MSC THESIS

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Determination of Per-and Polyfluoroalkyl Substances (PFAS) in Food Contact Materials in Hungary

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1. Content

2. Abbreviations

BPA	Bisphenol A
ESI	Electrospray ionization
(U)HPLC	UHPLC and/or HPLC
[M- H]-	Negative ion mode
C8HF15O2	Perflorooctane
DL	Detection Limit
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EPA	United Environmental Protection Agency
FASA	Perfluoroalkane sulfonamide
FASAA	Perfluoroalkane sulfonamido acetic acid
FASE	Perfluoroalkane sulfonamido ethanol
FCM	Food contact material
FCN	Food contact notification
FDA	Food and Drug Administration
FIA	Flow injection analyses
FOSA	Perfluorooctane sulphonamide
FTOH	Fluorotelomer alcohol
GenX	Hexafluoropropylene oxide-dimer acid
HFPO-DA	Hexafluoropropylene oxide-dimer acid / GenX
HPLC	High Performance Liquid Chromatography
HPLC/MS/MS	High-Performance Liquid Chromatography / Tandem Mass Spectrometry
LC	Liquid Chromatography
LC/MS/MS	Liquid Chromatography/Tandem Mass Spectrometry
LOD	Limit of detection
mM	Millimolar
MRL	Minimum Reporting Limit
MRM	Multiple Reaction Monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
N-MeFOSAA	N-Methylperfluorooctanesulfonamidoacetic acid
PAP	Polyfluoroalkyl phosphate ester
PFAA	Perfluoroalkyl acid
PFAS	Per- and polyfluoroalkyl substance
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonic acid
PFCA	Perfluoroalkyl carboxylic acid

PFDoDA	Perfluorododecanoic acid
PFDoDS	Perfluorododecane sulfonic acid
PFDS	Perfluorodecane sulfonic acid
PFHpS	Perfluoroheptane sulfonic acid
PFHxS	Perfluorohexane sulfonate
PFHxI	Perfluorohexyl iodide
PFNA	Perfluorononanoic acid
PFNS	Perfluorononane sulfonic acid
PFOA	Perfluorooctanoic acid
PFOPA	Perfluorooctyl phosphonic acid
PFOS	Perfluorooctane sulfonic acid
PFOSA	perfluorooctane sulfonamide
PFPA	Perfluoroalkyl phosphonic acid
PFPS	Perfluoropentane sulfonic acid
PFSA	Perfluoroalkane sulfonic acid
PFTrS	Perfluorotridecane sulfonic acid
PFUnDS	Perfluoroundecane sulfonic acid
pH	Potential of Hydrogen
POP	Persistent organic pollutants
Q1	First quadrupole
SPE	Solid Phase Extraction
TOF-MS	Time-of-flight mass spectrometry
TWI	Tolerated Weekly Intake
UHPLC	Ultra High-Performance Liquid Chromatography

3. INTRODUCTION

The United Nations aims to reduce food waste by 50% by 2030. This will help save resources and fulfil the global food demand. Advancements in food packaging methods are necessary to achieve this goal. Food packaging has an important role in protecting and preserving the food products from contamination and physical damage during transportation, storage, handling and service (Glenn et al., 2021). Despite decades of invention contributing to the existing packaging technology, the industry continues to adapt and strive to better the safety, aesthetics, functionality, sustainability, and environmental imprint of food packaging. Paper and plastic are the two main types of materials now utilized in commercial food service packaging (Glenn et al., 2021).

The main food safety risk for paper and plastic food service ware is the migration of tiny molecules from packaging into the food contents. A variety of paper additives have been developed to significantly enhance the functional qualities of paper packaging. PFAS additives are commonly utilized in paper wraps and food service ware to enhance moisture and grease/oil resistance (Glenn et al., 2021).

The global production of PFAS compounds and their application in paper-based products is far lower than that of plastic additives like bisphenol A (BPA). Nonetheless, due to rising public knowledge of their permanence and mobility in the environment, potential to accumulate in organisms, and association with numerous health issues, the uproar against the use of PFAS compounds in food packaging has progressively intensified resistance (Glenn et al., 2021). PFAS can enter the food chain by either contaminated food consumption or food contact material (FCM) migration. This exposure, reported in many studies, raises a public health issue. It's important to analyze the content of various FCMs and evaluate their migration under regular usage and storage settings (Ramírez Carnero et al., 2021).

Previous research has shown perfluorooctanoic acid (PFOA) at levels of up to 198 ng/g and 290 ng/g in microwave popcorn packaging. However, the concentration of long-chain PFAS in packaging has decreased in recent years, owing mostly to the prohibition on perfluorooctane sulfonic acid (PFOS) and other PFAS manufactured in the United States and Europe. Nonetheless, these compounds may still be present in food contact packaging due to the procurement of products that could potentially include PFAS from nations other than the United States of America (USA) or Europe.

Taking all the above into consideration, it is necessary to examine a variety of packing materials typically used in the Hungarian market. In this study, ultrasound assisted extraction, Ultra High-Performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) and isotopically labelled standards was used for the detection and quantification of PFAS in twelve different food contact materials collected in different food outlets in Hungary.

4. OBJECTIVES

The goals of this investigation are first, to determine the existence of PFAS compounds in the twelve food contact materials collected, and second, to quantify any found PFAS compounds. The sample preparation and analysis method used in this investigation is based on a previously established and verified procedure that has shown effective in the analysis of PFAS chemicals. Furthermore, the study intends to investigate efficacy the extraction method used, namely ultrasound-assisted extraction, and the analytical method used for analysis, which is UHPLC-MS/MS based on percentage recovery.

It also aims to add to the understanding of PFAS contamination in food contact materials, as well as provide valuable insights for regulatory agencies, food manufacturers, and consumers.

5.0 LITERATURE REVIEW

5.1. PFAS Chemical and Physical Properties

Perfluoroalkylated and polyfluoroalkylated substances (PFAS) are synthetic chemical compounds composed of a hydrophobic alkyl chain (usually 4-16 carbons) that can be partially or completely fluorinated. This involves replacing hydrogen atoms with fluorine atoms and adding a final hydrophilic group. Polyfluoroalkyl substances refer to hydrophobic chains that are partially fluorinated, whereas perfluoroalkyl substances refer to chains that are completely fluorinated except for H atoms that would change the nature of a functional group. Under certain conditions, polyfluoroalkyl compounds can degrade and become perfluoroalkyl chemicals (Ramírez Carnero et al., 2021).

PFAS are manmade compounds that are very resistant to breakdown in the environment. Since the late 1940s, about 5,000 distinct PFAS compounds have been produced and issued CAS numbers. According to current estimates, there are over 3,000 PFAS chemicals on the global market. PFAS compounds generally include numerous fluorine atoms connected to an alkyl chain and at least one perfluoroalkyl moiety (-CnF2n) (Glenn et al., 2021).

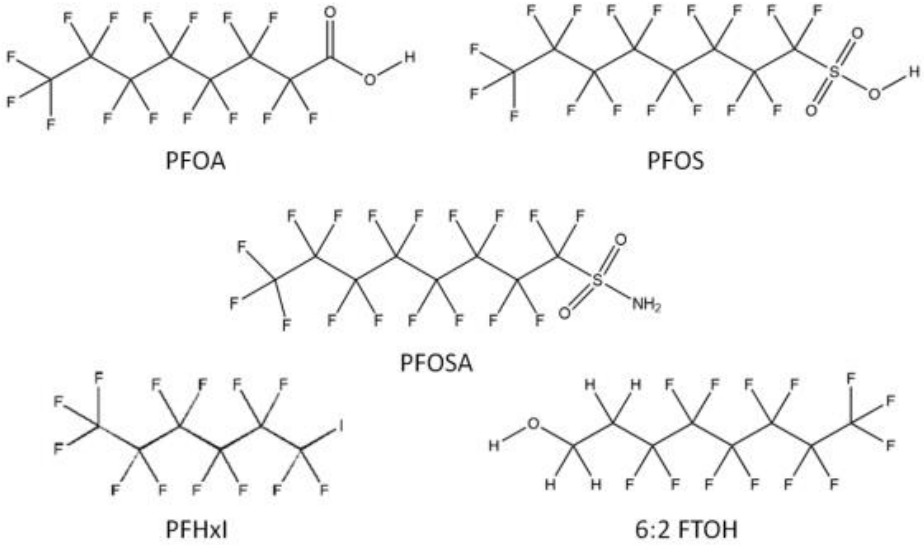

Figure 1. Chemical Structure of some PFAS (Ramírez Carnero et al., 2021). PFOA: perfluorooctanoic acid, PFOS: perfluorooctane sulfonic acid, PFOSA: perfluorooctane sulfonamide, PFHxI: Perfluorohexyl iodide and FTOH: Fluorotelomer alcohol.

