mL

$0.00055\ M\ =\ \frac{moles\ PFOA}{0.020\ L}$ → moles PFOA = 0.000011 moles

Mass of PFOA = $0.000011\ moles\ *\frac{414.1\ g}{mol} = 0.00456\ g$

For 0.55 mM solution: need 0.00456 g PFOA in 20 mL of water

GenX

Standard Concentration 1 = 0.55 mM

Molar mass of GenX = 330.06 g/mol
ρ = 1.85 g/mL

Mass of liquid in bottle = 5 g

$$5g * \frac{1\ mol}{330.06\ g} = 0.0151\ mol$$

$$5g * \frac{1\ mL}{1.85\ g} * \frac{1\ L}{1000\ mL} = 0.00270\ L$$

Concentration of GenX bottle = $\frac{0.0151\ mol}{0.00270\ L} = 5.59M$

$M_1V_1 = M_2V_2$

$$(5590\ mM\ GenX)(.1\ mL) = (Stock\ Concentration\ mM)(100\ mL\ of\ water)$$

Stock Concentration = 5.59 mM

$$(5.59\ mM\ GenX)(Volume\ mL) = (0.55\ mM)(30\ mL\ solution)$$

Volume of 5.59 mM GenX needed = 2.95 mL

Volume of water = 30 mL - 2.95 mL = 27.05 mL

Standard 1 = 2.95 mL of 5.59 mM GenX solution and 27.05 mL water

6:2 FTOH

Molar mass of FTOH = 364.1 g/mol
ρ = 1.651 g/mL

Mass of liquid in bottle = 5 g

$$5g * \frac{1\ mol}{364.1\ g} = 0.0137\ mol$$

$$5g * \frac{1\ mL}{1.651\ g} * \frac{1\ L}{1000\ mL} = 0.00303\ L$$

Concentration of FTOH bottle = $\frac{0.0137\ mol}{0.00303\ L}$ = 4.53 M

$M_1V_1 = M_2V_2$

$$(4530\ mM\ GenX)(.1\ mL) = (Stock\ Concentration\ mM)(50\ mL\ of\ water)$$

Stock Concentration = 9.07 mM

$$(9.07\ mM\ FTOH)(1\ mL) = (Concentration\ of\ Standard\ 1)(16.5\ mL)$$

Concentration of Standard 1 = 0.55 mM

Appendix A.2: Spiked Solution Concentrations

PFOA

PFOA Solution B:

Weight of PFOA powder = 2.4 mg = 2400 µg

Volume of methanol = 250 mL = 0.250 L

Concentration of Solution A = $\frac{2400\ \mu g}{0.250\ L} = 9600\ \mu g/L$

Need 200 mL of solution of 480 µg/L (Solution B):

$M_1V_1 = M_2V_2$

$$(9600\ \mu g/L)(Solution\ A\ V_1) = (480\ \mu g/L)(0.200\ L)$$

Solution A V_1 = 0.010 L = 10 mL

PFOA Solution B = 10 mL PFOA Solution A + 190 mL methanol

GenX

GenX Solution B:

Need 250 mL of solutions of 720 µg/L (Solution B)

$720\ \frac{\mu g}{L} * \frac{1\ g}{1 * 10^6\ \mu g} * \frac{1\ mol}{330\ g} * \frac{1000\ mmol}{1\ mol} = 0.00218\ mM$ = Concentration of Solution B

$$(Solution\ A\ Concentration)(250\ mL\ MeOH) = (5.59\ mM\ GenX)(Volume\ 0.1\ mL)$$

Solution A Concentration = 0.00224 mM

$$(0.00224\ mM)(Solution\ A\ volume) = (0.00218\ mM\ GenX)(250\ mL\ of\ solution)$$

Solution A volume = 243.3 mL

Methanol volume = 250 mL - 243.3 mL = 6.7 mL

GenX Solution B = 243.3 mL GenX Solution A + 6.7 mL methanol

6:2 FTOH

FTOH Solution B:

Molar mass FTOH = 364.1 g/mol

ρ = 1.65 g/mL

$$720\ \frac{\mu g}{L} * \frac{1\ g}{1\ *\ 10^6\ \mu g} * \frac{1\ mol}{364.1\ g} * \frac{1000\ mmol}{1\ mol} = 0.00198\ mM$$

$$(Solution\ A\ concentration)(250\ mL\ MeOH) = (9.07\ mM\ FTOH)(0.1\ mL)$$

Solution A concentration = 0.00363 mM

$$(0.00363\ mM)(Solution\ A\ volume) = (0.00198\ mM\ FTOH)(250\ mL)$$

Solution A volume = 136.4 mL

Methanol volume = 250 mL - 136.4 mL = 113.5 mL

FTOH Solution B = 136.4 mL FTOH Solution A + 113.5 mL methanol

Appendix B: Error Calculations

Appendix B.1: Concentration Error

Mass balance error = ± 0.00005g

Graduated Cylinder Error = ± 0.5 mL

100 µL - 1000µL Pipette error (assumed 1% error) = ± 1-10 µL

10µL - 50µL Pipette error (assumed 1% error) = ± 0.1-0.5 µL

PFOA Solution A Error (0.0232 mM):

Mass PFOA = 0.0024 ± 0.00005 g

Volume measured = 250 ± 0.5 mL

Concentration = $\frac{0.0024\ g}{250\ mL} * \frac{1\ mol}{414.1\ g} * \frac{1000\ mL}{L} * \frac{1000\ mM}{1\ M} = 0.0232\ mM$

$$Error\ = 0.0000232\ M * \left(\frac{0.00005/414.1\ g}{0.0024/414.1\ g}\ + \frac{0.5/1000\ L}{250/1000\ L}\right) * 1000$$
$$= 0.0232 \pm 0.000046\ mM$$

Appendix B.2: Dilution Error

$M_1V_1 = M_2V_2$

$$M_2\ = \frac{M_1V_1}{V_2}$$

PFOA Solution B Error:

M_1 = 0.0232 ± 0.000046mM

V_1 = 15 ± 0.5 mL

V_2 = 200 ± 0.5 mL

$$M_2 = \frac{0.0232\ mM\ *\ 0.01\ L}{0.200\ L} = 0.00116\ mM$$

$$Error\ = 0.00116\ mM * \left(\frac{0.000046\ mM}{0.0232\ mM} + \frac{0.0005\ L}{0.010\ L} + \frac{0.0005\ L}{0.200\ L}\right)$$
$$= 0.00116 \pm 0.000063\ mM$$

Percent error increases as concentration decreases.

Appendix B.3 Error Values for Spiking Solutions

Solution	Error
PFOA Solution A	± 0.000046 mM
PFOA Solution B	± 0.000063 mM
PFOA Solution C	± 0.000066 mM
GenX Solution A	± 0.000060 mM
GenX Solution B	± 0.000068 mM
Genx Solution C	± 0.000049 mM
FTOH Solution A	± 0.00012 mM
FTOH Solution B	± 0.000075 mM
FTOH Solution C	± 0.000046 mM

Appendix C: Photos of Sample Food Packaging

Figure C.1: McDonald's small French fry bag

Figure C.2: Burger King's small chicken nugget bag

Figure C.3: Burger King's Whopper wrapper

Appendix D: NMR Spectra

Link to NMR Zip Files: https://wpi0-my.sharepoint.com/:f:/g/personal/schase_wpi_edu/EhX7-aue72FJjWmyiWdDuJwB377_yW5_-YRgUpYdsv-erg?e=RN

