Packed Column Supercritical Fluid Chromatography: Applications in Environmental Chemistry
Dedication
To Steve
NICOLE RIDDELL

Packed Column Supercritical Fluid Chromatography: Applications in Environmental Chemistry
Abstract

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Although gas and liquid chromatography have emerged as dominant separation techniques in environmental analytical chemistry, these methods do not allow for the concurrent analysis of chemically diverse groups of persistent organic pollutants (POPs). There are also a small number of compounds which are not easily amenable to either of these traditional separation techniques. The main objective of this thesis was to address these issues by demonstrating the applicability of packed column supercritical fluid chromatography (pSFC) coupled to mass spectrometry (MS) in various aspects of environmental chemistry.

First, pSFC/MS analytical methods were developed for legacy POPs (PCDDs, PCDFs, and PCBs) as well as the emerging environmental contaminant Dechlorane Plus (DP), and issues relating to the ionization of target analytes when pSFC was coupled to MS were explored. Novel APPI and APCI reagents (fluorobenzene and triethylamine) were optimized and real samples (water and soil) were analyzed to demonstrate environmental applicability.

The possibility of chiral and preparative scale pSFC separations was then demonstrated through the isolation and characterization of thermally labile hexabromocyclododecane (HBCDD) stereoisomers. The analytical pSFC separation of the α-, β-, and γ-HBCDD enantiomers as well as the δ and ε meso forms was shown to be superior to results obtained using a published LC method.

Finally, technical mixtures of phosphorus flame retardants (RBDPP, BPA-BDPP, and DOPO; a group of related compounds which are challenging to analyze concurrently) were examined using multiple analytical techniques and pSFC was found to be the only method which facilitated the accurate determination of the components of all 3 mixtures.

This thesis confirms the potential of pSFC/MS as a fast, green, and cost effective means of separating and analyzing environmental contaminants.

Keywords: Supercritical Fluid Chromatography; Mass Spectrometry; Ionization; POPs; APPI; APCI; chiral; achiral; packed column.

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List of Papers

This thesis is based on the following papers, which hereafter will be referred to by their Roman numerals.


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Abbreviations

\( \alpha \) selectivity factor
\( \gamma \) obstructive factor
\( \sigma \) standard deviation
\( \sigma^2 \) variance
\( \% \text{RSD} \) percent relative standard deviation
\( ^1 \text{H} \) proton
\( ^{31} \text{P} \) phosphorus
ABPR automatic back-pressure regulator
APCI atmospheric pressure chemical ionization
API atmospheric pressure ionization
APPI atmospheric pressure photoionization
BFR brominated flame retardant
BPA-BDPP Bisphenol A bis(diphenyl phosphate)
CO\(_2\) carbon dioxide
CP critical point
cSFC capillary column supercritical fluid chromatography
CSP chiral stationary phase
DDT dichlorodiphenyltrichloroethane
\( D_M \) mobile phase diffusion coefficient
DOPO 9,10-Dihydro-9-oxa-10-phosphaphenanthrene-10-oxide
DP Dechlorane Plus
\( D_S \) solute diffusion coefficient
ECNCl electron capture negative chemical ionization
EFSA European Food Safety Authority
EI electron ionization
ESI electrospray ionization
FR flame retardant
GC gas chromatography
H plate height
HBCDD 1,2,5,6,9,10-hexabromocyclododecane
HCB hexachlorobenzene
HCH hexachlorocyclohexane
HFFR halogen-free flame retardant
HRGC high resolution gas chromatography
HRMS high resolution mass spectrometry
HSS  high strength silica
$k_1'$ and $k_2'$  retention factors of the two solutes
L  length of the column
LC  liquid chromatography
LSER  linear solvation energy relationship
MRM  multiple reaction monitoring
MS  mass spectrometry
MS/MS  tandem mass spectrometry
m/z  mass-to-charge ratio
N  theoretical plate count
NMR  nuclear magnetic resonance spectroscopy
NO$_2$  nitrogen dioxide
ODS  octadecylsilane
OH-PBDE  hydroxylated polybrominated diphenyl ether
OPFR  organophosphorus flame retardant
PAH  polycyclic aromatic hydrocarbon
PBDE  polybrominated diphenyl ether
$P_c$  critical pressure
PCB  polychlorinated biphenyl
PCDD  polychlorinated dibenzo-$p$-dioxin
PCDF  polychlorinated dibenzofuran
PDA  photodiode array
PFOS  perfluorooctanesulfonic acid
PFOSF  perfluorooctanesulfonyl fluoride
PGC  porous graphitic carbon
PI  photoinitiator
POP  persistent organic pollutant
PREG  polar retention effect on graphite
prep-TLC  preparatory thin-layer chromatography
pSFC  packed column supercritical fluid chromatography
$R^2$  linear regression
RBDPP  Resorcinol bis(diphenyl phosphate)
$R_s$  chromatographic resolution
SF  supercritical fluid
SP  solvation parameter
TBBPA  tetrabromobisphenol-A
$T_c$  critical temperature
TEA  triethylamine
Term A  multipath term
Term B/ $u$ longitudinal diffusion
Term C resistance to mass-transfer
TP triple point
UV ultraviolet

Abbreviations for individual PCDDs and PCDFs are provided in Table 3. IUPAC numbers for individual PCBs are provided in Table 4.
# Table of Contents

1.0 INTRODUCTION ............................................................................................................. 15  
1.1 Supercritical Fluids ..................................................................................................... 15  
1.2 Development of Supercritical Fluid Chromatography .............................................. 16  
  1.2.1 History .................................................................................................................. 19  
1.3 Theory ......................................................................................................................... 20  
1.4 Retention Behaviour .................................................................................................. 30  
1.5 Effects of Column Coupling in pSFC ....................................................................... 33  
1.6 Proposed Retention Mechanisms ............................................................................. 34  
  1.6.1 Retention Models ............................................................................................... 35  
2.0 SUPERCRITICAL FLUID CHROMATOGRAPHY OF POPs ..................................... 37  
  2.1 Applications of pSFC in Environmental Chemistry .................................................. 37  
    2.1.1 Polychlorinated Biphenyls .................................................................................. 38  
    2.1.2 Polycyclic Aromatic Hydrocarbons ................................................................... 39  
    2.1.3 Halogenated Flame Retardants ......................................................................... 39  
    2.1.4 Perfluoroalkyl Substances .................................................................................. 41  
    2.1.5 Pesticides ......................................................................................................... 42  
    2.1.6 Emerging Contaminants .................................................................................... 43  
  2.2 Coupling pSFC with Mass Spectrometry .................................................................. 44  
3.0 AIM OF THE THESIS ................................................................................................. 47  
4.0 ANALYSIS OF ENVIRONMENTAL CONTAMINANTS OF CONCERN .......... 49  
  4.1 Analysis of Legacy Persistent Organic Pollutants ...................................................... 49  
    4.1.1 pSFC/MS/MS Analysis of PCDDs, PCDFs, and PCBs ....................................... 52  
    4.1.2 Optimization of the Atmospheric Pressure Ionization of PCDDs, PCDFs, and PCBs ................................................................. 56  
  4.2 Analysis of Emerging Environmental Contaminants ............................................... 58  
    4.2.1 Achiral pSFC/MS Analysis of the Polychlorinated Flame Retardant Dechlorane Plus ................................................................. 59  
    4.2.2 Chiral pSFC/MS Analysis of the Polybrominated Flame Retardant HBCDD ................................................................................... 61  
    4.2.3 pSFC/MS Analysis of Halogen-Free Phosphorus Flame Retardants ............. 64  
5.0 CONCLUSIONS AND FUTURE WORK ..................................................................... 68  
6.0 ACKNOWLEDGEMENTS ........................................................................................... 70  
7.0 REFERENCES ............................................................................................................ 72
1.0 Introduction

Supercritical fluids were first defined by Andrews in 1869 (Smith, 1999), but over the past 40 years their use for extraction of analytes from complex matrices and in separation chemistry has experienced steadily increasing interest. In terms of chromatography, applications for packed column supercritical fluid chromatography (pSFC) using carbon dioxide (CO$_2$) as the mobile phase have been extensively investigated and developed for the separation of pharmaceuticals and related compounds. Although the potential for expansion into the field of environmental analytical chemistry exists, as yet it has been largely unexplored. Suitable separation techniques already exist for most environmental contaminants of concern, but the development and application of pSFC methods could expand the breadth of analytical techniques available to laboratory researchers and also increase the range of target analytes. In addition, the coupling of pSFC to suitable methods of ionization and detection could provide a means of analyzing compounds that are not amenable to commonly used separation technologies such as liquid chromatography (LC) and/or gas chromatography (GC) and possibly facilitate the concurrent analysis of compounds which must currently be analyzed separately using these traditional techniques. With these objectives in mind, the application of pSFC coupled with mass spectrometry (MS) for the analysis of environmentally relevant contaminants will be investigated. The unique properties of supercritical fluids and the potential application range of this technology could result in fast, affordable screening methods encompassing many different groups of environmental contaminants. However, before this can be accomplished the development of suitable chromatographic methods must be undertaken and an understanding of how supercritical fluids may impact analyte separation and ionization attained.

1.1 Supercritical Fluids

For chromatographic applications such as pSFC, the selection of a supercritical fluid (SF) for use as a mobile phase is an important aspect of the separation that involves many considerations. A mobile phase must be used above its critical temperature ($T_c$) and critical pressure ($P_c$) in order to be classified as supercritical. If either parameter exceeds its critical value, a phase change is not observed when the other parameter passes through its critical value (Chester and Pinkston, 2004). At the critical temperature of a substance, the vapour and liquid phases have identical densities and a
distinction between the liquid and gas phases ceases to exist (Chester and Pinkston, 2004, White and Houck, 1986). The availability of instrumentation that can maintain a substance in its supercritical state can often limit mobile phase selections. For a compound to exist in its supercritical state, the column outlet must be maintained at a pressure above its critical pressure. This is accomplished using a back-pressure regulator located after the column (Lesellier, 2009). The following SFs have been investigated as potential pSFC mobile phases: hydrocarbons (e.g. hexane, pentane, and butane), nitrous oxide (capable of oxidizing organic substances), ammonia gas (dissolves silica based materials), haloalkanes (environmentally persistent, ozone depleting, and expensive), water (chemically aggressive), and carbon dioxide (inexpensive, highly available, and relatively inert) (Smith, 1999). Carbon dioxide has become most widely utilized SF for pSFC because of its achievable critical parameters, inertness, and low toxicity.