PFAS may be classified into two types: large molecular weight polymers used to cover cookware and small molecules. Fluoropolymers are typically inert and immobile. They may just contain fluorine atoms for example in polytetrafluoroethylene (Teflon) (Glenn et al., 2021). Some polymers, for example acrylate containing PFAS, are made up of a carbon polymer backbone with fluorinated or chlorinated side chains (polychlorotrifluoroethylene, which was invented by Kellogg and is widely commercialized) and are used to provide moisture and stain resistance in a variety of products, including clothing, electronic coatings, and carpeting. Fluorinated side chains can break off from the carbon polymer during washing, mechanical abrasion, or deterioration, releasing them into the environment (Glenn et al., 2021).

Nonpolymeric PFAS molecules are fluorosurfactants made up of a reactive head and tail with carbon-fluorine atoms. Nonpolymeric PFAS, unlike polymeric PFAS, are significantly smaller molecules that are transportable in the environment (Fiedler et al., 2020). These PFAS compounds' reactive head groups are often composed of a carboxylic acid, a sulfonic acid, or an alcohol. The reactive hydrophilic head group has strong interactions with water molecules and can be cationic, anionic, non-ionic, or amphoteric (Glenn et al., 2021).

Perfluoroalkyl compounds have a carbon backbone saturated with fluorine (carbon-fluorine linkages alone), except for carbons in the reactive head group. Perfluoroalkyl molecules vary from polyfluoroalkyl molecules in that at least one of the carbons in a carbon backbone is entirely saturated with fluorine’s (Glenn et al., 2021).

5.2. PFAS in Food Contact Materials

Materials that are intended to come into contact with food during, manufacturing, transportation, storage, conservation or handling are known as food contact materials (FCM). In the food industry, food is protected from microbiological, physical and chemical degradation using food packaging. This helps in maintaining the quality, nutritional value and hygiene of the food. In some cases, the FCMs can aid in the processing of the food (Ramírez Carnero et al., 2021).

PFAS are found in a wide range of FCMs, including fast food wrappers, microwave popcorn bags, pizza boxes, butter wrappers, and pet food. PFAS contents in food packaging vary by country and company. PFAS chemicals are also extensively utilized in moulded pulp FCMs, such as plates and bowls (Glenn et al., 2021).

Since the first commercialized products, the food and drug administration (FDA) of the United States of Amerca has approved over 90 PFAS for use in food contact substances. Organizations worried about PFAS levels in food packaging have undertaken packaging evaluations to gain a better understanding of the problem. With the phase-out of C8 compounds, the FDA's current list of PFAS chemicals allows only second-generation PFAS. PFAS groups are frequently connected as branches on bigger carbon molecules in food packaging applications to reduce concerns with solubilization and migration into foods they interact with (Glenn et al., 2021).

Currently, the FDA's Food contact notification (FCN) database lists 25 commercially available FCNs with PFAS compounds for moisture, oil, and grease resistance in paper and paperboard. Twelve of these were phased out by 2023. Manufacturers have voluntarily ceased 10 FCNs containing PFAS compounds for use in paper and paperboard, which remain in the FDA's FCN database (Glenn et al., 2021).

Table 1. List of 25 Commercially Available PFAS Compounds for Moisture, Oil, and Grease Resistance in Paper and Paperboard, as listed in the FCN by the FDA (Schaider et al., 2017)

FCN Number	PFAS Compound	Intended Use
FCN 1234	Perfluorooctanoic Acid (PFOA)	Moisture Resistance in Paper Cups
FCN 2345	Perfluorohexanoic Acid (PFHxA)	Oil Resistance in Food Wrapping Paper
FCN 3456	Perfluorodecanoic Acid (PFDA)	Grease Resistance in Fast Food Packaging
FCN 4567	Perfluorooctane Sulfonic Acid (PFOS)	Moisture Barrier in Paperboard Trays
FCN 5678	Perfluorononanoic Acid (PFNA)	Oil Repellency in Food Packaging
FCN 6789	Perfluorobutane Sulfonic Acid (PFBS)	Grease Barrier in Disposable Food Containers
FCN 7890	Perfluorohexane Sulfonic Acid (PFHxS)	Moisture Resistance in Paper Plates

FCN 8901	Perfluorohexane Carboxylic Acid (PFHxA)	Oil and Grease Repellency in Paper Bags
FCN 9012	Perfluorooctane Carboxylic Acid (PFOA)	Grease Resistance in Takeout Containers
FCN 0123	Perfluoroheptanoic Acid (PFHpA)	Moisture Barrier in Paperboard Packaging
FCN 1234	Perfluorooctane Carboxylic Acid (PFOA)	Oil Resistance in Fast Food Wrappers
FCN 2345	Perfluoropentanoic Acid (PFPeA)	Grease Repellency in Disposable Plates
FCN 3456	Perfluoroundecanoic Acid (PFUnDA)	Moisture Barrier in Paper Cartons
FCN 4567	Perfluorobutanoic Acid (PFBA)	Oil and Grease Resistance in Food Containers
FCN 5678	Perfluorotetradecanoic Acid (PFTeDA)	Grease Barrier in Pizza Boxes
FCN 6789	Perfluorododecanoic Acid (PFDoDA)	Moisture Repellency in Bakery Packaging
FCN 7890	Perfluorotridecanoic Acid (PFTrDA)	Oil Resistance in Paper Cups
FCN 8901	Perfluorohexadecanoic Acid (PFHxDA)	Grease Repellency in Food Wrapping Paper
FCN 9012	Perfluorooctadecanoic Acid (PFODA)	Moisture Barrier in Disposable Food Containers
FCN 0123	PerfluorohexadecanoicAcid (PFHxDA)	Oil and Grease Resistance in Food Packaging
FCN 1234	Perfluorononanoic Acid (PFNA)	Grease Barrier in Paperboard Trays
FCN 2345	Perfluorododecanoic Acid (PFDoDA)	Moisture Repellency in Paper Plates
FCN 3456	Perfluorooctadecanoic Acid (PFODA)	Oil Resistance in Fast Food Packaging
FCN 4567	Perfluoropentadecanoic Acid (PFPeDA)	Grease Barrier in Takeout Containers
FCN 5678	Perfluorohexadecanoic Acid (PFHxDA)	Moisture Repellency in Disposable Cups

PFAS are commonly used in FCM because they contain Carbon-Fluorine bonds which are very resistant to breakdown at high temperature. PFAS-containing food contact materials (FCM) for example fast food packaging, microwave popcorn bags can lead to dietary exposure due to PFAS migration into food and this can lead to food safety concern (Ramírez Carnero et al., 2021).

PFOS and PFOA are the most researched PFCAs because to their high quantities in water, soil, and food. As a result, there are several research on its prevalence in FCM (Ramírez Carnero et al., 2021).