Appendix D.1: Standard Curve Solution Samples

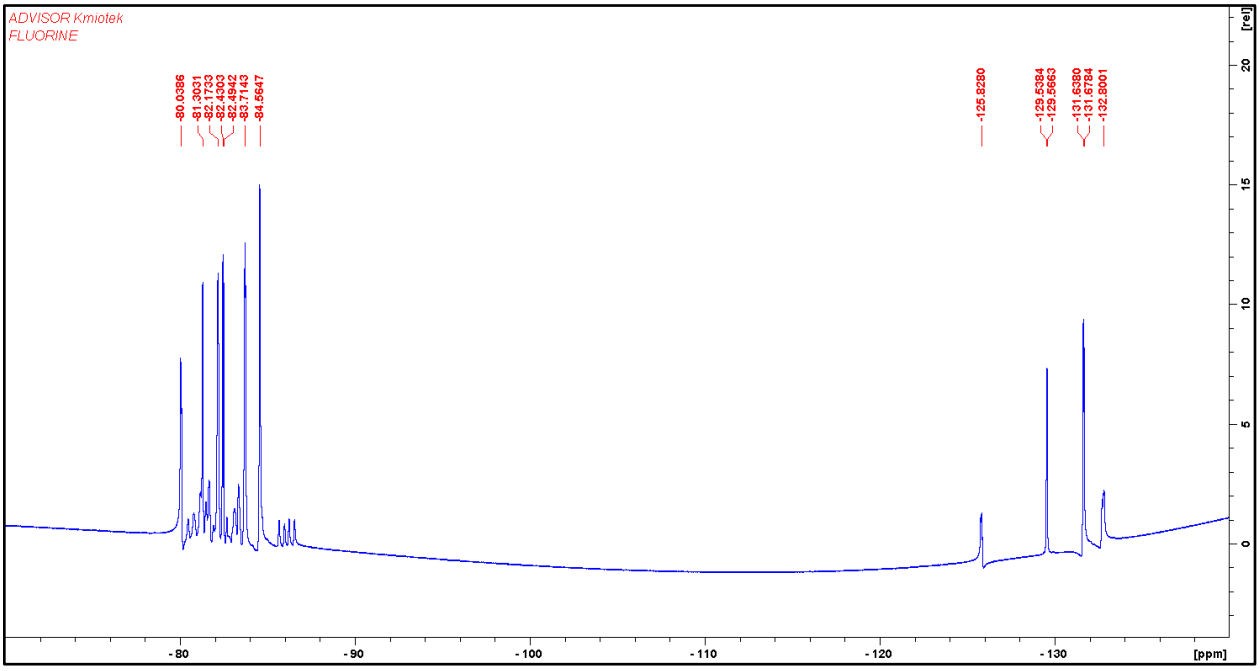


Figure D.1.1: 0.55 mM GenX standard in purified water

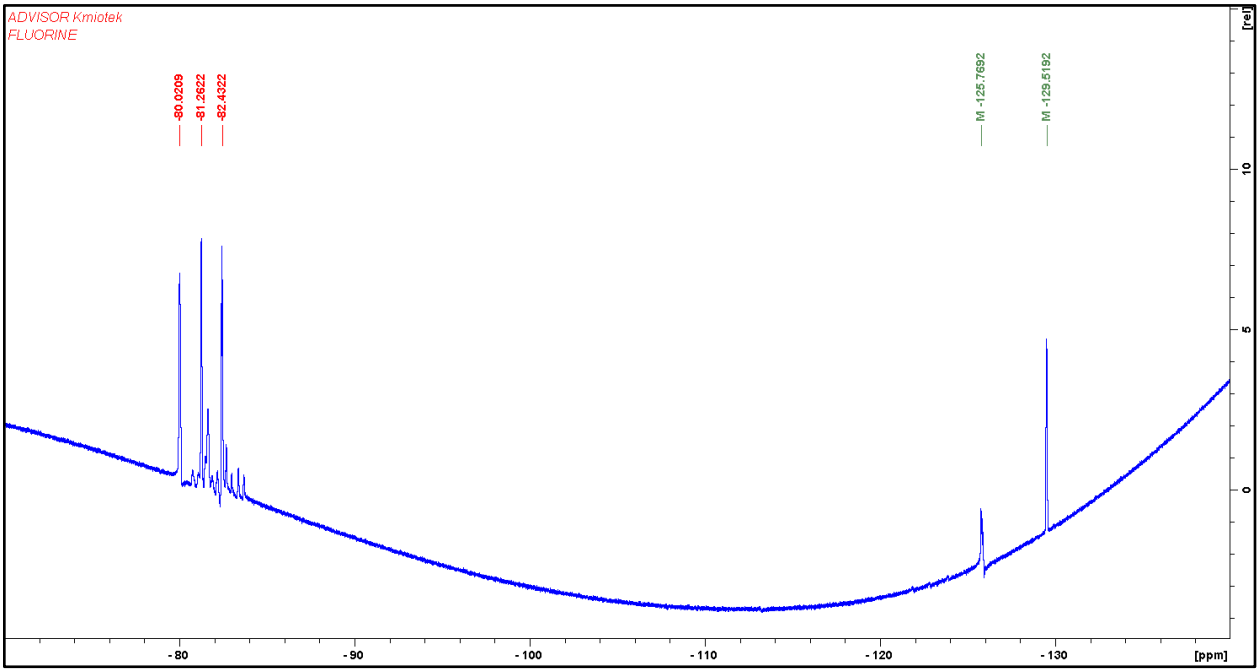


Figure D.1.2: 0.11 mM GenX standard in purified water

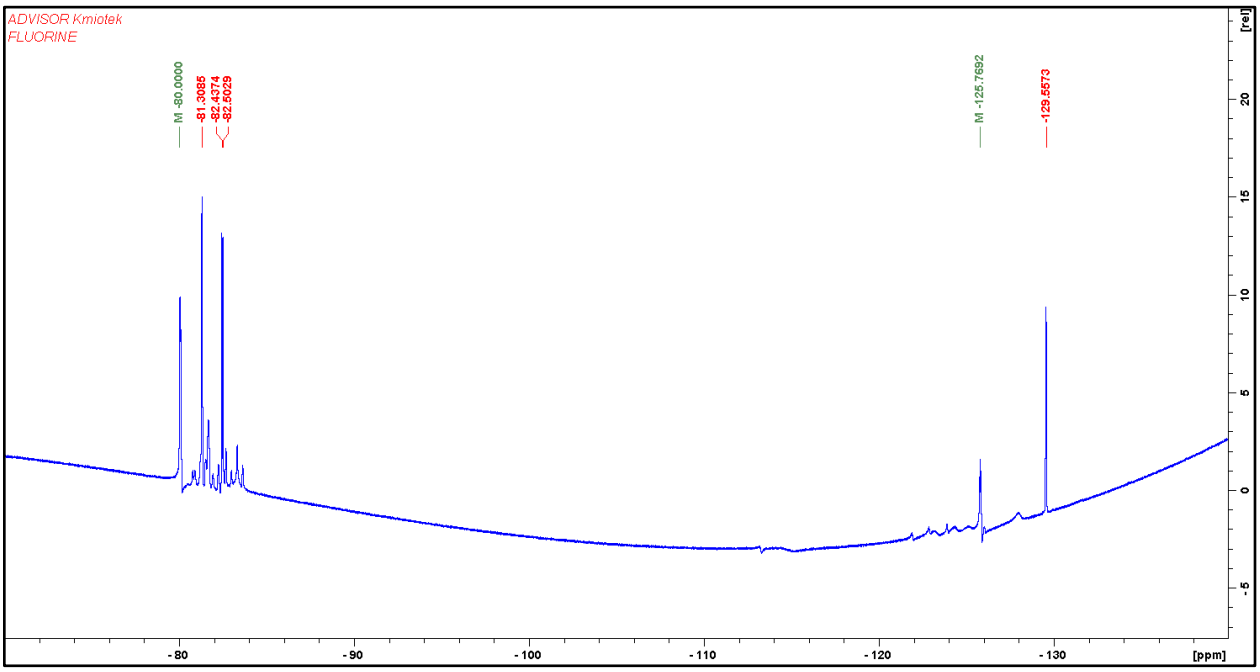


Figure D.1.3: 0.055 mM GenX standard in purified water