Table 1: Critical parameters [temperatures (°C) and pressures (atm)] for substances investigated for pSFC mobile phases.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Critical Temperature (°C)</th>
<th>Critical Pressure (atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>234.8*</td>
<td>29.6*</td>
</tr>
<tr>
<td>Butane</td>
<td>152.8*, 152.0‡</td>
<td>36*, 37.5‡</td>
</tr>
<tr>
<td>Propane</td>
<td>96.8*</td>
<td>42.0*</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>36.5‡</td>
<td>72.5‡</td>
</tr>
<tr>
<td>Ammonia</td>
<td>132.5‡</td>
<td>112.5‡</td>
</tr>
<tr>
<td>CHF3</td>
<td>25.9‡</td>
<td>46.9‡</td>
</tr>
<tr>
<td>Water</td>
<td>374.0‡</td>
<td>227.0‡</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>31.3‡</td>
<td>72.9‡</td>
</tr>
</tbody>
</table>

* denotes reference (Stull, 1947) and ‡ denotes reference (Janda et al., 1993)

1.2 Development of Supercritical Fluid Chromatography
Open tubular, or capillary column supercritical fluid chromatography (cSFC), was initially limited to non-polar analytes because when carbon dioxide was employed as a mobile phase it exhibited poor solvating power (similar to that of short chain aliphatic hydrocarbons) (Taylor, 2010, Wu, 2004). However, many substances that contain single polar functional groups were found to be surprisingly soluble in supercritical CO2 (e.g. formic acid, acetic acid, aniline, phenol, etc.) (Berger, 1997). The use of
packed columns and mobile phases modified with organic solvents and additives greatly expanded the range of target analytes that could be separated using pSFC (Tarafder, 2016). The introduction of polar stationary phases and their routine employment in pSFC for the separation of pharmaceuticals also expanded the applicability of this technique. A variety of stationary phases covering a wide polarity range are now available facilitating the application of pSFC in new areas/fields (Taylor, 2010). This is partially due to the fact that many columns designed for liquid chromatography systems can also be used in conjunction with pSFC instrumentation if they are mechanically able to withstand the back-pressure associated with the system.

Supercritical fluids are highly compressible and their densities can be manipulated over a wide range by adjusting the pressure. This equates to variable solvating power and selectivity that is tunable to the requirements of specific analytes (Pourmortazavi et al., 2014, White and Houck, 1986). However, when modifiers are added, the mobile phase composition actually becomes more important than the pressure or density of the carbon dioxide in determining the retention of analytes (Taylor, 2009). The eluting strength of a solvent modified CO2 mobile phase in pSFC is dependent on the stationary phase utilized; however, in general, the effects of pressure and temperature on eluting strength when employing a binary solvent system are significantly less compared to the effects observed with pure CO2 (Lesellier and West, 2015). Fornstedt et al. recently utilized a chemometric approach to demonstrate that, in pSFC, the cosolvent fraction and pressure parameters had the greatest impact on analyte retention factors while the cosolvent fraction and column temperature parameters had greater impact on analyte selectivity (Asberg et al., 2014, Fornstedt and Majors, 2015).

One of the most interesting aspects of supercritical fluid chromatography is the fact that the supercritical fluid acts as both a substance carrier, similar to mobile phases used in gas chromatography, and can also dissolve the analytes being chromatographed like the solvents in liquid chromatography (Taylor, 2009). This unique behaviour results in dramatic improvements in chromatography through alteration of the mobile phase by either variation of the physical state of the fluid or by adding organic modifiers and additives (Wu, 2004). For instance, it has been found that the separation and peak shape of very polar and/or ionic compounds can be improved if additives capable of ion pairing are added to the cosolvent. As an example, ammonium acetate has been used as an ion
pairing agent in pSFC of ionic analytes. It was noted that neutral compounds which exhibited satisfactory chromatography in the absence of ammonium acetate were virtually unaffected by its presence, but polar or ionic compounds, which displayed significant peak tailing and late elution when only methanol was used as the cosolvent, showed improved chromatographic behaviour in the presence of an additive (Taylor, 2010).

The use of a solvent modified carbon dioxide mobile phase in pSFC results in a mobile phase with a low fluid viscosity compared to LC which dramatically reduces the pressure drop observed when packed columns are employed. A typical inlet pressure in pSFC is 170 bar (2465 psi) and pressure drops of only 20 bar (290 psi) are commonly observed (Lesellier, 2009). An increase in flow-rate or column length results in minimal back-pressure changes which allows for a high number of theoretical plates to be achieved if long columns are utilized (Lesellier, 2009).

Some advantages of pSFC compared to traditional LC for the separation of analytes include:

- The lower viscosity and higher diffusivity of supercritical mobile phases relative to liquids leads to improved chromatographic peak shape and resolution, faster run times, and higher throughput.
- Carbon dioxide is an inert, environmentally friendly, mobile phase that has a low associated cost and high availability.
- The use of longer and/or coupled columns with the same or orthogonal stationary phases is possible.
- The conditions can be tuned so that the selectivity matches that of reversed phase LC (Taylor, 2009).
- Mobile phase density in SFC has been shown to have influence in shape selectivity (Chester and Pinkston, 2004).

The primary disadvantage associated with pSFC is the complex behaviour of SFs during chromatographic separations when the operation conditions (e.g. temperature, pressure, and composition) are varied (Pourmortazavi et al., 2014). However, the relatively low viscosity associated with SFs and a diffusivity midway between that of a gas and a liquid makes this technique complementary to both GC and LC (Gere et al., 1982) and potentially applicable to a wider range of compounds.
1.2.1 History

The critical phenomenon was first observed by French physicist Baron Charles Cagniard de la Tour in 1822 (Perrut, 1994) and supercritical fluids were later defined by Thomas Andrews in 1869, but the idea of using supercritical fluids for chromatographic separations wasn’t noted until 1958 by James Lovelock (Smith, 1999). Klesper et al. was the first group to successfully demonstrate the effectiveness of pSFC in 1962 when they described the separation of thermo-labile porphyrin derivatives using supercritical chlorofluoromethanes (Taylor, 2009, Klesper et al., 1962). Unfortunately, pSFC was plagued with problems associated with back-pressure regulation, inconsistent flow-rates, sample injection, automation, and a lack of stationary phases. Interest in pSFC increased in the early 1980s when Hewlett-Packard introduced instrumentation specifically designed for this purpose and subsequent studies by Berger and Gere focused on the development and expansion of pSFC technology and applications. However, it wasn’t until the early 1990s when the focus of pSFC shifted to pharmaceuticals and agrochemicals that its popularity increased (Taylor, 2009).

One of the most significant contributions to the field of pSFC made by Berger and his research team was the observation that there are differences between supercritical fluids, dense gases, and liquids, but they are not as dramatic as one might assume. In fact, Berger noted that the solvent characteristics of the fluids used in pSFC are not unique to the fluid in its supercritical state. The characteristics are present whether the fluid is technically defined as a liquid, a dense gas, or a supercritical fluid; that is, they are consistent under both super- and sub-critical conditions (Taylor, 2009). Other significant outcomes of Berger’s research include:

- Demonstrating that the use of very long columns with large pressure drops was feasible.
- Deconvoluting the relationship between supercritical fluid density and solvent strength.
- Introducing the use of mobile phase additives and studying their effects on analyte retention and peak shape.
- Demonstrating that pSFC was broadly applicable to pharmaceuticals and small drug-like compounds.
- Developing a gas-liquid separation technology which facilitated the quantitative recovery of analytes during preparative work (Taylor, 2009).
1.3 Theory

Giddings was the first separations scientist to introduce the concept of unified chromatography and suggest that all modes of chromatography could be dealt with using a single unified theory. This idea was critical for the adaptation of the van Deemter equation for application to all modes of chromatography (Silva et al., 2015). Martire later developed the unified theory of adsorption chromatography in which the mobile phase is described as an ideal gas, a moderately non-ideal gas, a supercritical fluid, or a liquid (Chester and Pinkston, 1990, Martire, 1987). This theory illustrated that the temperature, pressure, and composition of the mobile phase can be adjusted and set to values to achieve separations not possible at ambient temperature or pressure (Chester and Pinkston, 2004). Arguably, the most important concept that the use of supercritical fluids in separation science has brought forth is the recognition that there is unity in all separations methods (GC, LC, and pSFC) and that a continuum exists from gases to liquids (Smith, 1999, Wu, 2004). A supercritical fluid is simply defined as an element or compound above its critical pressure and critical temperature. For any pure compound, there is a transition known as the critical point (CP; Figure 1) where for temperatures below the T_c or pressures below the P_c, two phases (liquid and vapour) exist, however when temperatures exceed T_c or pressures are above P_c, only one phase exists (Perrut, 1994). Similarly, at the triple point (TP), the three phases of the compound (solid, liquid, and gas) coexist in thermodynamic equilibrium (Saito, 2013). Above the critical point, low pressures and/or high temperatures result in a supercritical fluid having low density and behaving similar to a highly viscous gas. Since the solvation capacity is low and the diffusion rates are lower than that of a gas, the separation process needs to be decelerated to maintain efficiency. As the pressure is raised (or the temperature is reduced), the fluid becomes denser and behaves more like a liquid. The solvation strength increases, but the diffusion rate decreases. It is important to note that conditions do not exist where a supercritical fluid can have the solvation strength of a liquid and the high diffusion rate of a gas at the same time (Smith, 1999). In the region around the critical point, the properties of the fluid change dramatically with temperature and pressure variations. Typically, more robust methods utilize higher back-pressures in order to achieve increased analyte solubility. An advantage associated with using higher back-pressures is the lower influence of pressure changes on the properties of the eluent. In fact, it has been demonstrated that there is usually no significant change in properties
(or boundary effect) when going from super- to sub-critical temperature conditions at moderate or high pressures (Smith, 1999, Lauer et al., 1983). Contrary to LC, as the temperature is raised in pSFC (at constant pressure) the retention time initially increases due to a reduction in eluent density. It has been reported that extreme temperature increases can overcome this effect and result in a decrease in retention as the volatility of the analyte increases and comes into play (Smith, 1999).

An empirical correlation between the solubility of a solute in a supercritical fluid and the temperature and pressure applied to the system was proposed by Chrastil that has proven to be relatively reliable. This equation (Equation 1) predicts that the solubility of a solute will increase with increasing density (or pressure) at constant temperature and that solubility may increase or decrease when the temperature is increased at constant pressure (Perrut, 1994, Chrastil, 1982).

Figure 1: A generalized phase diagram illustrating physical states at varying temperatures and pressures.
Equation 1

\[ C = \rho^a \exp\left(\frac{a}{T} + b\right) \]

where:
- \( C \) is the solubility of the solute in the supercritical fluid
- \( a, b, \) and \( \alpha \) are empirical constants
- \( \rho \) is density (pressure)
- \( T \) is temperature

It has been shown experimentally that highly compressed supercritical fluids are able to dissolve large amounts of low-volatility substances (Vanwasen et al., 1980). Cluster theory was proposed by Kajimoto as a method of explaining the unpredicted solvating power of supercritical fluids (Morita and Kajimoto, 1990). Kajimoto described the behaviour of molecules in gas, liquid, and supercritical states using intermolecular potential and average molecular energy. Statistical thermodynamics state that energetically lower states are strongly favoured at lower temperatures. In the liquid state at low temperatures, each molecule experiences an attractive intermolecular potential that keeps them in close proximity to their neighbour since the potential well is larger than the average kinetic energy per molecule. In the gas state at high temperatures, molecules possess large amounts of kinetic energy which allow them to easily overcome attractive intermolecular potentials. In the supercritical fluid region near the critical temperature, some molecules are able to move freely while others are trapped in weak clusters. The kinetic energy associated with each molecule fluctuates and clusters form when the molecular kinetic energy is smaller than the attractive intermolecular potential of adjacent molecules. The clusters are able to change in size and constitution due to molecular collisions. When a solute molecule is introduced into the system, if the solute-solvent attraction is stronger than the solvent-solvent interaction, the solute molecule will be surrounded by the solvent molecules. This cluster formation is believed to be a major cause of enhanced solubility in supercritical fluids (Saito, 2013).