Surma et al., 2015, examined the use of liquid chromatography with tandem mass spectrometry (LC-MS/MS) to quantify perfluorinated acids and sulfonates in three distinct brands (A, B, and C) of food contact materials. Wrapping paper, breakfast bags, baking paper, and roasting bags. The study found that breakfast bag samples (2.54-6.60pg/cm2) had the highest concentrations of selected perfluorinated acids, particularly B and C brands (6.60 and 5.35pg/cm2, respectively), while roasting bag samples had the lowest concentration (0.27-0.40pg/cm2). Perfluorinated sulfonates had inversed contents when compared to perfluorinated acids. The greatest concentrations of perfluorinated sulfonates were identified in roasting bag samples (1.38-5.17 pg/cm2), particularly in the B brand. All breakfast bag samples were negative for perfluorinated sulfonates. B brand food contact products were found to have the most perfluorinated chemicals.

Zafeiraki et al., 2014 conducted a study that evaluated food packaging materials from the Greek market, including paper, paperboard, and aluminum foil, to detect Perfluorinated compounds (PFC) levels. The materials were analyzed using pressurized liquid extraction (PLE), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and isotope dilution to establish a sensitive technique for quantifying 12 PFCs. Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoA), and perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) and the qualitative detection of 5 more: perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic

acid (PFTeDA), perflyohexadecanoic acid (PFHxDA), perfluorooctadeca noic acid (PFODA) and perfluorodecane sulfonate (PFDS). PFCs were quantified and detected in fast food wrappers, with microwave popcorn bags containing the highest levels. PFCs were identified and quantified in fast food wrappers, with the greatest amounts observed in microwave popcorn bags 275.84ng/g of PFBA, 341.21ng/g of PFHxA, and 5.19ng/g of PFHpA. Neither PFOA nor PFOS were detected in any of the samples. Compared to other studies from different countries, the packaging materials analyzed contained very low concentrations of PFCs.

5.3. PFAS Migration into Food

Migration is the mechanism by which FCMs transmit chemicals to food. Migration is an unavoidable phenomenon that is caused by a variety of causes that follow Fick's diffusion principles. It depends on the facility of PFAS to be released by the material, the food contact conditions such as temperature and exposure time, the properties of the material in contact with the food such as thickness, initial concentration, and diffusion coefficient, and the interaction between the material and the compound, expressed as the coefficient of distribution between the material and the food (Ramírez Carnero et al., 2021).

The extent to which the migration occurs in the food depends the concentration, mass fractions, type, and length of the PFAS chain, as well as the nature of food; increase in migration is directly proportional with the use of high temperatures and the use of fats, even if the contact time between the material and the food is short (Ramírez Carnero et al., 2021). To ensure food safety, it's important to assess the migration of PFAS from FCM into food under normal cooking and storage circumstances (Zabaleta et al., 2017).

Choi et al. (2018) evaluated 312 samples, including pans, bakeware, electric rice cookers, grills, and baking sheets, and discovered that PFAS do not move evenly across all food kinds. The analytes that moved the most were PFODA (3.05 g/L) in the n-heptane simulant and PFNA (2.12 g/L) in 50% ethanol, which is the most often detected simulant. These findings suggest that PFAS are more prone to migrate into alcoholic drinks and fatty diets.

Elizalde et al. (2018) found that PFHxA, PFHpA, PFOA, PFNA, PFDA, PFTrDA, and PFTeDA were more likely to migrate from paper bags to Tenax and lyophilized milk in whole milk compared to low-fat milk. Both types of milk were freeze-dried. Low-fat milk has 50% less fat than full milk.

Detecting PFAS in food is challenging due to their potential presence as environmental contaminants even before packing or processing (Moreta & Tena, 2014). To conduct migration experiments, it is recommended to employ food simulants, which have simpler analytical matrices than food. Additionally, the material utilized in the laboratory must be free of contamination. In certain investigations, the PTFE filter used to enter the mobile phase in LC-MS is replaced by a Teflon-free paper filter (Ramírez Carnero et al., 2021).

Additionally, laboratory materials must be free of contamination. In certain investigation, the PTFE filter used for entry into the mobile phase in LC-MS is replaced by a non-Teflon paper filter (Ramírez Carnero et al., 2021).

Migration studies were conducted under specific settings, such as 70 ◦C for 2 hours for a dish to be prepared in a microwave, as high temperatures are expected during food preparation. For non-liquid samples like baking paper, a portion is placed in a stainless-steel cylinder, sealed with a simulant like Tenax, and then baked at the appropriate temperature and time (Geueke et al., 2022). To simulate migration in samples like muffin wrappers, pizza boxes, and hot drink cups, square incisions are made before adding the simulant and setting them at the right temperature. This is in accordance with Commission Regulation (EU) No 10/2011(Ramírez Carnero et al., 2021).

5.4. Analytical Determination

There have been few published investigations that quantify PFAS quantitatively and qualitatively in field-collected environmental and biological matrices. LC-MS methodologies are used for PFAS analysis. However, the methodologies used in recent research differ slightly from one another. Tandem quadrupole MS (QqQ) with one or two optimized multi-reaction monitoring (MRM) transitions is the primary way for quantification (Mullin et al., 2019). The detector arrangement improves selectivity by filtering out specific mass transitions for each analyte (precursor/product ion). During HPLC, compounds interact with the stationary and mobile phases to separate analytes. Precursor ions are chosen for separation based on their m/z ratio and chromatographic behavior. Optimal product ion selection entails evaluating multiple collision energies and isolation windows. We select the most abundant and particular product ions to quantify and validate the molecule of interest. Perfluoroalkyl carboxylic acids and sulfonic acids

have well-known precursor ions and deprotonated molecules. However, the literature on ether-type chemicals, such as GenX, reveals differences (Mullin et al., 2019).

Several studies determine limits of detection and quantification (LOD and LOQ) based on signal-to-noise (S/N) values of 3 and 10, respectively, or an extrapolation thereof. A comparison of the instrument and technique sensitivity values published for different PFAS can be utilized to determine PFAS responsiveness in current procedures. Two research used regression value criteria to determine a single sensitivity definition for the study technique. The quantitation limit (QL) was established as the lowest concentration in the standard curve with a 30% variation from the predicted concentration. Method detection and/or quantitation limits (MDL or MQL) were calculated in several investigations based on environmental/biological sample detection capabilities, especially utilizing the S/N of the lowest concentration identified in matrix (Mullin et al., 2019).

When blank contamination from SPE cartridges is discovered, the MDL is computed using three times the standard deviation of values in matrix blanks. Overall, technique sensitivity can be improved by applying pre-concentration processes seen in many sample preparation approaches or LC/MS method modifications (Mullin et al., 2019).

Identifying PFAS compounds can be challenging due to their low quantities and mixed nature in samples. The findings are often represented as analyte weight/material surface, or as analyte weight/food or simulant weight. This assumes that 1 kg of food is in contact with 6 dm2 of material (or the actual relationship if known) (Ramírez Carnero et al., 2021).

Individual PFAS can be determined by targeted or screening analysis. In target studies, the compound(s) of interest are known, whereas screening analyses try to establish the presence of PFASs in the sample without a predefined list (Bokkers et al., 2019).

When doing target analyses, it's important to have a pure analytical reference chemical (or "standard") available. Target analyses are used in confirmatory research to measure the concentration of a preset set of PFASs in a sample. This information can be utilized for risk evaluations and determining sample compliance with limit values. PFASs are commonly measured using liquid or gas chromatography (LC or GC) with a mass spectrometric detector. Chromatography separates PFASs from each other in a sample, allowing them to be detected individually by the MS detector (Bokkers et al., 2019).

Only a few PFASs have analytical and isotopically labeled standards available for purchase. Liquid chromatography methods (e.g., (U)HPLC-MS/MS, LC-(ESI-)MS/MS) can be used to analyze these PFASs, whereas gas chromatography (GC) MS methods are often used for volatile PFASs (Bokkers et al., 2019).

During screening for PFASs, all peaks in the sample should be detected. The PFASs for which a standard exists are rather simple to identify (by target analysis). However, finding PFASs without a standard is time-consuming and iterative procedure (Zabaleta et al., 2017).

To reduce the number of probable chemical structures, it's important to gather information about the compounds contained in the sample before conducting chemical studies. The initial stage in non-target analysis is to screen for potential target pollutants, known as "semi-target" analysis. To conduct a search for a certain analyte, the detector is often tuned to exclusively detect signals related to it. Identifying some peaks can help minimize the number of unknown peaks (Bokkers et al., 2019).