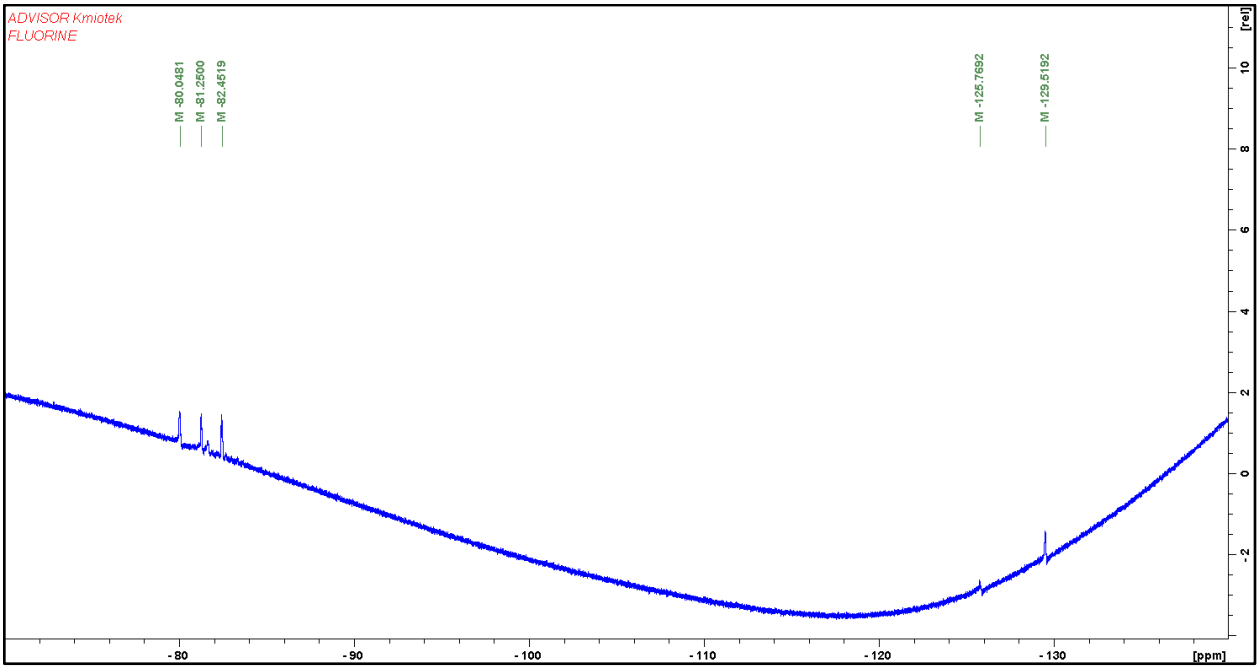


Figure D.1.4: 0.011 mM GenX standard in purified water

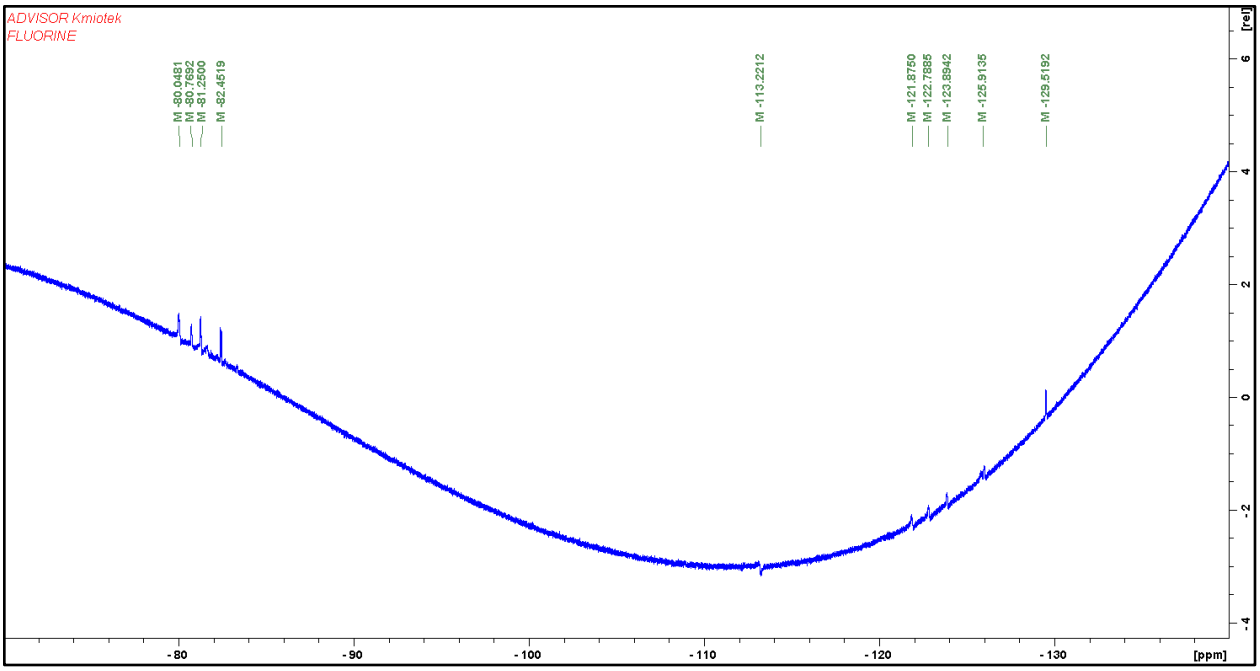


Figure D.1.5: 0.55 mM FTOH standard in purified water

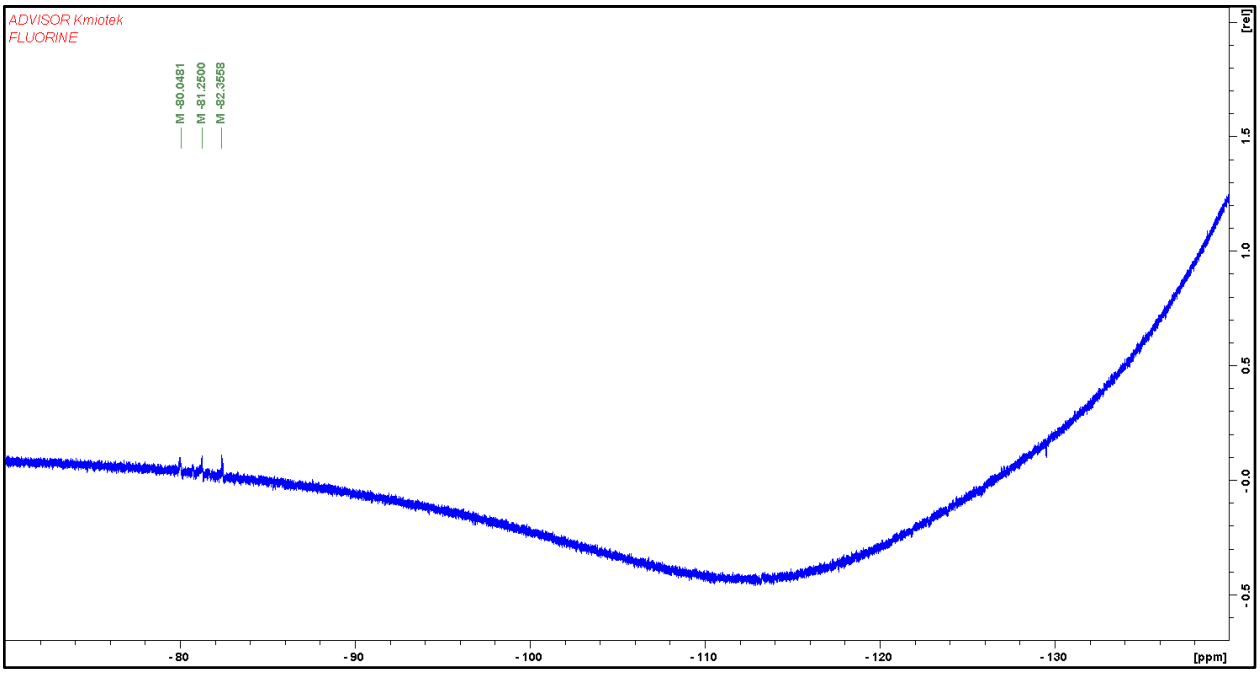


Figure D.1.6: 0.11 mM FTOH standard in purified water