The main difference affecting solute retention behaviour in SFC versus LC and GC lies in the chemical nature of the mobile phase and the resulting thermodynamic differences and physico-chemical interactions that occur between the solute and the mobile phase as well as the solute and the stationary phase (Guiochon and Tarafer, 2011). In order to understand how the properties of a supercritical fluid will affect chromato-
graphic separations, it is necessary to investigate their impact on plate height and column variables as defined by the van Deemter equation (Equation 2).

Equation 2

\[ H = A + \frac{B}{u} + (C_s + C_m)u \]

where:
- \( H \) = the plate height (cm)
- \( u \) = the linear velocity of the mobile phase (cm/s)
- \( A \) = the multipath term
- \( \frac{B}{u} \) = the longitudinal diffusion term
- \( C \) = mass-transfer coefficients \( (C_s + C_m) \)

Since chromatographic peaks are ideally Gaussian in shape, the width of the peak, and thus the efficiency of a column, can be related to the variance \( (\sigma^2) \) or the standard deviation \( (\sigma) \) of a measurement. Plate Height \( (H) \) is a measure of the efficiency of the column and can be defined in terms of the variance per unit length of column: \( H = \sigma^2/L \) (Skoog et al., 1998). Each variable of the van Deemter equation affects plate height and requires individual examination to determine its impact on \( H \) when a supercritical fluid is utilized during packed column chromatography. Ideally, all variables affecting plate height should be minimized.

Term A (described in Equation 3) is known as the multipath term and is often referred to as eddy diffusion. As analytes move through a column which is packed with stationary phase, they may traverse many different paths at random. This can cause broadening of the solute band because separate paths of varying lengths result in variable residence times on the column for molecules of the same species. This effect is directly proportional to the diameter of the particles making up the column packing therefore smaller particles result in a lower contribution of term A to plate height (Skoog et al., 1998).
Equation 3

\[ A = 2\lambda d_p \]

where:
- \( d_p \) is the diameter of the packing material (cm)
- \( \lambda \) is a constant that depends on the quality of the packing

At low linear velocities, band broadening can be offset by ordinary diffusion which results in the transfer of molecules through multiple flow paths and an overall averaging of residence times. At moderate or high velocities, there is not enough time for diffusion averaging to occur (Skoog et al., 1998). Since supercritical CO\(_2\) has a lower viscosity than solvents traditionally utilized in liquid chromatography, columns with smaller particle sizes as well as longer columns can be utilized without observing excessive backpressures resulting in a decrease in the value of this term.

Term \( B/u \) (described in Equation 4) is known as longitudinal diffusion and is a band broadening process in which analytes diffuse from the concentrated center of a solute band to the more dilute regions before and after the zone center (Skoog et al., 1998).

Equation 4

\[ \frac{B}{u} = \frac{2\gamma D_M}{u} \]

where:
- \( D_M \) is the diffusion coefficient in the mobile phase \((\text{cm}^2\text{s}^{-1})\)
- \( u \) is the linear velocity of the mobile phase \((\text{cm}\text{s}^{-1}) = L/t_M \)
- \( \gamma \) is a constant that depends on the quality of the packing.

The constant \( \gamma \) is called the obstructive factor because longitudinal diffusion is hindered by the column packing (for packed columns, the value of this term is \(~0.6\)). Longitudinal diffusion is directly proportional to the mobile phase diffusion coefficient \((D_M)\) which is a constant equal to the rate of migration under a unit concentration gradient. Therefore, this effect is also much less pronounced when diffusion coefficients are low with values generally ordered as: gases > supercritical fluids > liquids. Longitudinal diffusion is inversely proportional to the linear velocity since the residence time of an analyte on the column is shorter when the flow rate is high because diffusion has less time to occur (Skoog et al., 1998).
The lower fluid viscosities of supercritical fluids compensate for higher diffusion coefficient by allowing the use of higher mobile phase flow-rates (Lesellier and West, 2015).

Term C (described in Equation 5) encompasses the resistance to mass-transfer. Since analytes take a certain amount of time to equilibrate between the stationary and mobile phase, if the velocity of the mobile phase is high and the analyte has a strong affinity for the stationary phase, the analyte in the mobile phase will move ahead of the analyte in the stationary phase resulting in a broadening of the solute band. The higher the velocity of mobile phase, the worse the broadening becomes. There are two mass-transfer coefficients because the equilibrium between the mobile phase and stationary phase is established so slowly that a chromatographic separation operates under non-equilibrium conditions. Both coefficients are directly proportional to the linear velocity because the faster the mobile phase moves, the less time there will be for equilibrium to be approached (Skoog et al., 1998). The benefits of utilizing small particles on the mass transfer of analytes observed in LC (through reduced pore distance for analyte diffusion as well as decreased diffusion time) can be extended to super- and sub-critical fluids (Lesellier and West, 2015). Solute diffusion coefficients ($D_S$) are greater in supercritical fluids compared to the liquid phase due to higher analyte diffusivity resulting in narrower chromatographic peaks, the ability to use higher average linear velocities ($u$), and increased speeds of analysis (White and Houck, 1986).
Equation 5

\[(C_s + C_m)u = \left( \frac{f_S(k')d_f^2}{D_S} \right) + \left( \frac{f_M(k')d_p^2}{D_M} \right) u \]

where:
- \(f_S(x)\) is a function of the stationary phase
- \(k'\) is the retention factor
- \(d_f\) is the thickness of the stationary phase (cm)
- \(D_S\) is the diffusion coefficient in the stationary phase (cm² s⁻¹)
- \(f_M(x)\) is a function of the mobile phase
- \(k'\) is the retention factor
- \(d_p\) is the diameter of the packing particle (cm)
- \(D_M\) is the diffusion coefficient in the mobile phase (cm² s⁻¹)

and:
- \(u\) is the linear velocity of the mobile phase (cm s⁻¹)

The van Deemter equation provides a comprehensive explanation of plate height as well as the factors affecting band broadening, but when determining the efficiency of a chromatographic column, the number of theoretical plates (N) must also be taken into account (see Equation 6). In short, the efficiency of a chromatographic separation increases as the plate count (N) becomes greater and the plate height (H) becomes smaller. These two terms are related to each other by the length of the column packing (L) (Skoog et al., 1998).

Equation 6

\[N = \frac{L}{H} \]

where:
- \(N\) is the plate counts,
- \(L\) is the length of the column packing (cm),
- \(H\) is the plate height (cm)

Since the columns employed for packed column pSFC systems can be the same as those used in conventional liquid chromatography systems in terms of dimension and particle size, the efficiency terms that will have the
greatest effect on changes to the plate height from that observed in LC relate to the diffusivity and viscosity of the mobile phase (Table 2). For instance, when gaseous mobile phases are employed in GC, the rate of longitudinal diffusion can be reduced by lowering the temperature due to the dependence of the $D_M$ term on this experimentally controlled variable. This effect is normally not noticeable in LC because diffusion is so slow that longitudinal diffusion has little effect on the plate height (Skoog et al., 1998). However, since supercritical fluids have faster diffusion rates than liquids, the relevance of the longitudinal diffusion term on band broadening when this separation method is employed increases. It has been stated that the separation efficiency in pSFC strongly depends on the rate of solute diffusion in the mobile phase (Chester et al., 1994). Fortunately, since SFs have viscosities similar to those of a gas, a lower column pressure drop is observed during pSFC analysis compared to the same column in LC. This allows for high mobile phase velocities and smaller particle sizes to be employed in pSFC which reduce the magnitude of the other terms in the van Deemter equation (White and Houck, 1986). The contributions of other band-broadening factors such as sample solvent/mobile phase mismatch and extra-column volumes (e.g. tubing sizes) in pSFC analyses are not entirely understood. De Pauw et al. found that reducing the internal diameter of connection capillaries from 250 μm to 65 μm in a standard pSFC system configuration did not improve the efficiency associated with early eluting analytes. However, the authors reported that plate counts were increased if the viscosity, elution strength, and polarity of the sample solvent were matched to the mobile phase (De Pauw et al., 2015).

Table 2: A comparison of the diffusivity and viscosity values of gases, supercritical fluids, and liquids.

<table>
<thead>
<tr>
<th></th>
<th>Diffusivity (cm$^2$/s)</th>
<th>Viscosity (g/cm x s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAS</td>
<td>$10^{-4}$</td>
<td>$10^4$</td>
</tr>
<tr>
<td>SUPERCRITICAL FLUID</td>
<td>$10^{-4}$ to $10^{-3}$</td>
<td>$10^{-4}$ to $10^{-3}$</td>
</tr>
<tr>
<td>LIQUID</td>
<td>$&lt; 10^{-5}$</td>
<td>$10^2$</td>
</tr>
</tbody>
</table>

In order to optimize the efficiency of a chromatographic separation, one must alter experimental conditions in order to separate the components of a mixture in the shortest amount of time possible. Optimizations usually focus on reducing band broadening and/or altering the relative migration rates of the analytes. The resolution ($R_s$) of a column provides a quantita-
tive measure of its ability to separate two analytes. Equation 7 relates the resolution to the number of plates in the column (N), the selectivity factor (α), and the retention factors of the two solutes (k’₁ and k’₂). To obtain the highest possible resolution, these three terms must be maximised (Skoog et al., 1998).

Equation 7

\[ R_S = 0.25 \sqrt{N} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k'_2}{1 + k'_2} \right) \]

where:
N is the number of theoretical plates
α is the selectivity factor = \( \frac{k'_2}{k'_1} \)
k’₂ is the retention factor for the second analyte = \( \frac{t_R - t_M}{t_M} \)
t_R is the retention time of the analyte
t_M is the dead time

The resolution equation can be divided into three parts. The first section (\( \sqrt{N} \)) relates to kinetic effects that lead to band broadening. The number of theoretical plates (N) can be increased by simply lengthening the column, but this can also lead to an increase in retention time and increased band broadening. Alternatively, the plate height can be reduced by reducing the size of the stationary phase particles or by reducing the viscosity of the mobile phase which leads to an increase in the diffusion coefficient in the mobile phase.

The second \( (\alpha - 1)/\alpha \) and third \( k'_2/(1 + k'_2) \) terms relate to the thermodynamics of the analytes being separated. The quotient containing the selectivity factor (α) depends only on the properties of the two analytes, while the quotient containing the retention factor (k’₂) depends on the properties of both the solute and the column (Skoog et al., 1998).

In LC, selectivity can be manipulated to improve separations by changing mobile phase composition, changing column temperature, changing composition of stationary phase, or by using additives. In GC, selectivity is primarily a function of the stationary phase with separations governed by polarity and the ability to form hydrogen bonds or weak electron donor-acceptor complexes. However in pSFC, selectivity is a function of both the stationary phase and the mobile phase with the mobile phase affecting the retention of solutes as a function of its pressure and polarity.
(White and Houck, 1986). The retention factor \(k'\) is traditionally optimized by changing the temperature in GC or the composition of the mobile phase in LC, but it can be manipulated by altering the composition of the mobile phase, temperature, and/or pressure in pSFC.