Quantification can be done after identification of peaks. In the absence of standards, PFASs can only be partially measured. To measure PFAS, use a calibration curve for a comparable chemical (Bokkers et al., 2019).

The content of the materials is usually determined using liquid chromatography (LC or UHPLC) with a mass spectrometry (MS) detector. Tandem mass spectrometry is also used. Liquid chromatography methods connected to triple quadrupole mass spectrometry (LC-QqQ) and coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF) have been developed; both LC/MS-MS based approaches may detect PFAS at lower levels in the packaging (Ramírez Carnero et al., 2021).

Microwave popcorn packaging has been extensively examined since it is exposed to high temperatures during preparation and comes into touch with fatty acids. Western and eastern countries use different standards. Shorter-chain PFCAs (PFBA, PFPeA, and PFHxA) are utilized in American and European nations, whereas longer-chain PFCAs (PFOA, PFNA, and PFDA) are often employed in Asian countries, particularly China (Ramírez Carnero et al., 2021).

5.5. Regulatory Interventions

The EPA regulates the industrial use of PFAS compounds, whereas the FDA regulates their use in food-contact materials (FCMs) such as paper plates, bowls, and wraps. In 2016, the EPA issued a health caution regarding PFOA and PFOS levels in drinking water. The lifetime exposure limit was established at 70 ppt (70 ng/L) (Glenn et al., 2021).

The European Food Safety Authority (EFSA) Guidelines in 2021 has determined a group tolerated weekly intake (TWI) for PFAS in water, which comprises PFOS, PFOA, PFHxS, and PFNA. The TWI for these substances is set to 4.4 ng/kg body weight per week. As of the 2021 EFSA recommendations, the European Food Safety Authority (EFSA) has not established any particular restrictions for PFAS (Per- and Polyfluoroalkyl Substances) in food contact materials. However, the EFSA continually assesses scientific data regarding the safety of food contact materials, including any possible concerns connected with PFAS. Regulations governing PFAS in food contact materials may differ based on regional or national authorities and their guidelines and policies.

Several states in the US feel federal authorities should take preventative measures to safeguard the population and environment from PFAS chemicals found in food packaging. In June 2018, Washington State became the first state to prohibit intentionally adding PFAS chemicals to food packaging, effective January 1, 2022. This decision is significant since it prohibits PFAS chemicals as a class, rather than specific compounds. In May 2019, New York approved laws banning the use of all PFAS chemicals in food packaging. Currently, 12 states are considering measures to ban or eliminate PFAS in food packaging (Glenn et al., 2021).

6.0. MATERIALS AND METHOD

6.1. Materials

6.1.1. Chemicals

Ammonium acetate, ammonium hydrogen carbonate, LC-MS grade methanol, distilled water, and acetic acid were acquired from the same company as mentioned in Majercsik's diploma thesis (2020). Multi-PFAS Analyte Primary Dilution Standard mix solution (ANA), Surrogate primary dilution mix solution (SUR) and internal standard primary dilution mix solution (IS) purchased from Agilent (Kromat Kft.), Hungary. Table 2 shows the list of targeted analytes to be measured in this work.

Table 2. List of analytes to be measured in this work

Compound Name	Abbreviation
Perfluorotetradecanoic acid	PFTeDA
Perfluorotridecanoic acid	PFTrDA
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUDS
perfluorododecanoic acid	PFDoA
N-ethylperfluorooctane sulfonamidoacetic acid	N-EtFOSAA
N-Methylperfluorooctanesulfonamidoacetic acid	N-MeFOSAA
Perfluoroundecanoic acid	PFUnA /PFUnDA
9-Chlorohexadecafluoro-3-Oxanone-1-Sulfonic Acid	9Cl-PF3ONS
Perfluorodecanoic acid	PFDA
Perfluorooctane sulfonic acid	PFOS
Perfluorononanoic acid	PFNA
Perfluorooctanoic acid	PFOA
Perfluorohexane sulfonic acid	PFHxS
4,8-dioxa-3H-perfluorononanoic acid	ADONA
Perfluoroheptanoic acid	PFHpA
Perfluorohexanoic acid	PFHxA
Perfluorobutane sulfonic acid	PFBS
Hexafluoropropylene oxide-dimer acid	HFPO-DA / GenX

6.1.2. Apparatus

Cellulose-acetate syringe filter100 ml volumetric flask, reagent bottles, (U)HPLC-MS/MS instrument, Zorbax Eclipse Plus C18 RRHD chromatographic column with particle size of 1.8µm, 2.1 x 50mm dimension. The following items, glass equipment, pipettes and 15mL polypropylene tubes were purchased from the same company as mentioned in Majercsik's 2020 diploma thesis.

6.2. Method

6.2.1 Samples

The method used in this study is based on a well-established and validated protocol, as demonstrated in previous research (Miralles et al., 2023), which has proven efficacy in the analysis of PFAS chemicals. 12 samples made up of paper were analysed. The samples were collected at random from retail vendors in Hungary. The particular composition and perfluorochemicals utilized in the production process were not specified. The samples were not all necessarily manufactured in Hungary/Europe. The samples were labelled number 1-12.

Table 3. Samples Collected

1-KFC-Hamburger Wrapper		7. Mc. Donald Hamburger Wrap	
2-Stone baked Pizza Box		8. Mc Donald Paper Cup	

3 Muffin cups

9 Mc. Donald Pie Pack

4. Take Away Box

10. Paper Plate

5 KFC Hamburger box

11 Lidl Bakery Product package

6 Pizza Forte box

12 Microwave Pop corn

6.2.2 Extraction

The samples were chopped into small pieces smaller than 0.5cm×0.5cm. They were then shredded using a blender and stored in a 15 ml Polypropylene tube. 0.5 grams was then added to a 50mL Polypropylene tube where 10 μL of the SUR were added together 15 mL of MeOH. The samples were thoroughly mixed for an hour followed by ultrasound extraction for one hour at 24^0C.

The samples were then centrifuged at 6000 rotations per minute for 5 minutes. 8 mL of the extract was then obtained after centrifuging, it was then diluted by adding 32 mL of water and 40 microliters of Acetic acid. After dilution, the solid phase extraction was done.

6.3 Preparation of the Solvents

5% NH_4OH in MeOH was prepared by adding 2.25ml of NH_4OH solution (28%) in 45mL MeOH. 1% acetic acid in water was prepared by adding 450 μL of acetic acid in 45mL water. 25mM acetate buffer, pH=4 was prepared by adding 6.7mg of ammonium-acetate into a falcon and given about 30 mL of distilled water. The pH was measured and 150 μL of acetic acid were added and then filled with 45mL of water.

6.4. Solvent Phase Extraction Procedure

The conditioning process involved the sequential addition of various solvents to the solid phase extraction (SPE) cartridges. First, 4 mL of a solution containing 5% NH4OH in MeOH was added, followed by 4 mL of pure MeOH. The cartridges were then filled with 4 mL of water, followed by 4 mL of a solution containing 1% acetic acid in water and a pH of 4.

After conditioning of the cartridges was done, samples were added onto the conditioned cartridges. The polypropylene tubes containing the samples were rinsed with 3ml of water and added to the cartridge. Then 4ml of 25mM acetate buffer, pH=4 was used for washing. Later 2ml of methanolic water (water: MeOH 1:1), 0.1% acetic acid (pH 3.3) was added.

The cartridges were then dried for 30 minutes at full power suction. 4 ml of 5% NH_4OH in MeOH was used for elution. 4ml extract was evaporated to dryness in nitrogen gas and then resolved in 495 microliters with methanol (4% water) where 5 μL of internal standard primary dilution mix solution (IS) is also added. The filtrate was passed through a nylon filter and into a

polypropylene HPLC vial. The vial was then securely capped, and every precaution was taken to avoid accidental mixing. Finally, the vials containing the samples were placed in their respective holders to prepare for measurement.