Appendix D.2: Spiked Samples

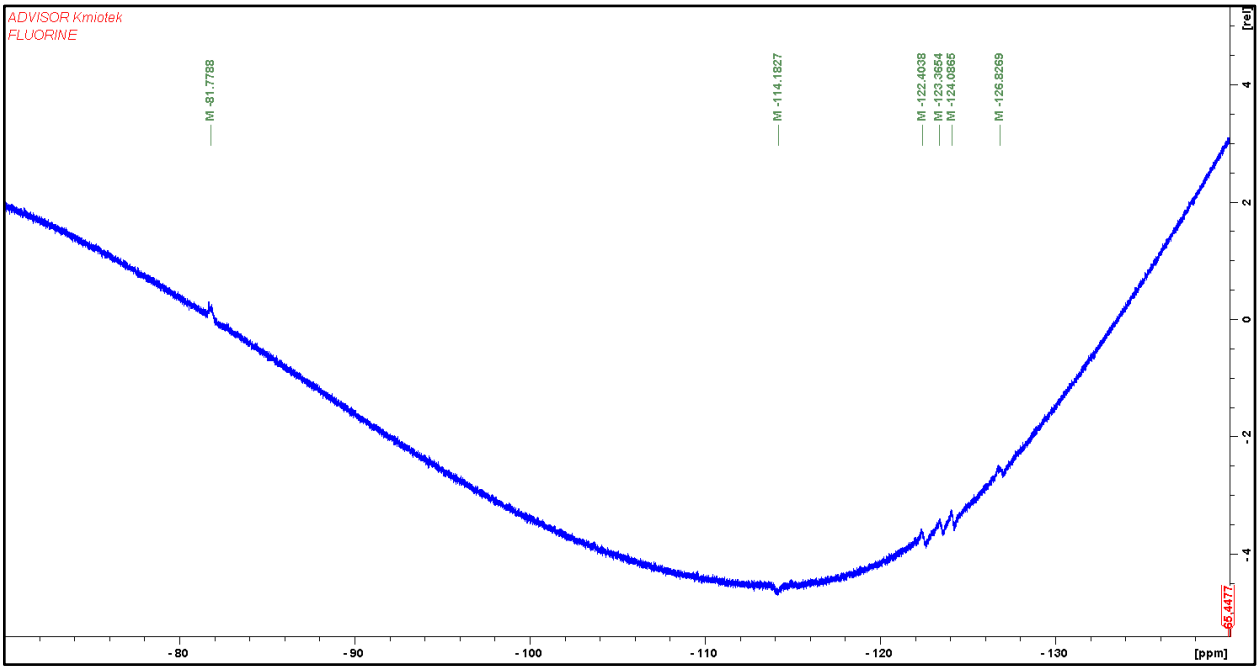


Figure D.2.1: 0.0016 mM PFOA-spiked McDonald's sample in acetone

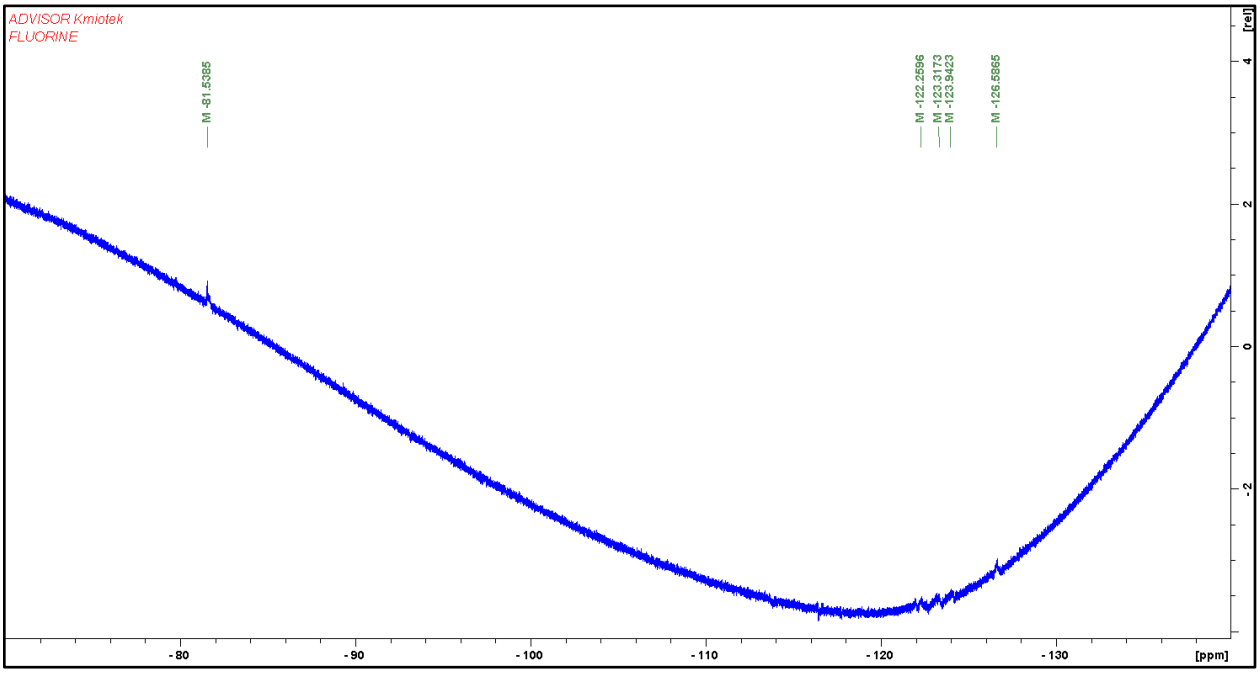


Figure D.2.2: 0.0016 mM PFOA-spiked Burger King sample in acetone

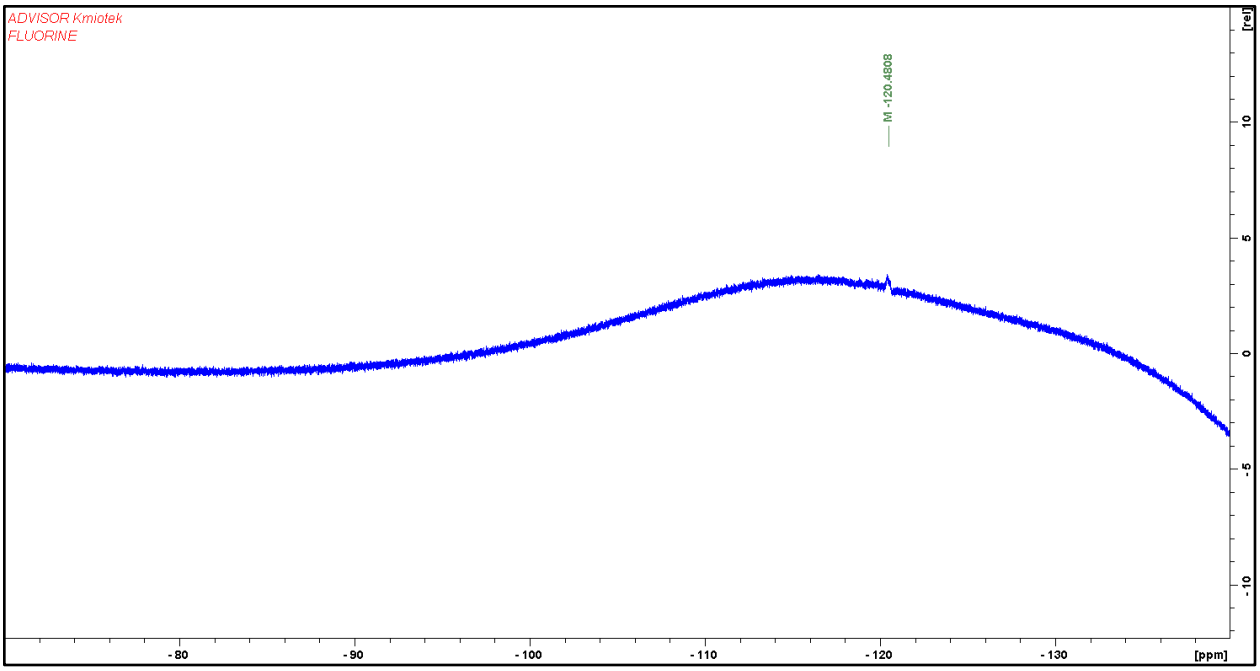