The importance of the intermediate properties of supercritical fluids compared to those of gases and liquids can be seen when their effects on chromatographic separations are examined. Since supercritical fluids have faster diffusion rates than liquids, it is possible to achieve greater optimum mobile phase velocities and therefore shorter analysis times than in LC. Of course, as previously stated, SFs have lower viscosities than liquids therefore longer columns (with smaller particles) can be utilized without resulting in excessive backpressure allowing higher efficiencies to be achieved. Finally SFs have greater solvating power than gases resulting in lower operating temperatures and allowing target analytes to have higher molecular masses.

Retention mechanisms in pSFC are more complicated than in GC or LC because they are a function of temperature, pressure, mobile phase density, mobile phase composition, as well as the stationary phase and many of these variables are interrelated (Wu, 2004). For instance, when the flow-rate is increased during a pSFC analysis, the change in system pressure also increases the density of the supercritical fluid. This results in an increase in the strength of the eluting solvent which favours a decrease in the retention time of the analytes being separated (Lesellier, 2009). Migration of analytes through the chosen column in pSFC occurs as a function of mobile phase density and polarity. At increased system pressures, intermolecular distances between the mobile phase and solutes are decreased which results in increased intermolecular interactions (White and Houck, 1986). The dependence of analyte retention behaviour on mobile phase density can be manipulated in order to optimize selectivity by designing methods that employ pressure or density gradients. Density gradients can also be created from the pressure drop across the column. The often unaccounted for effects of radial and longitudinal temperature profiles in pSFC (arising from viscous heating and/or decompression cooling) can also have a drastic effect on retention and band broadening. In pSFC, a temperature decrease due to a large decompression cooling effect can be significant if the pressure drop across the column is significant (i.e. a longitudinal gradient). However, when higher pressures are utilized (e.g. 600 bar) viscous heating becomes more relevant (i.e. a radial gradient). Both of these processes are largely dependent on the pressure and temperature.
settings of the system as well as the mobile phase composition (Pauw et al., 2014). Such undesired gradients may have adverse effects on retention and efficiency if they are not identified and compensated for properly. For instance, if the pressure drop across a packed column is significant and results in expansion of the mobile phase which in turn leads to a significant temperature drop due to the Joule-Thomson effect, a thermally insulated column can be utilized to increase the efficiency and resolution of the separation, but the operator loses temperature control (Wu, 2004). It has been reported that working under subcritical conditions and/or utilizing columns with reduced particle sizes to offset the cooling effect with frictional heating can also minimize this phenomenon. Columns with smaller internal diameters may also promote faster temperature exchanges between the centre of the column and the oven (Lesellier and West, 2015).

1.4 Retention Behaviour

In GC, retention of analytes is dependent on two factors; first on the vapor pressure of the solute, and thus on the temperature of the system, and secondly on the solute’s interaction with the stationary phase. Conversely, LC retention behaviour depends on solute partitioning between the mobile phase and stationary phase, so the composition of the mobile phase becomes a much more important factor (Guiochon and Tarafder, 2011). The retention behaviour exhibited during pSFC is much more complicated and dependent on the design of the analytical separation. When polar stationary phases are employed, a normal-phase retention behaviour is induced. Similarly, when non-polar stationary phases are employed, a reversed-phase retention behaviour is induced (Lesellier, 2009). Although certain retention behaviours can be promoted, analytes do not behave or chromatograph in a predictable manner during pSFC analyses based on previously observed LC behaviour. The absence of water in the mobile phase elevates interactions between analytes and the stationary phase compared to what occurs during liquid chromatography (Lesellier, 2009).

When supercritical CO₂ is utilized as the mobile phase, its non-polar character favours the solubility of hydrophobic compounds in the mobile phase. Modifiers (also called cosolvents) can be added to the mobile phase in order to alter the chromatographic behaviour of compounds. This is accomplished by changing compound solubility and inducing selectivity changes by modifying intermolecular interactions such as hydrogen bonding (Lesellier, 2009). It is believed that a low percentage of the modifier acts by deactivating free silanols on the surface of the stationary phase
(this effect varies depending on the choice of cosolvent), while higher percentages of cosolvent change the polarity of the bulk eluent (Roth, 2004). Their introduction can have a transient effect or induce solvent adsorption onto the stationary phase, which can modify its apparent polarity (Lesellier, 2009). Berger reported that decreasing the polarity of the stationary phase was not an effective means of improving the separation of polar solutes in pSFC. He found that polar modifiers used with polar stationary phases were required to produce acceptable resolution and symmetrical peak shapes. It is believed that interactions between the solute and the mobile phase approach those between the solute and the stationary phase only when the solvent strength is sufficiently increased resulting in an effective elution of the analyte (Wu, 2004).

The addition of organic modifiers (such as methanol or acetonitrile) to the mobile phase as cosolvents can have a profound effect on pSFC separations. Problems associated with mixed solvent-CO₂ phases may arise; most commonly because the chosen elution conditions lead to phase separations which result in poor chromatographic performance (changes in retention time, selectivity, baseline noise, peak shape, and column efficiency) (Smith, 1999, Page et al., 1992). It is often the case that separations are performed below one of the critical parameters (T_c or P_c) without the realization of the operator. This is due to the fact that conditions may no longer be “supercritical” if the temperature and pressure are held constant, but the percentage of the cosolvent is increased (Chester and Pinkston, 2004). In general the addition of modifiers results in an increase in critical values, but this depends on the proportion and nature of the modifier (Lesellier, 2009). Phase diagrams are far more complex for mixed or binary mobile phases than for pure compounds. In fact, van Konynenburg and Scott analyzed such systems and concluded that the relationship between the pressure and temperature under which the gas and liquid phases are at equilibrium is completely dependent on the composition of the mixture and the molecular interactions that exist between the components (Guiochon and Tarafder, 2011, Konynenburg and Scott, 1980). Fortunately, as long as a transition between phases does not occur (i.e. the supercritical fluid separates into gaseous CO₂ and liquid modifier; see Figure 2), separations can be carried out without consequence (Wu, 2004). To avoid phase separations, an operator may utilize a temperature inferior to the critical temperature and perform their desired separations in a subcritical state. In a subcritical state, fluid compressibility is reduced,
therefore the variation in fluid density between the column inlet and outlet due to a pressure drop is limited (Lesellier, 2009).

![Figure 2: Phase transitions of carbon dioxide](image)

(A = liquid/gaseous CO₂, B = phase mixing, C = CO₂ at the critical point, D = supercritical CO₂)

It should be noted that when binary systems are utilized and separations are performed outside of the supercritical region, the solvating power of the mobile phase (and thus retention) may no longer be altered by changing the pressure. The addition of large percentages of cosolvent causes both the working temperature and pressure to be well below their critical values and therefore the density of the mobile phase will not change to a great extent with pressure variations. Under such circumstances, the mobile phase is simply a mixture of liquefied CO₂ and organic solvent (Saito, 2013).

Solvent strength is a nonlinear function of the modifier concentration because clustering of modifier molecules can result in increased local concentrations of the modifier in the supercritical fluid. An enhancement in the solvent strength of the mobile phase through addition of a modifier can be tuned by changing the modifier composition (Wu, 2004, Berger, 1997). Variations in the density of the supercritical fluid resulting from the addition of organic cosolvents have been found to have only minor effects on retention changes. The introduction of additives to the cosolvent (typically at low levels) can also have a profound effect on its polarity. Additives can affect the retention of analytes by partitioning to the stationary phase and altering its polarity, increasing the physical thickness of the stationary phase by causing a swelling effect, altering the density of the mobile phase, blocking active sites on the stationary phase, and changing the solubility of the analytes in the mobile phase (Wu, 2004, Roth, 2004, Tarafder, 2016). Interestingly, changing the percentage of modifier
has been reported to have an effect on the amount of additive absorbed on
the stationary phase with lower quantities of additive absorbed at higher
modifier concentrations (Berger, 1997).

The volatility of a compound may also have an effect on its retention if
pure CO₂ is utilized and the separation is conducted at a high temperature
(above 60°C) because the properties of the supercritical fluid are close to
those of a gas under these conditions. However, typical separations using
packed columns are conducted using modifiers and the fluid is often in a
subcritical state. Under these circumstances, a higher fluid flow resistance
is present and the backpressure necessary to reach the sub- or super-
critical state results in a fluid density that is closer to those of liquids
(Lesellier, 2009).

1.5 Effects of Column Coupling in pSFC

It is becoming common practice to increase the separation capacity of
complex chromatographic analyses by coupling two columns with orthog-
onal stationary phases. This technique requires the use of a specialized
modulator in GC to focus the analytes of interest as they elute from the
first column and then re-inject them onto the second column (Skoczynska
et al., 2008). Often stationary phases can be selected that result in separa-
tions that would be otherwise unobtainable in one dimension. The effect
of column coupling in pSFC is complicated by the pressure changes that
occur during the course of the separation. Theoretically, the coupling of
two identical columns results in the increase in the length of the column
packing and therefore in the number of theoretical plates. However in
pSFC, column coupling also increases the flow rate resistance at the inlet
and the average pressure experienced in the coupled columns. The result-
ing modification in the density of the supercritical fluid results in a change
to the apparent void volume as well as the eluting strength of the mobile
phase in all of the coupled columns except the one at the end which is only
subjected to the pressure imposed by the back-pressure regulator. This
results in decreased retention times in the first column due to the greater
internal pressure created by the presence of serially coupled columns
(Lesellier, 2009).

The usefulness of column coupling is especially evident in enantiosepa-
rations where the coupling of achiral columns with chiral stationary phas-
es was introduced to contend with the limited achiral selectivity of com-
monly used chiral stationary phases (Terfloth, 2001). In this specific ap-
application of tandem-column coupling or two-dimensional pSFC, it has
been found that the best separations are obtained if the achiral column is followed by the enantioselective one (Kalikova et al., 2014).

1.6 Proposed Retention Mechanisms

The retention mechanisms that have been proposed for pSFC involve five basic interactions: dispersion, dipole-dipole, donor and acceptor hydrogen bonding, and charge transfer. When a non-polar stationary phase is employed, the absence of water in the mobile phase results in a modification of the relative interactions responsible for the separation of analytes compared to that observed in LC. The chemical nature of carbon dioxide favours stationary phase – solute interactions and minor associations (dipole-dipole and charge transfer) become significant in the retention of compounds resulting in an increase in the importance of stationary phase selection (Lesellier, 2009).

For instance, when alkyl bonded phases are employed in pSFC, an increase in the hydrocarbon character of the analytes results in increased retention, whereas polar groups (alcohols, acids, etc.) or double-bonds decrease retention. Stationary phases containing an embedded polar group have been shown to have high shape selectivity and porous graphitic carbon (PGC) columns have been shown to be effective in separating aromatic isomers. It is believed that the flat electron rich surface of graphite gives rise to the retention of acidic compounds such as phenols. Interestingly, on PGC columns, the retention of compounds follows a reverse-phase mode for hydrophobic analytes and a normal-phase mode for polar analytes. This retention behaviour is known as the PREG effect (polar retention effect on graphite). Unfortunately, this stationary phase can only be used for analyses targeting the separation of small compounds because the elution of large molecules requires the use of a mobile phase with very high eluotropic strength (usually involving the use of chlorinated solvents) (Lesellier, 2009).