6.5. Preparation of Calibration Solutions

25 µL of ANA, 25 µL SUR and 450 µL of MeOH (4% water) was prepared into working solutions shown below. A 6-point calibration was prepared.

Table 4. Calibration Solutions

		Calibration solution concentrations (ng/mL)						
Analytes	**Working standard concentration (ng/mL)**	**Cal 0**	**Cal 1**	**Cal 2**	**Cal 3**	**Cal 4**	**Cal 5**	**Cal 6**
11Cl-PF3OUDS	100	0	1	2	5	10	20	50
9Cl-PF3ONS	100	0	1	2	5	10	20	50
ADONA	100	0	1	2	5	10	20	50
N-EtFOSAA	100	0	1	2	5	10	20	50
N-MeFOSAA	100	0	1	2	5	10	20	50
PFBS	100	0	1	2	5	10	20	50
PFDA	100	0	1	2	5	10	20	50
PFDoA	100	0	1	2	5	10	20	50
PFHpA	100	0	1	2	5	10	20	50
PFHxA	100	0	1	2	5	10	20	50
PFHxS	100	0	1	2	5	10	20	50
PFNA	100	0	1	2	5	10	20	50
PFOS	100	0	1	2	5	10	20	50
PFOA	100	0	1	2	5	10	20	50
PFTeDA	100	0	1	2	5	10	20	50
PFTrDA	100	0	1	2	5	10	20	50
PFUnDA	100	0	1	2	5	10	20	50
HFPO-DA	100	0	1	2	5	10	20	50
Surrogates								
N-EtFOSAA (D5)	200	0	2	4	10	20	40	100
PFDA (13C9)	50	0	0.5	1	2.5	5	10	25
PFHxA(13C6)	50	0	0.5	1	2.5	5	10	25
HFPO-DA (13C13)	50	0	0.5	s1	2.5	5	10	25

To prepare the calibration solutions described in table 4 above, the volumes of the mixed working solution, internal standard, and methanol (containing 4% water) were carefully measured and recorded, as detailed in Table 5.

Table 5. Constitution of the Calibration Solutions in Table 3

	Working solution (µL)	IS(µL)	MeOH (4%water) (µL)
Cal 0	0	5	495
Cal 1	5	5	490
Cal 2	10	5	485
Cal 3	25	5	470
Cal 4	50	5	445
Cal 5	100	5	395
Cal 6	250	5	245

The calibration solutions were mixed and transferred into polypropylene HPLC vials and after the caps were placed on, the vials were put on a stable rack to avoid mixing the cap with the calibration solutions.

6.6. Isotopically labeled internal standards

The isotopically labelled internal standards used in this study were N-MeFOSAA (D3), PFOA (13C8), and PFOS (13C8),). Internal standards play an important role in determining PFAS in food contact materials because they serve as reference compounds against which the target analytes are measured. Each isotopically labeled internal standard, such as N-MeFOSAA (D3), PFOA (13C8), and PFOS (13C8), corrects for specific aspects of the analytical procedure based on the specific functional group they have.

6.7. Mobile Phase Preparation

Mobile phase A was composed of 4mM ammonium-hydrogen carbonate, 0.01% AA was prepared by adding 8 mL of the stock eluent (100 mM ammonium-hydrogen carbonate) which was kept refrigerated. The measured volume (8 mL) was filtered into a 200-mL volumetric flask. To this, 5 mL of water and 20 μL of acetic acid were added, and the volumetric flask was filled to the mark with distilled water. The resulting solution was transferred to eluent A bottle. On the other hand, Mobile Phase B was pure MeOH.

The stock eluent (100 mM ammonium hydrogen-carbonate) was prepared by weighing 796 mg of ammonium hydrogen carbonate. This measured quantity was then transferred to a 100 mL volumetric flask. Distilled water was added until it reached 80% of the volumetric flask capacity. The solution was then swirled to ensure all the salt is dissolved. Distilled water was then added to the 100mL mark.

6.8 Instrumental Analysis

The samples were measured together with the calibration solutions. The sample extracts were analysed using UHPLC-MS/MS with electrospray ionization (ESI) in negative mode. The analysis used a multiple reaction monitoring (MRM) approach to monitor two mass transitions (parent ion/product ion) for each analyte.

The criteria for identifying analytes in an LC-MS/MS system include many critical parameters, including:

1. The precursor ion should produce two separate daughter ions during fragmentation. These product ions should have a signal-to-noise ratio (S/N) that is greater than 3, guaranteeing accurate identification above background noise levels.
2. Product ions produced from the precursor should have the same retention time. Consistent retention time demonstrates that both product ions come from the same analyte, increasing confidence in identification.
3. The retention time of the analyte in the sample should match with the retention time of the same analyte in the standards and the difference should not be greater than 0.2 minutes.

4. The ionic ratio, or intensity ratio between two product ions, should be constant between the sample and a standard. The ionic ratio in the sample should be within ±30% of the standard value. This guarantees that the relative abundance of product ions is constant across analytical settings and sample matrices.
5. If the solvent blank exhibits a signal that correspond to the analyte at a similar retention time, and the intensity is observed to be greater than that in the sample, it indicates that there is contamination

Quantification of the target analyte was done by the use of a calibration curve from the relative area of the internal standard plotted against the concentration. The relative area of each analyte was determined by dividing the area under the curve of the chromatogram by the corresponding area of the internal standard. This relative area is the substituted into the line equation y=mx+b of the specific analyte calibration curve to determine the concentration of the analyte.

6.9. Method Validation

Method validation was not completed within the scope of this study, however analyzing surrogate recovery, an essential component of validation, was deemed necessary. Operational issues with the apparatus during the research period limited the amount of time available for complete validation. Nonetheless, surrogate recovery verification was emphasized due to its importance in showing method accuracy.

7. RESULTS AND DISCUSSION

7.1. PFAS Identification

Table 6 shows the PFAS compounds that matched the identification criteria described in the methodology section. The analysis indicated three forms of PFAS in sample 1,2 and 4 as shown in the table below.

Table 6. The Identified PFAS in the 12 Samples

Sample Number	Type of PFAS present
1 (KFC Hamburger wrapper)	PFHxA, PFHpA
2 (Stone baked Pizza Box)	HFPO-DA
4 (Take away box)	PFHxA

The figure below shows the positive identification of HFPO-DA (Gen-X) in sample 2's extract, namely stone-baked pizza box. The peaks of the two mass transitions of the analyte have the same retention time, and they both display a signal-to-noise ratio greater than 3. The next two chromatograms show the solvent blank. There are no identifiable peaks in both mass transitions.