Figure D.2.3: 0.0022 mM GenX-spiked Burger King sample in purified water

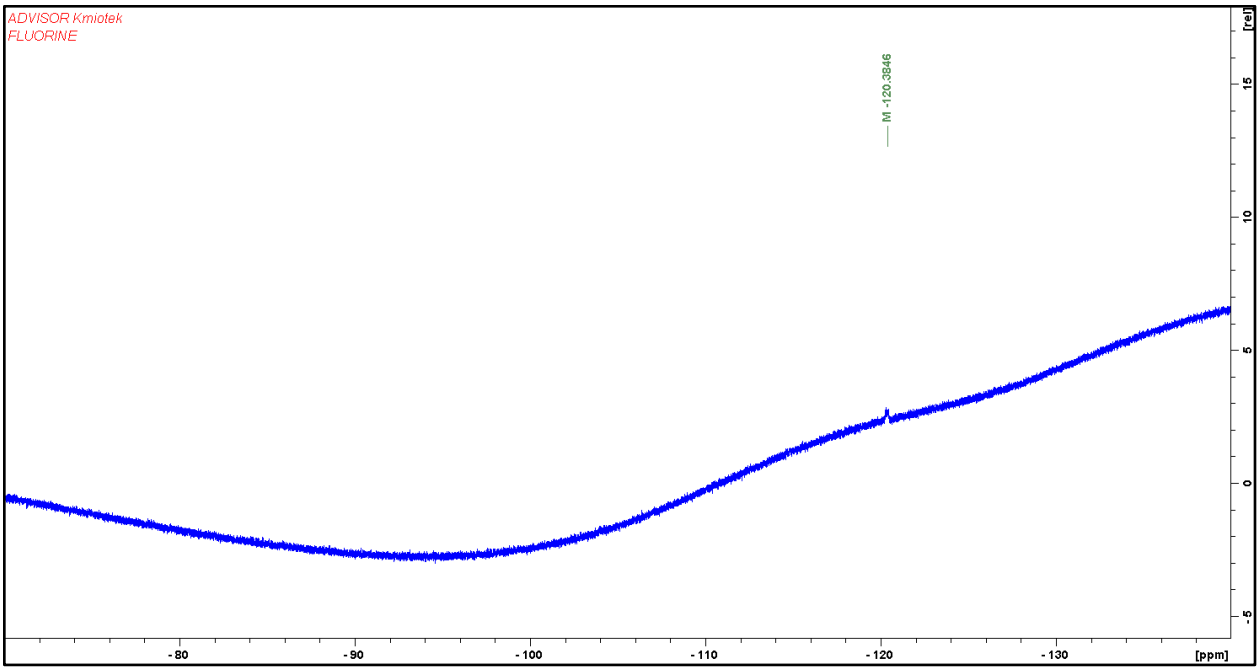


Figure D.2.4: 0.0013 mM GenX-spiked Burger King sample in purified water

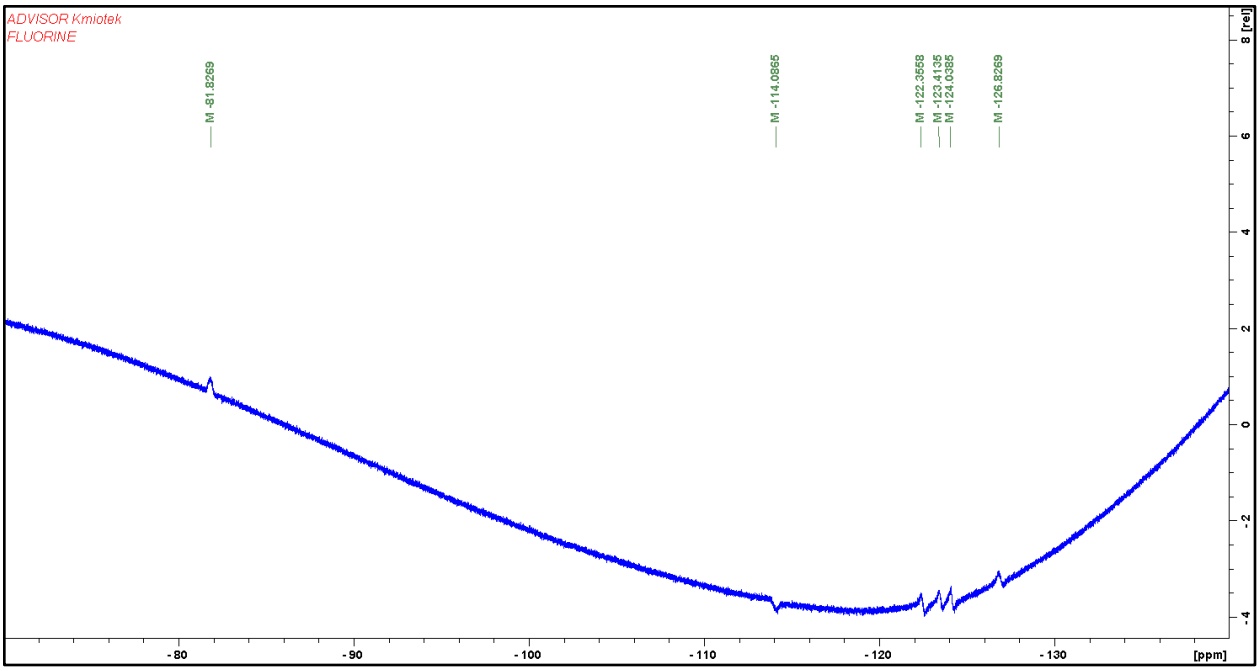


Figure D.2.5: 0.0022 mM GenX-spiked McDonald's sample in acetone

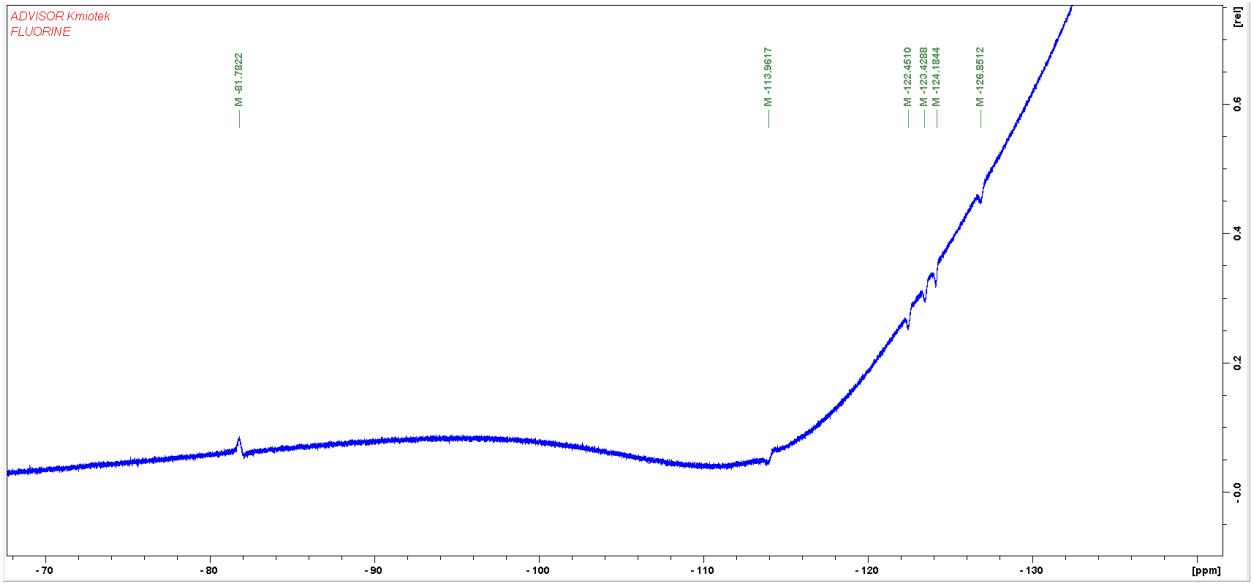