In chiral separations, the chemo- and enantio-selectivity of the chiral stationary phase (CSP) is the most important factor in determining the effectiveness of the separation. The chiral recognition mechanisms occurring during pSFC separations are presumably similar to those taking place during LC analyses and one method does not appear to provide superior enantio-resolution over the other (De Klerck et al., 2012). However, certain CSPs are considered better suited for pSFC due to the chemical properties of the mobile phase and the mechanism of separation. For instance, Xiao et al. have reported that the formation of inclusion complexes be-
tween cyclodextrin CSPs and chiral analytes can be limited by the low polarity of the carbon dioxide mobile phase in pSFC (Xiao et al., 2012). On the other hand, chiral polysaccharide-based stationary phases, specifically cellulose and amylose CSPs, have been widely used for pSFC enantioseparations due to the broad enantioselectivity and chiral recognition ability that they have demonstrated in association with this separation technique (da Silva and Collins, 2014, Chankvetadze, 2012). The heterogeneous surfaces of polysaccharide-based CSPs provides multiple binding sites and can result in both non-specific interactions that determine retention as well as enantioselective interactions which determine separation (Khater and West, 2014).

1.6.1 Retention Models

There are three main retention models used to explain retention behaviour in pSFC: empirical, thermodynamic, and extrathermodynamic. Empirical models correlate retention behaviour with functional group similarities, differences, and the regiochemistry of compounds containing the same functional groups. As an example, Berger linked the surface area of diol coated silica particles to the retention of organic acids, amines, aminophenols, and amides (Wu, 2004). In thermodynamic models, it is postulated that variables such as diffusion coefficients, solute adsorption properties, and the enthalpy and entropy of transfer of the solutes from the mobile phase to the stationary phase influence the retention of analytes during a chromatographic separation. These models can be used to explain a variety of chromatographic and physicochemical parameters including the effect of pressure, temperature, modifier, and solute distribution (Wu, 2004). Extrathermodynamic models include different types of linear free energy relationships, for which, although they can be stated in terms of thermodynamic parameters, a thermodynamic principle does not exist that states the relationship should be true (e.g. dispersive, dipole-dipole, dipole-induced dipole, and hydrogen bonding acidity/basicity interactions). A popular extrathermodynamic model is the linear solvation energy relationship (LSER) which can be used to identify different intermolecular interactions that contribute to various retention behaviours in different chromatographic systems (Wu, 2004). In the LSER model, the selection of a cosolvent may be used to either promote or suppress a specific type of molecular interaction in order to effect separation of analytes (Roth, 2004). A variant of LSER is the Solvation Parameter (SP) model which estimates the contribution of cavity formation and intermolecular interac-
tion on the retention of molecules in a two-phase separation system. In this model, system constants are used to determine the contribution of defined intermolecular interactions on neutral molecule retention (Poole, 2012). West and Lesellier published multiple papers on the characterization of different stationary phases in subcritical fluid chromatography using the solvation parameter model (West and Lesellier, 2006d, West and Lesellier, 2006a, West and Lesellier, 2006b, West and Lesellier, 2006c, West and Lesellier, 2012, Khater et al., 2013). Ebinger and Weller noted that the selectivities of many silica based columns can change over time when additives such as ammonium acetate are utilized; they attributed this observation to a change in the silanophilicity of the stationary phases and developed a non-traditional column ranking system which can be utilized for column selection in similar systems (Ebinger and Weller, 2014). These references provide useful information when undertaking column screening during method optimization.

Unfortunately, the complexity of supercritical fluid systems makes the application of LSER and/or SP models difficult. For instance, as the pressure is increased in a pSFC system, the CO₂ becomes denser and the dispersion interactions between the solutes and the mobile phase increase. However, an increase in temperature at constant pressure results in a decrease in the dispersion interactions between the solutes and the stationary phase as well as those between the solutes and mobile phase due to a decrease in the CO₂ density (Wu, 2004). The effect of pressure variations on mobile phase density and eluent strength make the application of retention mechanism models to gradient systems in pSFC complicated (Tytėtė et al., 2015).
2.0 Supercritical Fluid Chromatography of POPs

High resolution gas chromatography and liquid chromatography are established separation methods utilized in the environmental monitoring of persistent organic pollutants (POPs). Although most environmental contaminants of concern can be analyzed by one of these techniques, supercritical fluid chromatography provides an analytical tool complementary to both LC and GC that increases the analyst’s ability to tackle difficult analytical problems (Combs et al., 1997). For instance, when performing non-targeted analyses of environmental samples, pSFC allows for the analysis of non-volatile, polar, adsorptive, and/or thermally labile solutes using one analytical method (White and Houck, 1986). Samples that previously required derivitization prior to analysis by GC may be screened without additional preparative steps with potentially low yields. Also, the use of affordable and widely applicable instrumentation capable of analyzing a broad range of compounds could increase the occurrence of environmental contaminant monitoring in developing countries.

2.1 Applications of pSFC in Environmental Chemistry

The innovations associated with modern society have resulted in a number of specifically designed compounds possessing the chemical properties required to meet the very particular requirements of an application. Unfortunately, investigations relating to the toxicity and persistence of these compounds are not always comprehensive (Howard and Muir, 2010, Howard and Muir, 2013) and their environmental fate and impact is only discovered after they’ve been in use for extended periods of time. The lack of transparency in the composition of industrial formulations also makes it difficult for scientists to easily identify the source of novel contaminants without exhaustive investigation. Additionally, many environmental pollutants are formed and released as unintentional by-products of industrial or combustion processes (Booth and Gribben, 2005, Liu et al., 2014, Organtini et al., 2014, Tue et al., 2016, Zhang et al., 2016a). Access to an analytical technique, such as pSFC, that is applicable to a wide range of chemical classes and functionality could result in the development of comprehensive screening methods for environmental samples. It has been recognized that, although standard techniques (GC and LC) allow for the trace analysis of most known POPs, the need for fast, low cost, and environmentally friendly techniques still exists (Xu et al., 2013). The availability of improved instrumentation (Berger, 2015) has renewed interest in
pSFC as an analytical separation technique in environmental chemistry and literature precedent for this avenue of research does exist.

2.1.1 Polychlorinated Biphenyls

Applications of pSFC for the separation of polychlorinated biphenyls (PCBs) have been reported in the scientific literature. PCBs are legacy environmental contaminants which were industrially produced to be used mainly in transformers and condensers as dielectrics (Bogdal et al., 2014). In 1990, Cammann et al. investigated the retention of PCBs using packed cyanopropyl and octadecylsilane (ODS) columns with carbon dioxide and nitrous oxide mobile phases. They reported that the retention was highly governed by the shape and electron configuration of the PCB congener with coplanar PCBs being more strongly retained than non-planar PCBs with the same substitution level. They also noted a nearly linear relationship between the retention factor and increasing chlorine substitution for the coplanar PCBs investigated, but this relationship did not extend to non-planar PCBs. The authors postulated that the difference in retention on the ODS stationary phase between ortho- and non-ortho-substituted PCBs was due to the weakening of Van der Waals interactions between the analytes and the stationary phase with increasing out-of-plane orientation. Similar observations were noted for the cyanopropyl stationary phase with slightly better separation achieved (Cammann and Kleibohmer, 1990). In 1991, the same authors published an article detailing the analysis of technical PCB mixtures (Aroclor 1260 and a mixture of Aroclor 1221, 1240, and 1254) using an ODS column and a CO$_2$ mobile phase. They utilized density programming (through negative temperature programming) to achieve maximum resolution and utilized the developed conditions to analyze PCBs in a sediment sample using ultraviolet (UV) detection (Cammann and Kleibohmer, 1991).

The enantiomeric separation of 18 chiral polychlorinated biphenyls was reported in 2012 by Zhang et al. using a polysaccharide-type chiral stationary phase (Sino-Chiral OJ), a mobile phase consisting of 100% CO$_2$, and UV detection. The authors reported that resolution decreased with increasing temperature and the addition of an organic modifier (in this case ethanol, methanol, and isopropanol) resulted in a significant loss of resolution and decreased retention (Zhang et al., 2012).
2.1.2 Polycyclic Aromatic Hydrocarbons

Another prevalent group of POPs that have been investigated using pSFC are polycyclic aromatic hydrocarbons (PAHs). PAHs are organic lipophilic compounds comprised of fused aromatic rings that are highly hydrophobic. They are released into the environment mainly through industrial and combustion processes (Singh et al., 2016). The complex nature of PAH mixtures makes their analysis challenging, but their separation by pSFC was promoted by the petroleum industry which utilized this technology for the determination of hydrocarbon classes in feedstocks as well as simulated distillation (Shariff et al., 1997). The use of pSFC with CO$_2$ as the mobile phase and flame ionization detection to determine hydrocarbon types in petroleum liquids was first reported by Norris and Rawdon in 1984 (Norris and Rawdon, 1984). However, the separation of PAHs by pSFC predated this paper by 2 years; Gere et al. reported the separation of PAH standards on 10, 5, and 3 μm particles in 1982. In this work the authors indicated that particle size influenced the efficiency of the column with improved results observed for smaller particles (Gere et al., 1982). Further work was carried out by various groups investigating the influence of temperature and pressure (Sie and Rijnders, 1967) and the addition of mobile phase modifiers (Levy and Ritchey, 1985, Levy and Ritchey, 1986, Barker et al., 1989). In 1994, Kot et al. reported the separation of the 16 PAHs listed on the United States Environmental Protection Agency’s target list (Kot et al., 1994) and Heaton et al. later developed a faster method for the analysis of the same mixture on a bonded C$_{18}$ silica (Heaton et al., 1994). The rapid pSFC analysis of the same 16 PAHs in reclaimed water using a 2-ethyl pyridine column following solid-phase extraction was recently reported by Zhang et al. (Zhang et al., 2016c). The authors utilized photodiode array (PDA) detection, but reported limits of detection ranging from 0.4 – 10 μg/L and an impressive separation time of 4 minutes. The method was also successfully applied to samples from a wastewater treatment plant to demonstrate environmental applicability.

2.1.3 Halogenated Flame Retardants

The incorporation of flame retardants (FRs) into commercial products has been a common practice since the first official FR patent was filed by Obadiah Wyld in 1735 (Wyld, 1735). His proposal to use a mixture of alum, ferrous sulphate, and borax to increase the fire retardancy of cotton initiated the development and application of a number of FRs, of which
many are halogenated. However, independent scientific research on FRs has led to a heightened concern regarding their fate in the environment as well as their potential health effects. Indeed, existing and emerging regulations are forcing industry to look for safer alternatives. Unfortunately, there are some currently used FRs, such as the derivatives of tetrabromobisphenol-A (TBBPA), that have proven to be analytically challenging resulting in the sluggish assembly of data for risk assessments. For example, between 2010 and 2012 the European Food Safety Authority (EFSA) had a panel advise on the monitoring of food and feed for brominated flame retardants (BFRs) and the derivatives of TBBPA were included in this review, however a lack of occurrence data meant that a risk assessment on these compounds could not be completed (EFSA, 2014). It is very difficult to achieve acceptable chromatography of the TBBPA derivatives using gas chromatography because these compounds are thermally labile and are prone to degradation. Separation methods utilizing liquid chromatography have been reported (Letcher and Chu, 2010), but the use of pSFC provides a viable alternative since the temperature required to maintain carbon dioxide in its supercritical state (31°C) at a pressure of 1100 psi is well below that which a compound would experience during a typical GC analysis (Taylor, 2009). Recently, effective separations of selected derivatives of TBBPA (tetrabromobisphenol A-bisallylether, tetrabromobisphenol A-bis(2,3-dibromopropylether), tetrabromobisphenol A-bis(2-bromoallylether) and tetrabromobisphenol A-bishydroxyethylether) were demonstrated using supercritical fluid chromatography-mass spectrometry and UV detection (Riddell et al., 2014).