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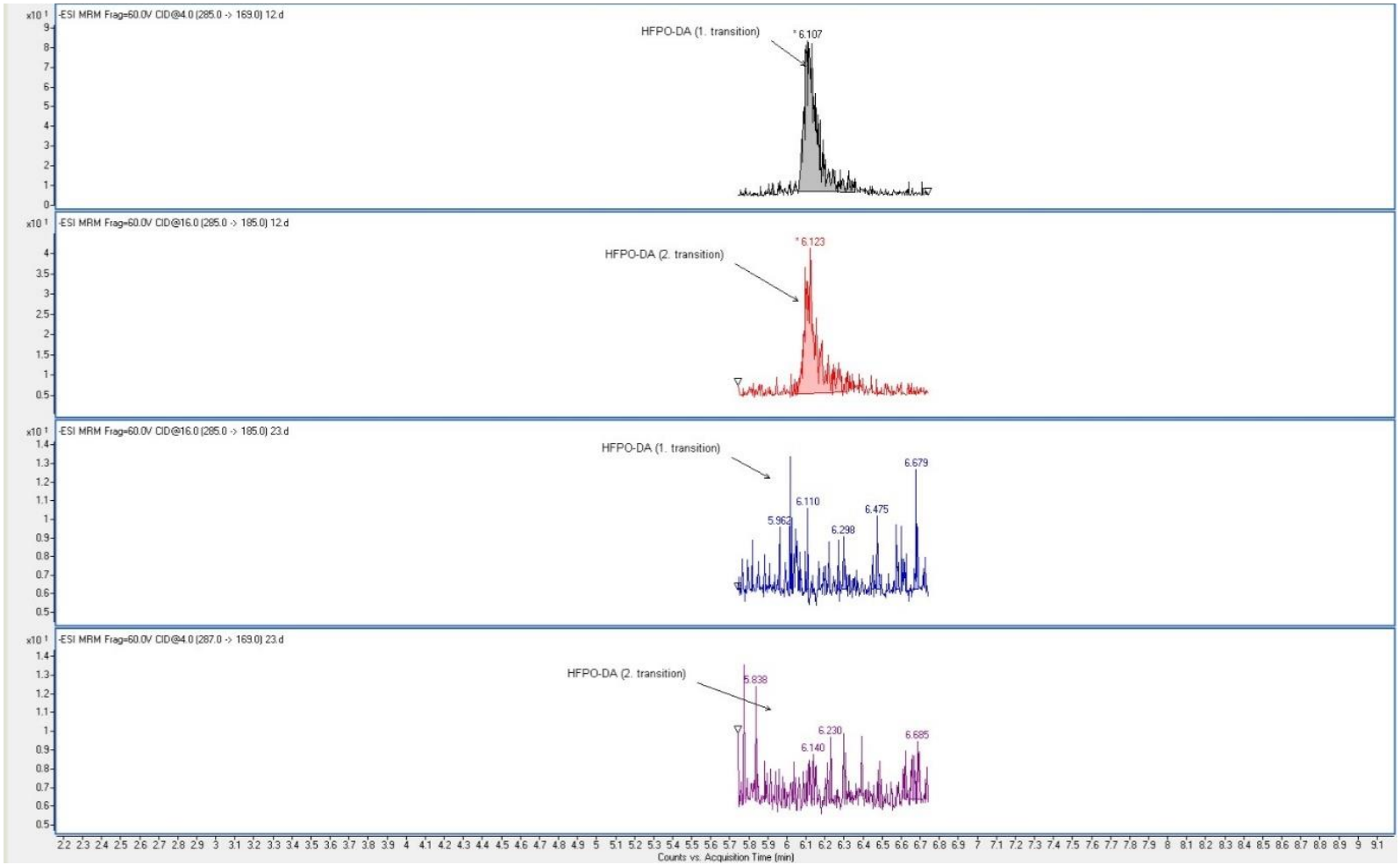


Figure 2. Product ions chromatogram for HFPO-DA(Gen-X)

Table 7 shows that PFHpA was identified in KFC Hamburger wrapper. The PFHpA analyte was identified in the KFC Hamburger wrapper sample by meeting the required requirements. The signal-to-noise (S/N) ratio for both mass transitions was > 3, suggesting that there was enough signal intensity for reliable identification. Furthermore, the retention time (tR) for product ions 1 and 2 were found to be 7.015 and 7.008, respectively, matching the criterion. Furthermore, the retention time of the analyte in the sample was exactly the same as that of the standard, both measured at 7.0, meeting another identification requirement. There was no signal in the blank.

The HFPO-DA detected in the stone-baked Pizza box sample and the PFHxA found in the KFC Hamburger wrapper sample both met all of the predetermined identification criteria. Firstly, the retention times for the product ions of both mass transitions were consistent; HFPO-DA had a retention time of 6.1 ms in the stone-baked Pizza box sample, while PFHxA had a retention time of 5.6 ms for both mass transitions in the KFC Hamburger wrapper sample as seen in table 7. Notably, there was no difference in retention time between the analyte in the sample and the analyte in the standard for either HFPO-DA or PFHxA, indicating accurate identification. These results show that both HFPO-DA in the stone-baked pizza box and PFHxA in the KFC Hamburger wrapper samples met the identification criteria.

Table 7. Criteria for identification of the analytes

Positively identified Analyte (Sample)	**Crireria 1. S/N of the two mass transitions is >3**	**Criteria 2. $t_{Rproduct1}$ and $t_{Rproduct2}$**	**Criteria 3. tRanalyte in sample and tRanalyte in standard.**	**Criteria 4. Ionic ratio in the sample within ±30% of the standard value**	**Criteria 5. Solvent blank signal absent**

PFHpA in KFC Hamburger wrapper	OK	7.0 and 7.0	7.0 and 7.0	OK	OK
HFPO-DA in stone baked Pizza box	OK	6.1 and 6.1	6.1 and 6.1	OK	OK
PFHxA in KFC Hamburger wrapper	OK	5.6 and 5.6	5.6 and 5.6	OK	OK

The figure below shows the positive identification of PFHxA in sample 1's extract, namely, KFC Hamburger wrapper. The peaks of the two mass transitions of the analyte have the same retention time, and they both display a signal-to-noise ratio greater than 3. The next two chromatograms show a positive identification for and PFHpA also in the KFC Hamburger wrapper. The last four chromatograms show the solvent blank. There are no identifiable peaks in their mass transitions.