Figure D.2.6: 0.0022 mM GenX-spiked Burger King sample in acetone

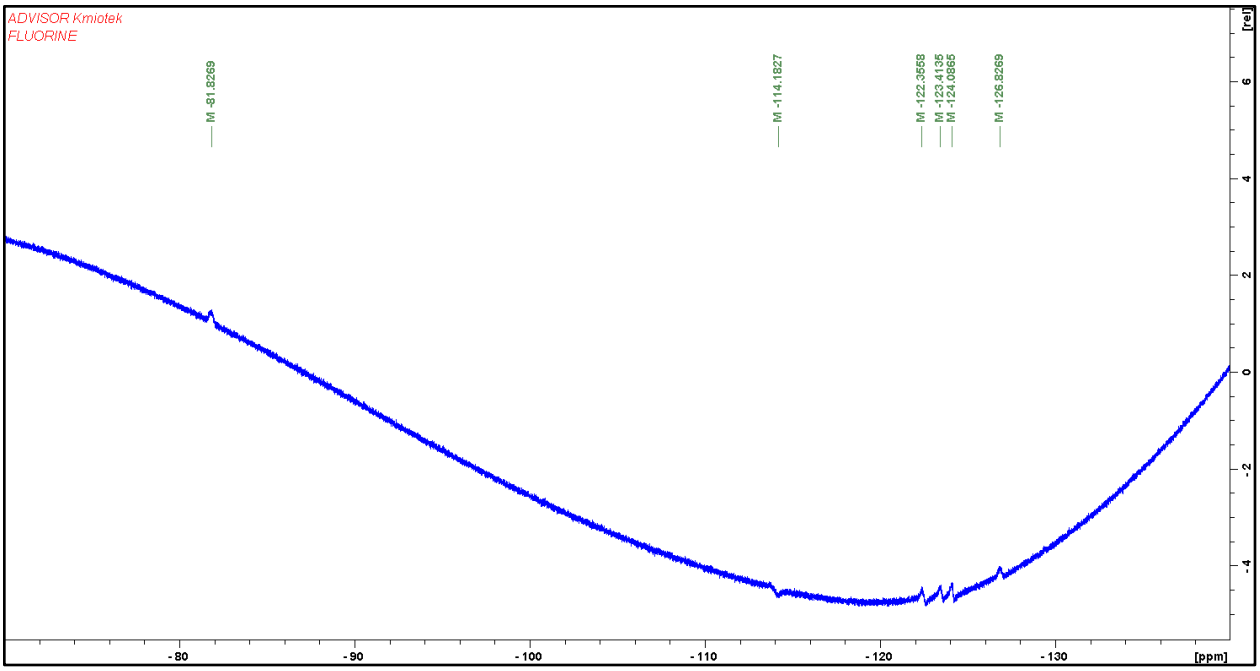


Figure D.2.7: 0.0013 mM GenX-spiked McDonald's sample in acetone

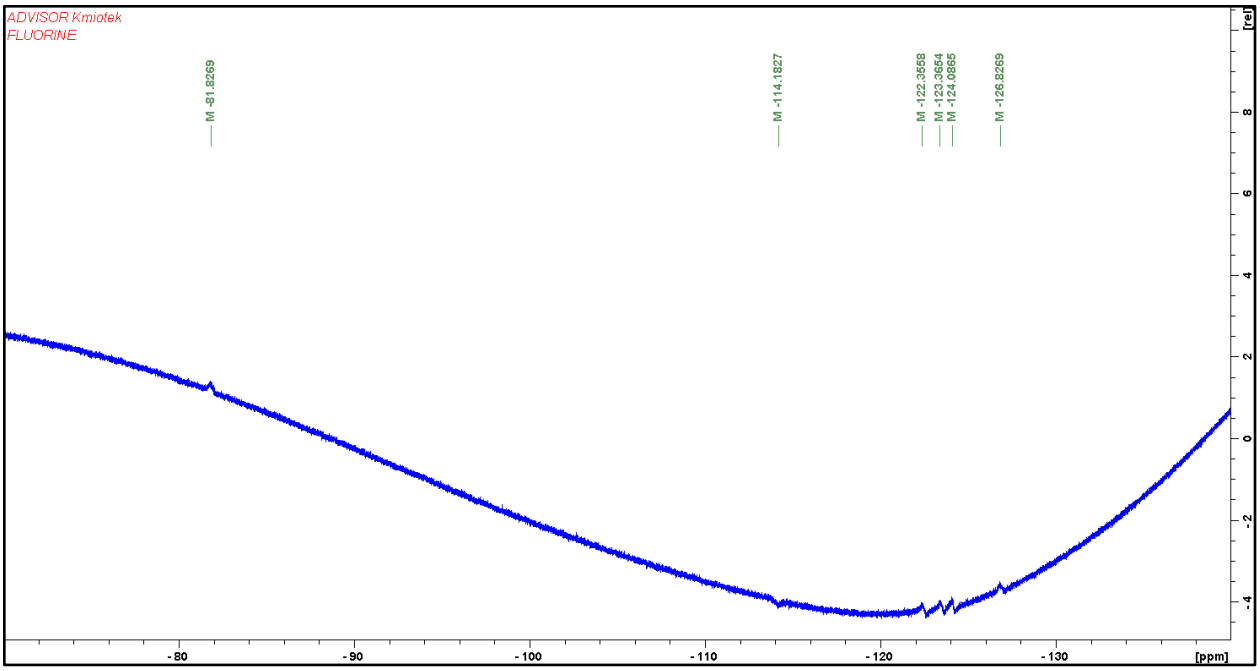


Figure D.2.8: 0.0012 mM FTOH-spiked McDonald's sample in acetone

Appendix D.3: Non-Spiked Samples