The rapid baseline separation of three isomers of 1,2,5,6,9,10-hexabromocyclododecane (HBCDD), an additive brominated flame retardant mainly used in polystyrene foams, using pSFC and tandem mass spectrometry (MS/MS) was also recently reported (Mullin et al., 2015). The authors utilized a high strength silica (HSS) C\textsubscript{18} column along with a methanol modified CO\textsubscript{2} mobile phase to achieve baseline separation of the three main diastereomers present in the technical mixture in under 3 minutes. The environmental applicability of the reported method was confirmed through the analysis of human serum and whale blubber extracts.

Polybrominated diphenyl ethers (PBDEs) are another class of additive flame retardants which have been primarily analyzed by GC/MS. Although the lower brominated PBDEs were added to the Stockholm
Convention (a global treaty developed to protect human health and the environment from POPs) in 2009, the environmental impact of these compounds, and their metabolites (Marteau et al., 2012), continues to be an issue since their release can now be linked to the disposal of aging electronic equipment. Gross et al. recently reported the trace analysis of 22 PBDE metabolites, specifically hydroxylated polybrominated diphenyl ethers (OH-PBDEs), in human serum using pSFC/MS (Gross et al., 2016). The authors report that the developed method offered advantages over LC/MS and GC/MS analyses of the same metabolites in terms of required sample preparation, sample analysis time, and detection limits.

2.1.4 Perfluoroalkyl Substances

The unique properties of perfluoroalkyl substances have resulted in their incorporation into a multitude of products for a variety of purposes since they were first industrially manufactured in 1947 by the electrochemical fluorination process. They exhibit many advantageous characteristics and properties; they are chemically stable, have surface tension lowering properties, and create stable foams. As such, they have been utilized in metal plating, coating formulations, fire-fighting foams, polymer production, lubricants, and water repellents (Prevedouros et al., 2006). Unfortunately, the chemical stability of these compounds also makes them persistent in the environment. Some polyfluoroalkyl substances such as perfluoroalkane sulfonamidoethanols have been shown to degrade to perfluoroalkane sulfonates via biotransformation processes and abiotic oxidation, but the end-products of these processes are quite stable and have been detected in water, fish, birds, mammals, and humans worldwide (Houde et al., 2006). Indeed, the continued release of fluorinated precursors that degrade to persistent perfluoroalkyl acids has resulted in a significant and ongoing exposure route that affects both human and environmental health (D'Eon and Mabury, 2011). It has been reported that the bioaccumulation of perfluoroalkyl substances is directly related to chain length (Conder et al., 2008) and industry has responded by manufacturing perfluoroalkyl substances with shorter chains or ether linkages, but limited information on their degradation is currently available (Wang et al., 2013). Perfluoroalkyl substances are generally amenable to LC with MS detection, but conditions vary depending on the chemical functionality of the compounds being analyzed. For instance, perfluoroalkyl phosphonates often require the presence of a base such as sodium hydroxide or 1-methyl piperidine (Ullah et al., 2011) in a buffered
mobile phase to prevent excessive tailing and improve chromatographic resolution, but n:2 fluorotelomer alcohols are known to have ion suppression issues and adduct formation under similar conditions (Berger et al., 2004). Therefore these compound classes cannot be analyzed for simultaneously. Since analytical conditions for the pSFC analysis of multiple perfluoroalkyl substance families have not been reported in the literature yet, it is unknown if these limitations will also be present in this technique.

However, initial investigations into the applicability of pSFC as a separation technique for perfluoroalkyl substances have been promising; Adamasu et al. have reported that the separation of branched isomers of perfluoroalkane sulfonates and perfluoroalkyl carboxylates can be accomplished by pSFC if a cosolvent composed of methanol with 5% water and 10 mM ammonium formate and a LUX cellulose 3 column are employed (Admasu et al., 2013). This methodology has precedent in the literature; the addition of water to a pSFC cosolvent has been proposed as a method of optimizing the separation of non-ionic surfactants by Takahashi et al. (Takahashi et al., 2013), while the effect of ionic additives on the elution of sodium aryl sulfonates in pSFC has been previously investigated by Zheng et al. (Zheng et al., 2005).

2.1.5 Pesticides
The availability of modern pSFC instrumentation has also resulted in the recent publication of several studies relating to the chiral and achiral analysis of pesticides. In 2014, Chen et al. reported the chiral separation and MS/MS detection of neonicotinoid sulfoxaflor in vegetables and soil (Chen et al., 2014). The authors utilized a Chiralpak® IA-3 [amylose tris(3,5-dimethylphenylcarbamate)] stationary phase along with a 2-propanol/acetonitrile modified CO₂ gradient to baseline separate the stereoisomers of sulfoxaflor. In terms of achiral analysis, Vass et al. compared the separation achieved for 30 pesticide residues by pSFC using a C₁₈ column to that typically observed by LC or GC (depending on the target analyte) (Vass et al., 2016). At the time of publication, further optimization of their method was required, but the capability of analyzing both GC and LC amenable compounds using a single technique was illustrated. Further demonstrating the potential associated with pesticide analysis by pSFC, Ishibashi et al. published an impressive multi-residue method that involved the separation of 444 pesticides of varying polarity and molecular weight using a methanol (0.1% ammonium formate)
modified CO$_2$ gradient elution on an Inertsil ODS-EP column in 20 minutes (Ishibashi et al., 2015). The authors were able to use the developed method to analyze QuEChERS spinach extracts and confirm the presence of 373 pesticides at levels of 10 μg kg$^{-1}$. They demonstrated the potential for high throughput multiresidue pesticide analysis using pSFC/MS and electrospray ionization. Additional studies reporting the rapid and sensitive analysis of seven pyrethroid insecticides in vegetable samples (El-Saeid and Khan, 2015) and six herbicide residues in tobacco (Guo et al., 2015) were also published in 2015.

2.1.6 Emerging Contaminants

In recent years, interest in the application of pSFC for the rapid analysis of compounds with potential environmental or human health impact has grown. In 2014, Zhou et al. reported the determination of 17 disperse dyes (low molecular weight organic dyes of which many have been identified as allergens) in textiles by pSFC/MS (Zhou et al., 2014). The authors utilized a BEH column and a methanol modified CO$_2$ gradient to achieve separation of the structurally diverse allergenic dyes within 5 minutes. In the next year (2015), Salvatierra-Stamp et al. reported a pSFC method for the analysis of seven emerging contaminants with ranging functionality and use; two pharmaceuticals (carbamazepine and glyburide), three endocrine disruptors (17α-ethinyl estradiol, bisphenol A, and 17β-estradiol), one bactericide (triclosan), and one pesticide (diuron) (Salvatierra-Stamp et al., 2015). The authors utilized a 2-ethyl pyridine column and an acetonitrile modified CO$_2$ mobile phase to achieve baseline separation of all target analytes in less than 10 minutes. Unfortunately MS detection was not utilized for this study, but the authors were able to demonstrate the application of pSFC as a separation technique for diverse groups of compounds.

In 2016, Zhang et al. evaluated the use of pSFC/MS for the analysis of thirteen UV-ink photoinitiators from polyethylene food packaging (Zhang et al., 2016b). The particular photoinitiators (PIs) investigated are catalysts for a polymerization reaction associated with the UV curing of inks in carton board or plastic food packaging. The authors reported that residual PIs can migrate from the packaging material into food and pose a risk to consumers. In this study, the authors utilized an HSS C18 SB column with a methanol-acetonitrile modified CO$_2$ gradient to separate the target analytes in 4.5 minutes.
2.2 Coupling pSFC with Mass Spectrometry

The separation efficiency provided by known chromatographic techniques is very impressive, however in order to identify, characterize, and accurately quantify organic compounds, a separation method must be successfully coupled to a sensitive and selective detector (Schmid, 1990). Detection of compounds being analyzed using pSFC must occur in one of two scenarios: the measurement is performed directly in the supercritical medium (as with UV detection) or detection occurs after fluid decompression (as with MS detection) (Novotny, 1986). In the field of environmental chemistry, mass spectrometry is a widely used detection technique due to its inherent sensitivity and selectivity. The interfacing of GC and LC instrumentation to MS detectors with ionization capabilities relevant for environmental contaminants has been established, however pSFC introduces new challenges when attempting to do low level analytical work. Ideally, an interface should maintain chromatographic integrity, allow for multiple ionization methods, promote high analyte transport efficiency, limit thermal degradation, have high compatibility with multiple solvents, and be reliable and reproducible. Direct coupling between pSFC instrumentation and MS detectors has limitations associated with the amount of fluid that can enter the MS source before spectrometric performance is lost due to high ionization source pressures (Combs et al., 1997). A post-column split must be employed in order to obtain low source pressures and optimize sensitivity. The use of automated back-pressure regulators (ABPRs) in modern instrumentation also limits the split ratio since the regulator is located after the split. If the bleed to the MS is too large, the ABPR will not be able to maintain system pressures that keep the mobile phase in a sub- or super-critical state.

Atmospheric pressure ionization sources, specifically electrospray (ESI) and atmospheric pressure chemical ionization (APCI) are commonly used in environmental chemistry when coupled to LC systems. If modifications are made to the ionization conditions, ESI and APCI sources are also compatible with pSFC systems. For instance, since electrospray ionization occurs in the liquid phase with solute ions forming from liquid solution aerosols in electric fields, the addition of a proton-donating organic modifier to the CO$_2$ mobile phase is generally required in order to effect ion generation when pSFC is coupled to a mass spectrometer (Li and Hsieh, 2008, Combs et al., 1997). Alternatively, since APCI is a gas-phase process in which solvent molecules are ionized in a corona discharge and solute ionization occurs during molecular collisions of sample analytes
with solvent ions, additives are commonly required in order to improve sensitivity and/or promote the formation of certain ions. For instance, it has been reported that the addition of benzene as an APCI reagent causes benz[a]anthracene to undergo charge transfer since this polycyclic aromatic hydrocarbon has a lower ionization energy than benzene. However, if water is present during the ionization process the primary ionization mechanism changes to proton transfer (Combs et al., 1997).