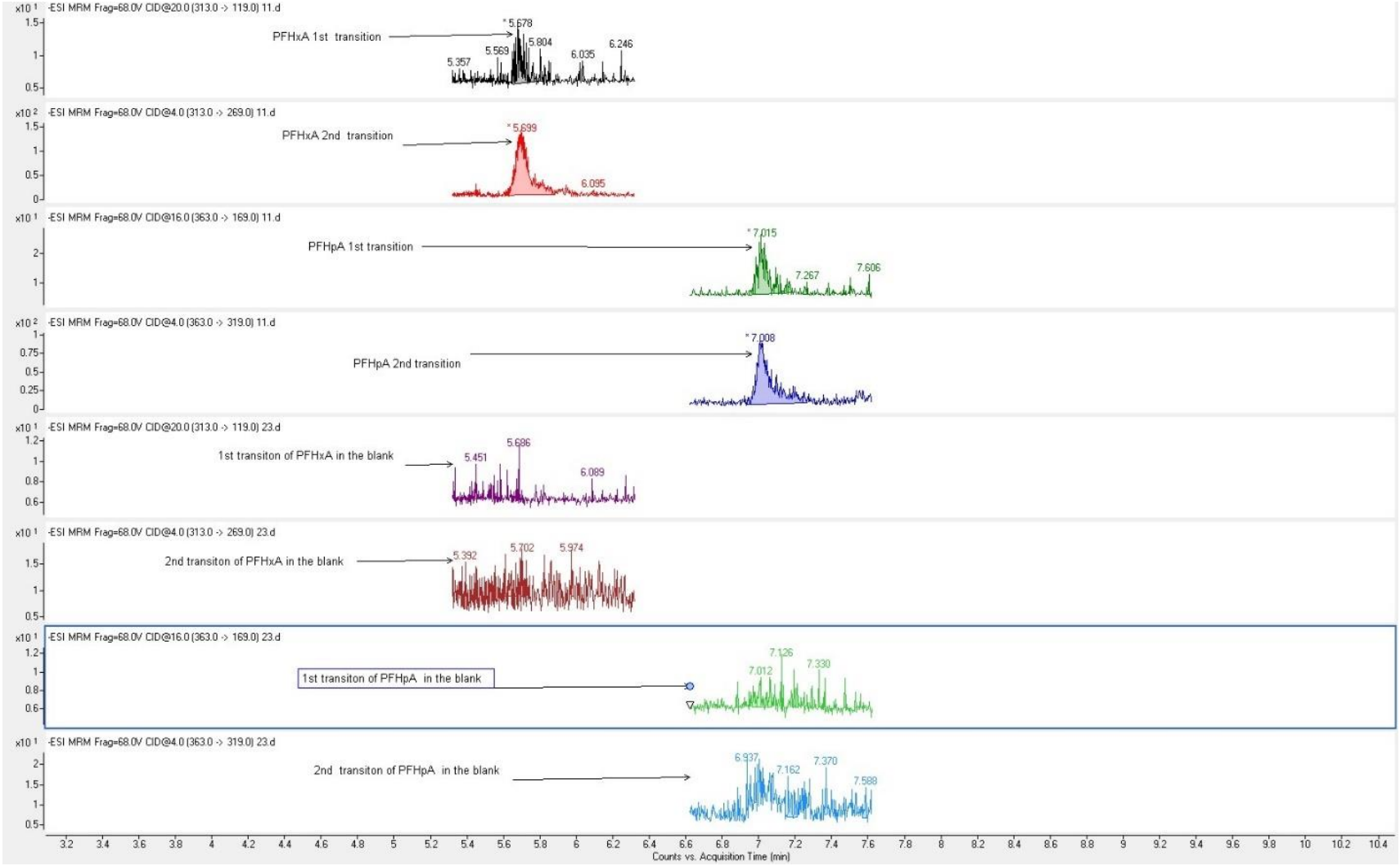


Figure 2. Product ion Chromatogram for PFHxA and PFHpA

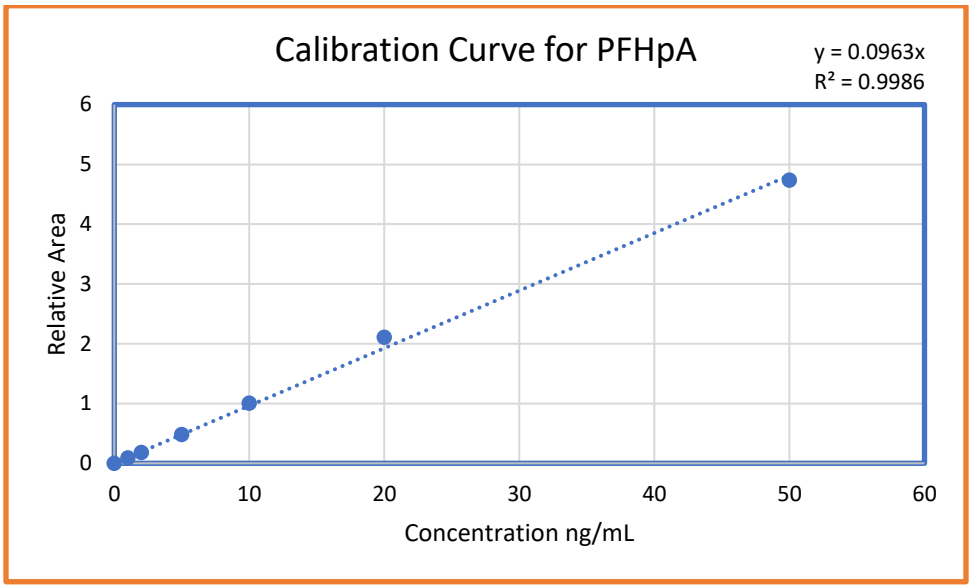
The physical and chemical properties of PFHxA, HFPO-DA, and PFHpA made them detectable in the 3 food contact materials (FCMs) using UHPLC-ESI-MS/MS because they have favourable ionization efficiency under electrospray ionization (ESI), allowing for their sensitive detection by mass spectrometry techniques like UHPLC-ESI-MS/MS. PFHxA, HFPO-DA, and PFHpA are often monitored and regulated by regulatory bodies because to their potential health and environmental consequences, resulting in their inclusion in analytical methodologies for PFAS analysis in FCMs. They are also relatively stable under the chromatographic conditions used in UHPLC-ESI-MS/MS analysis, which ensured precise measurement with no major degradation.

Only the three forms of PFAS were discovered in the 12 distinct food contact materials (FCM) tests, which might be attributed to many factors. It's likely that the FCM samples had just a small number of PFAS chemicals. PFAS may have entered FCMs as additives or as impurities from production processes. If just a few PFAS compounds were utilized in the manufacturing of these products, or if contamination sources were restricted, only a few PFAS kinds may be detected. FCM samples may include additives, coatings, or pollutants that might have interfered with the measurement of the other PFAS compounds. The 12 analysed samples did not contain the two most common PFCs (PFOS and PFOA), which are commonly found in biological and environmental matrices such as food, biological fluids, water, and air. This contrasts with previous studies on food packaging materials, which found significant amounts of PFOA and PFOS (Miralles et al., 2023).

7.2. PFAS Quantification

Several parameters are taken into consideration during quantifying PFAS in food contact materials (FCMs) using UHPLC-ESI-MS/MS analysis. Before analysis, isotopically labeled internal standards were added to the sample. These internal standards contained chemical characteristics identical to the target analytes, but their masses differed. They aided in compensating for differences in sample preparation and instrument response, therefore enhanced quantification accuracy. A calibration curve was created by employing standard solutions with

known PFAS component concentrations. The curve established a linear link between the analyte concentration and the relative area. One of the calibration curves created is as shown below:



The peak area of the analyte PFHpA obtained after analysis was divided by the peak area of PFOA (13C8) which is the corresponding isotopically labeled internal standard. This value was then substituted in the equation of the calibration curve y=mx+b. Since b=0, x which is the concentration we are looking for is obtained by x= y/b. Y is the Relative area and b is the slope of the line.

The table below shows the concentration of the identified PFAS compounds and the concentration of the surrogate standards that were added before extraction to test for recovery.

Table 8. Concentration of the surrogates and detected PFAS compounds in the analytical sample (the liquid in vial where the injection happened)

Concentration of Surrogates and Detected PFAS Compounds in the Vial (ng/mL)							
	Concentration of SUR (ng/mL)				**Concentration of positively identified analytes (ng/mL)**		
Sample	N-EtFOSAA (D5)	PFDA (13C9)	PFHxA (13C6)	HFPO-DA(13C13)	PFHxA	HFPO-DA	PFHpA
1	17.87	4.68	4.13	4.05	0.071	<DL	0.15
2	69.84	17.29	15.16	15.84	<DL	2.24	<DL
3	16.49	3.87	3.31	3.51	<DL	<DL	<DL
4	30.36	10.18	15.54	17.34	0.78	<DL	<DL

5	36.44	8.82	7.15	7.13	<DL	<DL	<DL
6	80.78	20.17	16.86	17.07	<DL	<DL	<DL
7	30.05	8.09	6.61	7.98	<DL	<DL	<DL
8	69.44	17.54	14.64	17.79	<DL	<DL	<DL
9	29.70	8.00	6.68	7.15	<DL	<DL	<DL
10	69.25	18.90	18.36	18.67	<DL	<DL	<DL
11	63.84	19.58	17.25	18.16	<DL	<DL	<DL
12	26.35	16.41	14.99	4.88	<DL	<DL	<DL

The amounts of PFAS compounds varied noticeably between samples. Samples that had the highest concentration of the surrogate standards recovery could be due to optimal extraction.

The concentration of the PFAS detected in the samples was calculated with sample dilution to obtain the concentration in the original FCM.

Table 9. Concentration of Analyte in Original FCM

	Concentration in original FCM (ng/g)		
	HFPO-DA	**PFHxA**	**PFHpA**
1	<DL	0.07	0.78
2	2.24	<DL	<DL
4	<DL	0.78	<DL

The results in table 9 show show the concentrations of PFAS compounds detected in the 3 FCMs. Notably, the concentration of HFPO-DA in sample 2 (Stone baked Pizza Box) was found to be 2.24 ng/g, while PFHxA concentrations were 0.07 ng/g in sample 1 (KFC Hamburger wrapper) and 0.78 ng/g in sample 4 (Take away box). PFHpA was also measured at a concentration of 0.78 ng/g in sample 1.

In the context of PFAS analysis in food contact materials, surrogate recovery is critical for ensuring the validity and reliability of the analytical results, as well as providing insights into the performance of the analytical method and potential factors influencing the accuracy of PFAS quantification. The figure below shows the percentage recovery of the surrogate.