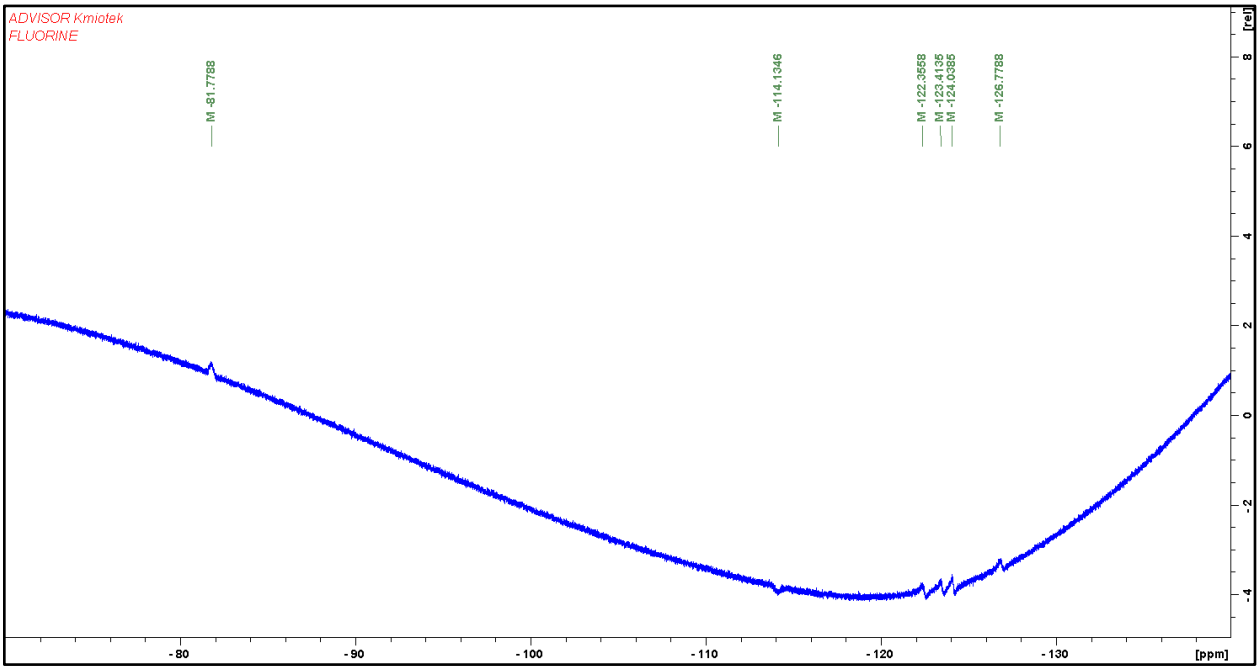


Figure D.3.1: Non-spiked McDonald's sample in acetone (replicate #0)

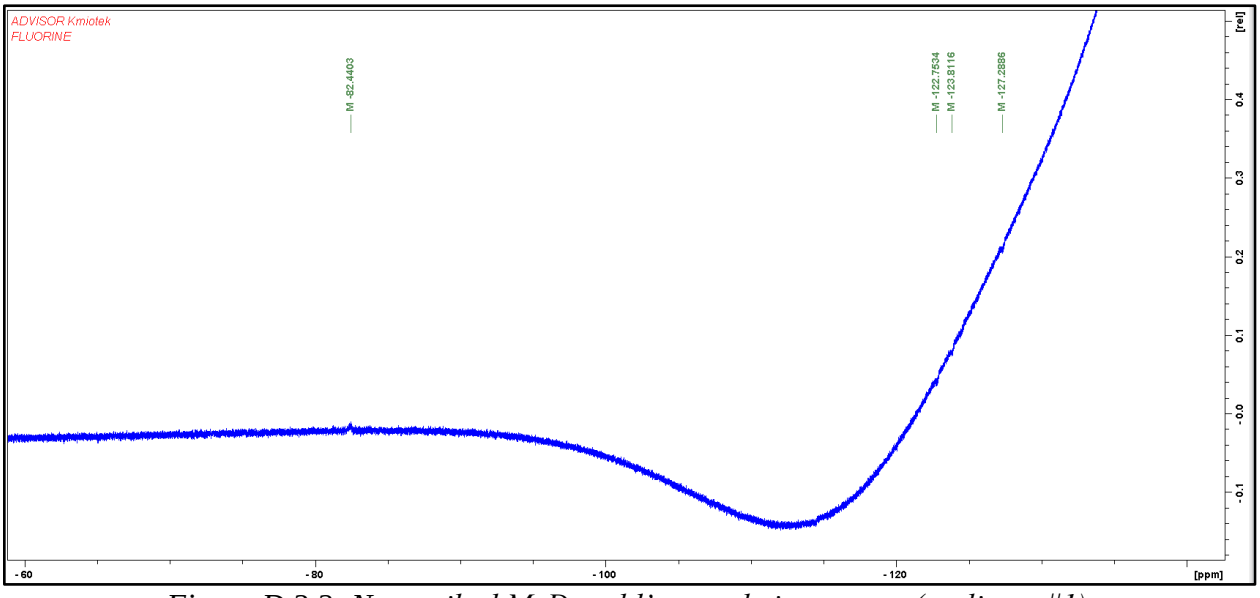


Figure D.3.2: Non-spiked McDonald's sample in acetone (replicate #1)

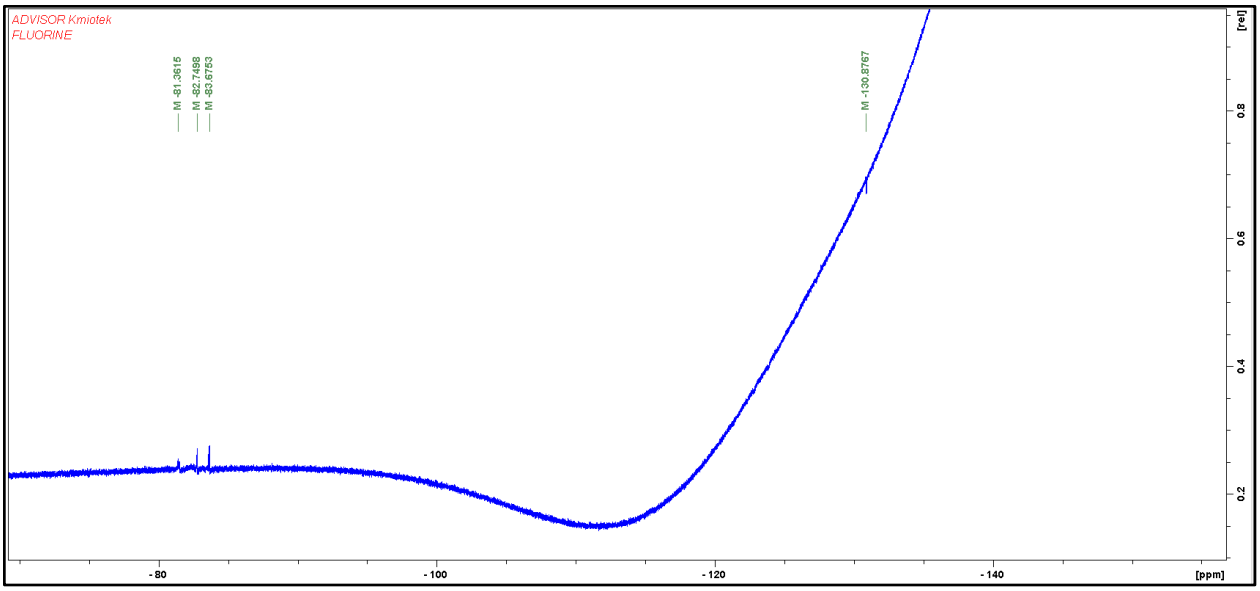


Figure D.3.3: Non-spiked Burger King sample in acetone (replicate #2)