Although electron ionization (EI) is commonly used for many halogenated aromatic environmental contaminants, there have been literature reports of such POPs being ionized using atmospheric pressure ionization (API) processes. In 1975, it was reported by Dzidic et al. that phenoxide ions, \([M-\text{Cl}+\text{O}]^-\), were formed during API mass spectrometry of chlorinated aromatic compounds. The authors proposed that this ionization process occurred in the API source in the presence of nitrogen containing 0.5 ppm oxygen and also in air. They reported that meta-substituted chloronitrobenzenes as well as highly substituted polychlorobenzenes formed the phenoxide ion, but ortho- and para-substituted chloronitrobenzenes formed mainly chloride and nitrophenoxide ions (Dzidic et al., 1975).

\[
M^- + O_2 \rightarrow [M-\text{Cl} + \text{O}]^- + \text{ClO}'
\]

\[
O_2^- + M \rightarrow [M-\text{Cl} + \text{O}]^- + \text{ClO}'
\]

The idea of oxygen enhanced negative chemical ionization was revisited by Guevremont et al. when they published a method detailing the identification of PCB congeners based on the collision induced fragmentation of their \([M-\text{Cl}+\text{O}]^-\) ions (Guevremont et al., 1987). Further work on this ionization process by Lépine et al. focused on chlorinated compounds, such as polychlorinated biphenyls, which undergo oxygen addition-induced dechlorination reactions during electron capture negative chemical ionization (ECNCI) (Lepine et al., 1996b). This process might be relevant for other atmospheric pressure ionization sources as well. In fact, the authors report that many chlorinated compounds show ions with mass-to-charge (m/z) ratios that are lower than the expected molecular ion cluster by 19. They proposed that these ions correspond to the addition of \(O_2\) to the molecule which subsequently loses \(\text{ClO}'\) producing an even electron anion corresponding to \([M-\text{Cl}+\text{O}]^-\). The mechanism associated with this reaction sequence has not been definitively determined, but the following
process proceeding through a radical intermediate has been proposed (Lepine et al., 1996a):

\[
\text{ArCl}_n^- + O_2 \rightarrow \text{ArO}_2\text{Cl}_n^- \rightarrow \text{ArOCl}_{n-1}^- + \text{ClO}^-
\]

In general, the intensity of the \([\text{M-Cl+O}]^-\) cluster increases with increasing oxygen levels until a reduced ion transmission is observed due to high source pressures. The oxygen pressure at which the \([\text{M-Cl+O}]^-\) anion reaches its highest intensity also appears to be dependent on the extent of chlorine substitution on the aromatic rings with the highly substituted aromatics being more stable and therefore requiring higher amounts of oxygen in the source to effect ionization (Lepine et al., 1996a). The API/MS analysis of halogenated aromatic compounds such as PBDEs, polychlorinated dibenzo-\(p\)-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and PCBs has generally been accomplished by monitoring the products of oxygen enhanced negative chemical ionization (\([\text{M-X+O}]^-\) where \(X\) is a halogen) (Dzidic et al., 1975, Guevremont et al., 1987, Lepine et al., 1996b, Luosujarvi et al., 2008, Moukas et al., 2014, Mitchum et al., 1982, Fernando et al., 2016).
3.0 Aim of the Thesis

The application of Supercritical Fluid Chromatography as a separation technique for legacy (Paper I and Paper II) and emerging persistent organic pollutants (Papers III, IV, and V) has been investigated. It was postulated that the unique properties of supercritical fluids, particularly carbon dioxide, and its use in the chromatographic separation of environmentally relevant compounds with varying functionality could result in the development of screening methods applicable to a wide variety of environmental contaminants. The challenges associated with the coupling of pSFC with MS detection are also addressed for the target analytes investigated (Papers II and III).

The overall aim of this work was to determine the applicability of pSFC as a separation technique for environmental contaminants of concern.

Specific aims of papers included in this thesis:

**Paper I:** Optimize the separation of 2,3,7,8-substituted polychlorinated dibenzo-\(p\)-dioxins, dibenzofurans, and polychlorinated biphenyls and compare the efficiency and resolution achieved against established protocols (i.e. high resolution gas chromatography). Demonstrate the application of pSFC/MS of PCDDs, PCDF, and PCBs at environmentally relevant levels.

**Paper II:** Investigate positive ion atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) for the generation of molecular ion clusters of PCDDs, PCDFs, and PCBs when pSFC/MS is utilized.

**Paper III:** Optimize ionization conditions for the non-aromatic environmental contaminant Dechlorane Plus (DP) and demonstrate the applicability of pSFC/MS at environmentally relevant levels. Investigate reaction mechanisms associated with the commonly observed \([M-Cl+O]^−\) ion cluster of DP.

**Paper IV:** Utilize preparative scale pSFC for the chiral separation and isolation of \(1,2,5,6,9,10\)-hexabromocyclododecane (HBCDD) stereoisomers and compare the resolution of the isolated HBCDD
stereoisomers on an analytical scale using pSFC/MS and established LC/MS methods.

**Paper V:** Investigate the composition of technical mixtures of halogen free organophosphate flame retardants (OPFRs); specifically, resorcinol bis(diphenyl phosphate) (RBDPP), bisphenol A bis(diphenyl phosphate) (BPA-BDPP), and 9,10-dihydro-9-oxa-10-phosphaphenanthrene-10-oxide (DOPO) using multiple analytical separation techniques including pSFC.
4.0 Analysis of Environmental Contaminants of Concern

4.1 Analysis of Legacy Persistent Organic Pollutants

Some of the most well-known legacy persistent organic pollutants include polychlorinated dibenzo-\( p \)-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls. These groups of compounds have well-known toxic effects (Ahlborg et al., 1992) and are highly regulated globally (Cleverly et al., 1989). PCDDs and PCDFs have historically been introduced into the environment through the incineration of municipal waste, the bleaching of pulp and paper, and as contaminants in industrial chemicals such as chlorophenols and PCBs (Buser et al., 1978). The elution orders of the 49 PCDD congeners and 87 PCDF congeners that are tetra- to octa-chlorinated on different, commonly used, GC stationary phases have been determined and the required conditions are readily available (Ryan et al., 1991). The analysis of PCDDs and PCDFs in environmental samples is typically performed by high resolution gas chromatography coupled with high resolution mass spectrometry (HRGC/HRMS) (U.S. EPA, 1994), but recently GC coupled with tandem mass spectrometry (GC-MS/MS) was accepted as a means of confirming compliance with established maximum regulatory limits of PCDDs/PCDFs and PCBs in foodstuffs (The European Commission, 2014).

On the other hand, PCBs were industrially produced to be used mainly in transformers and condensers as dielectrics, but were also found to be useful in other open applications such as additives for glues, dyes, and construction materials. The most recognized tradenames for PCB mixtures include Aroclor 1254, Aroclor 1260, and Clophen A50. There are 209 possible PCB congeners and their GC separation, as well as their IU-PAC numbering system, has been widely documented (Schulz et al., 1989, Zabelina et al., 2010, Rogers et al., 2004).

The most toxic PCDD and PCDF congeners are substituted in the 2, 3, 7, and 8 positions of the dioxin and furan skeleton (Figure 3) and are the most important congeners targeted for identification and quantification in environmental samples (Ryan et al., 1991). For PCBs, the congeners that are most commonly monitored are those that are present at high concentrations in common technical formulations (PCB-28, PCB-52, PCB-101, PCB-118 [which is also classified as a toxic congener], PCB-138, PCB-153, and PCB-180) as well as the most toxic or “dioxin-like” congeners (PCB-
77, PCB-81, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-156, PCB-157, PCB-167, PCB-169, and PCB-189). High PCB toxicity has been associated with those congeners that contain 0 chlorine atoms (coplanar PCBs) or 1 chlorine atom (mono-ortho PCBs) in a position ortho to the C-C bond in the biphenyl skeleton (Zabelina et al., 2010).

Other legacy POPs, as initially defined by the Stockholm Convention in 2001, but not investigated in this work include aldrin, chlordane, dieldrin, endrin, heptachlor, mirex, toxaphene, dichlorodiphenyltrichloroethane (DDT), and hexachlorobenzene (HCB) (Lohmann et al., 2007, Xu et al., 2013). The primary analytical method for quantification for all of these legacy compounds is currently HRGC/HRMS, but the use of pSFC as a separation technique for 2,3,7,8-substituted PCDDs and PCDFs (Table 3) as well as dioxin-like PCBs (Table 4) has been investigated (Papers I and II).
Table 3: IUPAC Names, Abbreviations, and CAS Registry Numbers for the Investigated 2,3,7,8-Substituted Polychlorinated Dibenzo-p-dioxins and Dibenzo furans.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Abbreviation</th>
<th>CAS Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-Tetrachlorodibenzofuran</td>
<td>2378-TCDF</td>
<td>51207-31-9</td>
</tr>
<tr>
<td>1,2,3,7,8-Pentachlorodibenzofuran</td>
<td>12378-PeCDF</td>
<td>57117-41-6</td>
</tr>
<tr>
<td>2,3,4,7,8-Pentachlorodibenzofuran</td>
<td>23478-PeCDF</td>
<td>57117-31-4</td>
</tr>
<tr>
<td>1,2,3,4,7,8-Hexachlorodibenzofuran</td>
<td>123478-HxCDF</td>
<td>70648-26-9</td>
</tr>
<tr>
<td>1,2,3,6,7,8-Hexachlorodibenzofuran</td>
<td>123678-HxCDF</td>
<td>57117-44-9</td>
</tr>
<tr>
<td>1,2,3,7,8,9-Hexachlorodibenzofuran</td>
<td>123789-HxCDF</td>
<td>72918-21-9</td>
</tr>
<tr>
<td>2,3,4,6,7,8-Hexachlorodibenzofuran</td>
<td>234678-HxCDF</td>
<td>60851-34-5</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-Heptachlorodibenzofuran</td>
<td>1234678-HpCDF</td>
<td>67562-39-4</td>
</tr>
<tr>
<td>1,2,3,4,7,8,9-Heptachlorodibenzofuran</td>
<td>1234789-HpCDF</td>
<td>55673-89-7</td>
</tr>
<tr>
<td>Octachlorodibenzofuran</td>
<td>OCDF</td>
<td>39001-02-0</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>IUPAC Number</th>
<th>CAS Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-Tetrachlorodibenzo-p-dioxin</td>
<td>2378-TCDD</td>
<td>1746-01-6</td>
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<td>1,2,3,7,8-Pentachlorodibenzo-p-dioxin</td>
<td>12378-PeCDD</td>
<td>40321-76-4</td>
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<tr>
<td>1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin</td>
<td>123478-HxCDD</td>
<td>39227-28-6</td>
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<tr>
<td>1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin</td>
<td>123678-HxCDD</td>
<td>57653-85-7</td>
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<tr>
<td>1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin</td>
<td>123789-HxCDD</td>
<td>19408-74-3</td>
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<td>1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin</td>
<td>1234678-HpCDD</td>
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<td>Octachlorodibenzo-p-dioxin</td>
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<td>3268-87-9</td>
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</tbody>
</table>