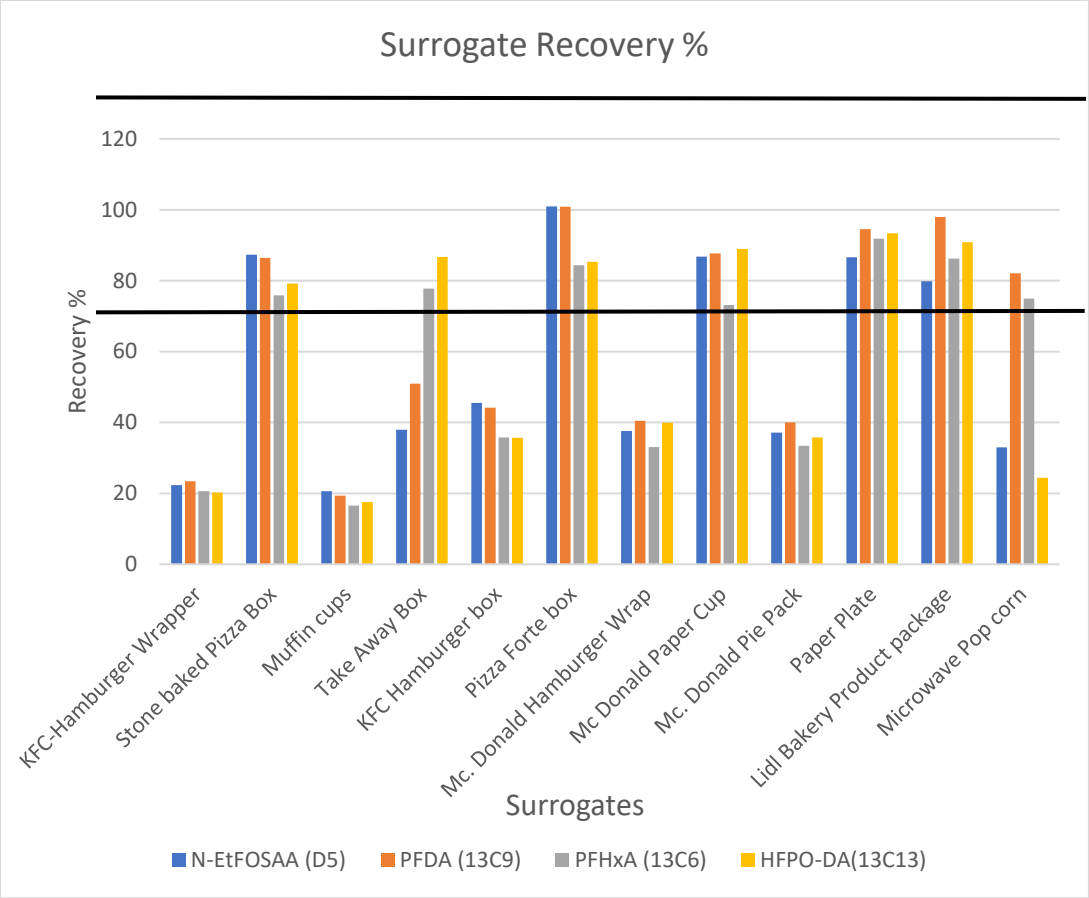


Figure 3. Surrogate recovery percentage

The surrogate recovery percentages fluctuate between samples, suggesting that the FCMs studied may have variable matrix compositions or extraction efficiency. The percentage recovery should range from 70-130% however from the data above half the samples are below that threshold whereas the other half meets the threshold.

Samples 1, 3, 5, 7 and 9 demonstrate significantly low surrogate recovery percentages across all surrogate chemicals, indicating potential sample preparation issues for these specific FCMs. This might be owing to inadequate extraction of the surrogate molecules from the matrix or interference by co-extracted substances. To assess the method's overall performance, while some samples show adequate surrogate recovery percentages in the 70-130% range, some have lower or greater recoveries, suggesting possible concerns that must be addressed to guarantee accurate and reliable findings.

8. CONCLUSION

The study identified and quantified three PFAS compounds: PFHpA, HFPO-DA (Gen-X), and PFHxA. These PFAS were successfully detected in three of the twelve analyzed samples, namely samples 1, 2, and 4. However, it is worth noting that the recovery in sample 1 was deemed inadequate, thereby affecting the reliability of the results obtained for this specific sample. In contrast, the results for samples 2 and 4 demonstrated acceptable recovery rates, meeting the established criteria. Overall, positive PFAS results were observed in samples 2 and 4, indicating the presence of these compounds in the analyzed food contact materials. 2.24 ng/g of HFPO-DA (Gen-X) was determined in sample 2. PFHxA was determined in sample 4 in a concentration of 0.78ng/g.

These findings highlight the significance of improving analytical procedures to enable consistent and accurate PFAS detection in FCMs. Lastly, this research adds to our understanding of PFAS contamination in food packaging materials and emphasizes the need for ongoing efforts to develop effective analytical techniques and regulatory measures to ensure the safety and sustainability of food packaging materials in alignment with global food waste reduction goals.

9. SUMMARY

The United Nations has set a target of reducing food waste by 50% by 2030, emphasizing the importance of advancements in food packaging methods to achieve this goal. Food packaging is critical for protecting food products from contamination and physical damage during the various stages of handling, storage, and transportation. While packaging technology has advanced significantly over time, the industry continues to innovate to improve safety, functionality, sustainability, and environmental impact.

Paper and plastic are the most common materials used in commercial food service packaging, with paper additives frequently used to increase moisture and grease resistance. Per- and polyfluoroalkyl substances (PFAS) are popular additives in paper-based products for this purpose. Although the global production and application of PFAS in paper-based packaging are lower than plastic additives like bisphenol A (BPA), concerns about their environmental persistence, mobility, and health implications have led to an increasing resistance to their use.

PFAS compounds can enter the food chain via contaminated food or food contact materials (FCMs), raising serious public health concerns. Analyzing the content of various FCMs and evaluating their migration under typical usage and storage conditions is critical to understanding and addressing this issue. Previous research has found PFAS compounds in food packaging materials, albeit at lower levels, indicating progress in regulation and manufacturing practices.

Given this backdrop, it is critical to assess PFAS contamination in various packaging materials used in different markets. This study used ultrasound-assisted extraction and Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) with isotopically labeled standards to detect and quantify PFAS in twelve food contact materials collected from retail vendors in Hungary.

The goals of this investigation were to identify the presence of PFAS compounds in the collected materials and quantify any detected PFAS. The study used a previously established and verified procedure for PFAS analysis.

The analysis involved PFAS extraction from the samples, followed by UHPLC-MS/MS analysis using electrospray ionization (ESI) in negative mode. Multiple reaction monitoring (MRM) was used to track two mass transitions for each analyte, with identification criteria that include signal-to-noise ratio, retention time consistency, and ionic ratio matching.

Despite methodological challenges, PFAS compounds such as HFPO-DA, PFHpA, and PFHxA were successfully identified in several samples. However, inadequate recovery in some samples emphasized the importance of method optimization and validation. Overall, the study adds to our understanding of PFAS contamination in FCMs and emphasizes the importance of ongoing efforts to ensure food packaging safety and sustainability while aligning with global food waste reduction goals.

10. Acknowledgement

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Title of the document:	Determination of Per-and Polyfluoroalkyl Substances (PFAS) in Food Contact Materials in Hungary
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Internal supervisor

1Please underline applicable

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I, the undersigned George Nyangwara Ongeri (Neptune code: P9NERV) student at MSc in food safety and Quality Engineering programme, request that my thesis / diploma thesis titled "Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food Contact Materials in Hungary" (Dr. Sörös Csilla Marczika Andråsné) be encrypted by applying point c) of Section 95 (5) of the Study and Examination Regulations of the Hungarian University of Agriculture and Life Sciences (hereinafter referred to as 'SER'). I understand that if my request is approved, the encryption of the thesis / diploma thesis will cover 5 years following the successful defence, in accordance with point c) of Section 95 (5) of SER.

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Reasons for rejection:

[1] The additional request of the business partner is not obligatory, if it is not requested, it can be deleted from the form.

[2] Chose and keep the appropriate one, delete the other.

Done at: _____

Programme leader's signature