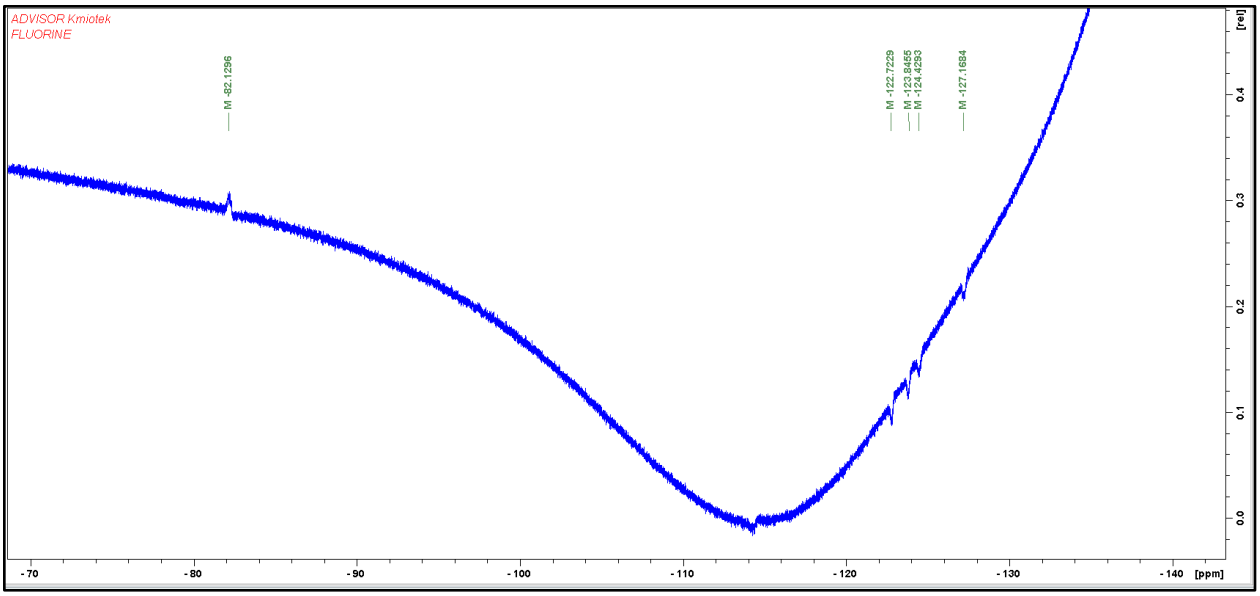


Figure D.3.4: Non-spiked McDonald's sample in methanol (replicate #2)

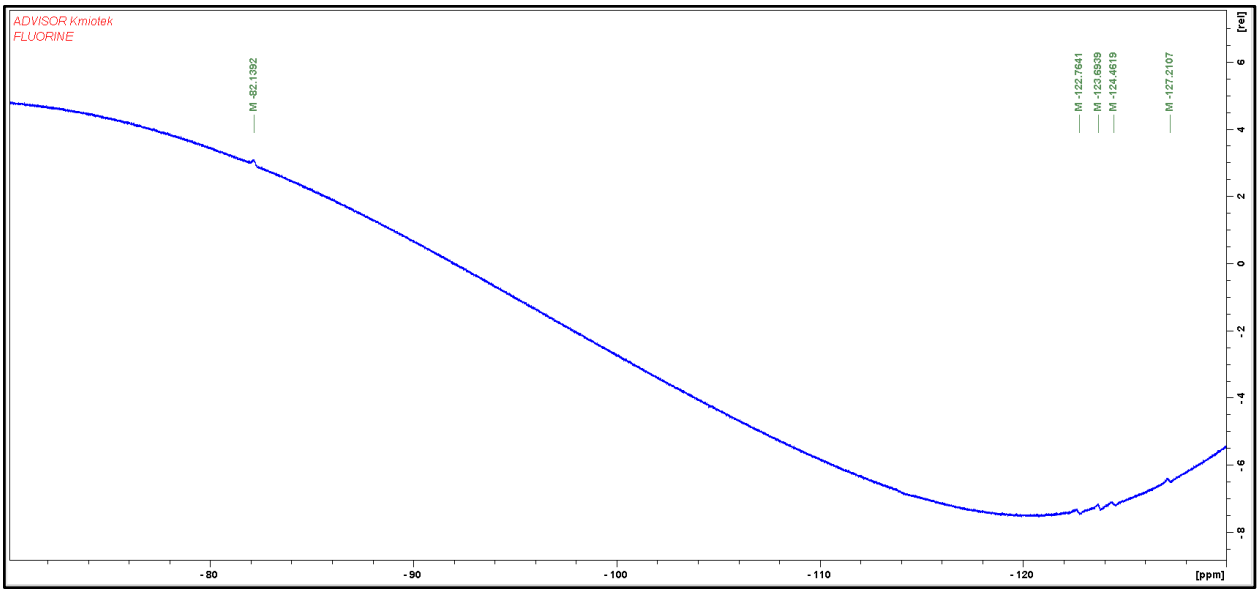


Figure D.3.5: Non-spiked Burger King Whopper sample in methanol (replicate #0)

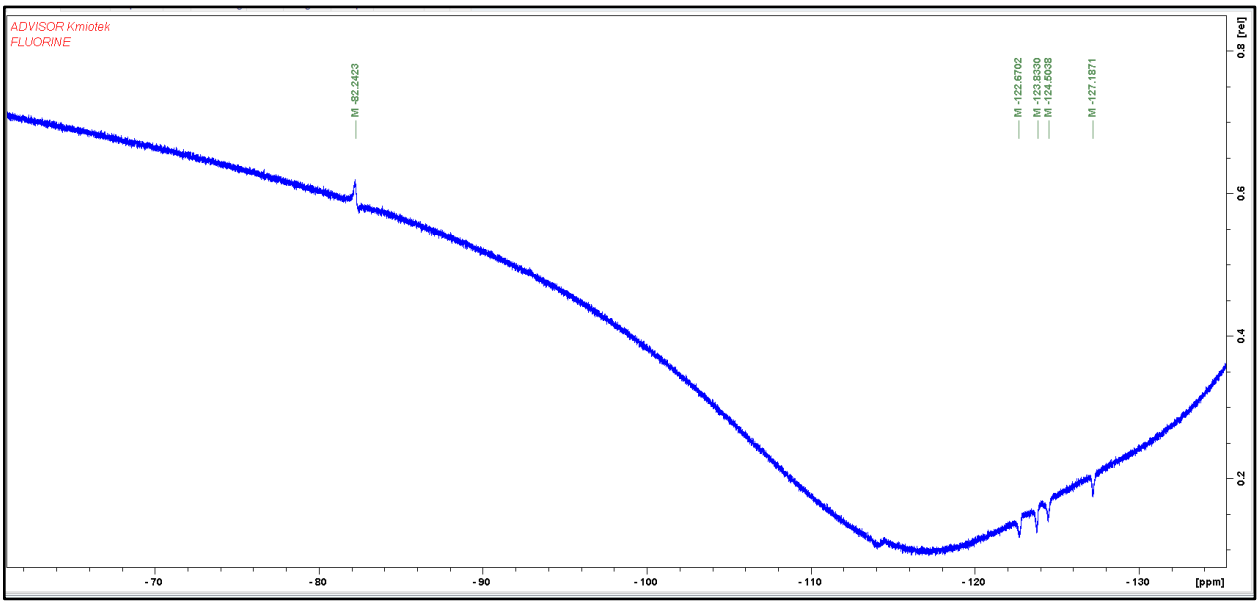


Figure D.3.6: Non-spiked Burger King Whopper sample in methanol (replicate #1)