Table 4: IUPAC Names, IUPAC Numbers, and CAS Registry Numbers of the Investigated Dioxin-like Polychlorinated Biphenyls.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>IUPAC Number</th>
<th>CAS Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,3',4,4'-Tetrachlorobiphenyl</td>
<td>77</td>
<td>32598-13-3</td>
<td>Coplanar</td>
</tr>
<tr>
<td>3,4,4',5-Tetrachlorobiphenyl</td>
<td>81</td>
<td>70362-50-4</td>
<td>Coplanar</td>
</tr>
<tr>
<td>2,3,3',4,4'-Pentachlorobiphenyl</td>
<td>105</td>
<td>32598-14-4</td>
<td>Mono-ortho</td>
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<tr>
<td>2,3,4,4',5-Pentachlorobiphenyl</td>
<td>114</td>
<td>74472-37-0</td>
<td>Mono-ortho</td>
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<tr>
<td>2',3,4,4',5-Pentachlorobiphenyl</td>
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<td>31508-00-6</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>3',3,4,4',5-Pentachlorobiphenyl</td>
<td>117</td>
<td>65510-44-3</td>
<td>Mono-ortho</td>
</tr>
<tr>
<td>2,3,3',4,4',5-Pentachlorobiphenyl</td>
<td>126</td>
<td>57465-28-8</td>
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</tr>
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<td>2,3,3',4,4',5-Hexachlorobiphenyl</td>
<td>156</td>
<td>38380-08-4</td>
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<td>2,3,3',4,4',5'-Hexachlorobiphenyl</td>
<td>157</td>
<td>69782-90-7</td>
<td>Mono-ortho</td>
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<tr>
<td>2,3,4,4',5,5'-Hexachlorobiphenyl</td>
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<td>52663-72-6</td>
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<td>3,3',4,4',5,5'-Hexachlorobiphenyl</td>
<td>169</td>
<td>32774-16-6</td>
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<tr>
<td>2,3,3',4,4',5,5'-Hexachlorobiphenyl</td>
<td>189</td>
<td>39635-31-9</td>
<td>Mono-ortho</td>
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</tbody>
</table>
4.1.1 pSFC/MS/MS Analysis of PCDDs, PCDFs, and PCBs

The application of pSFC coupled to MS for the analysis of legacy environmental contaminants was first evaluated by investigating the separation of 2,3,7,8-substituted PCDDs and PCDFs (Paper I). Preliminary pSFC method development involved column screening followed by cosolvent, temperature, pressure, and gradient optimizations on the most promising stationary phases. It was found that stationary phases with varying degrees of aromatic character exhibited the most promising elution profiles. Methanol was determined to be the most effective cosolvent that was also compatible with the selected ionization method: atmospheric pressure photoionization (APPI) for generation of molecular ion clusters [M]+. However, in order to obtain optimal response for all PCDD and PCDF congeners, the use of a fluorobenzene dopant was required. Therefore, the temperature and pressure parameters were optimized for a methanol modified CO2 gradient elution on a Torus 1-AA stationary phase (1.7 μm, 3.0 x 100 mm). The composition of the make-up solvent was optimized as a 5% solution of fluorobenzene in methanol.

Utilizing the developed pSFC method, separation of the PCDD and PCDF congener groups was achieved. Notable differences in the separation of the individual 2,3,7,8-substituted PCDDs and PCDFs compared to that obtained by HRGC using a 60 m DB-5 capillary column were observed. For instance, when using the described pSFC method PCDD congeners eluted from the column faster relative to similarly substituted PCDFs (see Figure 4). The first obvious difference being the relative elution order of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran with 2378-TCDD eluting before 2378-TCDF by pSFC. This is opposite to what is observed in HRGC analysis of these compounds. The relative elution order of PCDD and PCDF isomers using the developed pSFC method versus GC separation on a 60m DB-5 column is illustrated in Figure 4.
A selection of possible co-eluting non-2,3,7,8 substituted congeners were separated using the described pSFC method and the results were compared to the co-elutions observed on commonly used HRGC capillary columns, specifically DB-5, DB-225, and SP-2331 (see Table 5) to ensure adequate resolution using the described method. Of the known tetrachlorodibenzofuran isomers that co-elute with 2378-TCDF on a DB-5 HRGC capillary column, 1279-TCDF, 2348-TCDF, 2347-TCDF and 2346-TCDF were investigated. Of the TCDF isomers investigated, only 2347- and 2348-TCDF did not fully resolve from 2378-TCDF. Other co-elutions that were observed included 234678-HxCDF with 123489-HxCDF and 12378-PeCDF with 12348-PeCDF.
Table 5: A comparison of possible non-2,3,7,8-substituted PCDD/PCDF co-eluters on commonly used HRGC columns (Ryan et al., 1991) and a selected pSFC column under optimized separation conditions.

<table>
<thead>
<tr>
<th>2,3,7,8-Substituted PCDD/PCDF</th>
<th>HRGC: DB-5 (1° column)</th>
<th>HRGC: DB-225 (2° column)</th>
<th>HRGC: SP-2331 (3° column)</th>
<th>pSFC: Torus 1-AA(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDF</td>
<td>2347-TCDF</td>
<td>no co-eluters(^c)</td>
<td>2348-TCDF(^a)</td>
<td>2347-TCDF(^a)</td>
</tr>
<tr>
<td></td>
<td>2348-TCDF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2349-TCDF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2379-TCDF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2346-TCDF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23478-TeCDF</td>
<td>12489-TeCDF</td>
<td>no co-eluters</td>
<td></td>
<td>no co-eluters</td>
</tr>
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<td>1279-TeCDF</td>
<td>12348-TeCDF(^a)</td>
<td>13469-TeCDF</td>
<td>12348-TeCDF(^a)</td>
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<td>123678-HxCDF</td>
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<td>123789-HxCDF</td>
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<td>234678-HxCDF</td>
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<td>123789-HxCDD</td>
<td>123467-HxCDD</td>
<td>no co-eluters</td>
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\(^a\)Partially resolved
\(^b\)Congeners investigated: 2348-TCDF, 2347-TCDF, 2346-TCDF, 1279-TCDF, 12348-TeCDF, 123467-HxCDF, 123489-HxCDF, and 123467-HxCDD
\(^c\)In-house data

The resolution of the 2,3,7,8-substituted hexachlorinated PCDD/PCDF congeners was carefully examined. Through direct comparison of the pSFC and HRGC separations, it was evident that both the 2,3,7,8-substituted HxCDF and HxCDD congeners are better separated by HRGC. The height of the valley between 123678-HxCDF and 123478-HxCDF by HRGC is 13%, but this increases to 68% using pSFC. Similarly, HRGC affords baseline separation of 123789-HxCDF and 234678-HxCDF, but a valley of 24% was measured between these two isomers using pSFC. Also, the height of the valley between 123678-HxCDD and 123478-HxCDD is 24% by HRGC and 22% by pSFC using the developed method.

An HRGC column performance mixture, along with individual standards, was also analyzed to verify that adequate tetrachlorodibenzo-p-dioxin resolution could be achieved using the developed pSFC method. It was found that 2378-TCDD was separated from its nearest eluting congener (1234-TCDD) with a 14% valley. Another interesting finding was the partial separation of 1237-TCDD and 1238-TCDD using the pSFC method. These congeners co-elute on the DB-5 stationary phase, but are also
partially separated on DB-225 and SP-2331 during HRGC analysis of the same mixture.

In order to verify that other isomers, which are resolved by HRGC analysis, do not co-elute with 2,3,7,8-substituted congeners using the developed pSFC method, additional PCDD and PCDF standards would have to be tested or a sample containing all of the possible PCDD/PCDF congeners, such as in a fly ash extract, would have to be analyzed against any available standards. The use of a longer pSFC column, development of a method utilizing coupled columns, or an alternate stationary phase may also provide better resolution, but this requires further investigation.

PCBs are often analyzed concurrently with PCDDs and PCDFs, but when HRGC/HRMS is utilized there is significant overlap between the PCDD/PCDF and PCB windows. To examine the elution of mono-ortho and coplanar PCBs using the developed pSFC method, a solution containing the dioxin-like PCB congeners was analyzed and it was found that good separation could be achieved before the elution of 2378-TCDD (see Figure 5). The only complete co-elution observed is between PCBs 77 and 105, but since they belong to different homologue groups, tetrachlorinated and pentachlorinated respectively, they are separable using mass spectrometry. The increased retention of the coplanar PCBs on the Torus 1-AA column compared to the mono-ortho PCBs was also notable and the difference in retention of these congeners was attributed to decreased π-π interactions with increasing out-of-plane orientation.

U.S. EPA Method 8280B (U.S. EPA, 2007) is a low resolution mass spectrometric method for the analysis of PCDDs and PCDFs in water, soil, fly ash, and other matrices. In order to demonstrate the applicability of the developed pSFC method to environmentally relevant concentration levels, a calibration curve designed to be used with this regulated method was run to ensure that acceptable linearity could be achieved for all components. The method requires the percent relative standard deviation (%RSD) to be below 20% for each individual component and this was accomplished using the developed method when the data was collected in multiple reaction monitoring (MRM) mode (see Paper I, Table 2). A proficiency testing sample extract was also analyzed by both HRGC/HRMS and pSFC-MS/MS and the percent differences between the measured and reported results were found to be comparable with the average percent difference for the HRGC/HRMS data being 9.9% and that for the pSFC-MS/MS data being 21.0% (tabulated results are provided in the supporting information of Paper I). It should be noted that in order for the same
extract to be run on both the high resolution and low resolution detectors, its concentration was at the high-end of the HRGC/HRMS calibration and the low-end of the pSFC-MS/MS calibration (due to sensitivity limitations of the MS) and internal standards were only used in the generation of the HRGC/HRMS data since the spiked levels were too low to be detected using pSFC-MS/MS. The retention times of all of the PCDD and PCDF components were also found to be reproducible with an average standard deviation of 0.04 min over the course of the calibration.

Figure 5: Chromatograms illustrating the elution order and resolution of mono-ortho and coplanar PCBs (B; labeled with IUPAC number) in comparison to the 2,3,7,8-tetrachlorinated dibenzo-p-dioxin (A).

4.1.2 Optimization of the Atmospheric Pressure Ionization of PCDDs, PCDFs, and PCBs

A key aspect of method development included the optimization of ionization conditions for these legacy persistent organic pollutants. The utilization of positive ion atmospheric pressure chemical ionization and atmospheric pressure photoionization as well as charge transfer dopants/reagents facilitated the generation of molecular ion clusters. How-
ever, the coupling of pSFC to MS complicated atmospheric pressure ionization processes due to the large quantities of expanding carbon dioxide and presence of ionizable cosolvents (see Paper II).

The use of ABPRs in modern instrumentation limits the split to the MS detector since the regulator is located after the split. If the eluent split to the mass spectrometer is too large, the ABPR will not be able to maintain the system pressure required to keep the mobile phase in a sub- or supercritical state. If the ionization technique being employed is mass-flow sensitive (as in APCI and APPI) (Urban, 2016), the split ratio can directly affect MS sensitivity by limiting the amount of analyte reaching the detector. Conversely, a concentration-dependent ionization technique (such as ESI) (Urban, 2016) would be less affected by the split ratio and more influenced by the dilution factor arising from the addition of a make-up solvent.

The API/MS analysis of halogenated aromatic compounds such as PCDDs, PCDFs, and PCBs has generally been accomplished by monitoring the products of oxygen enhanced negative chemical ionization ([M-X+O]− where X is a halogen) (Dzidic et al., 1975, Guervremont et al., 1987, Lepine et al., 1996b, Luosujarvi et al., 2008, Moukas et al., 2014, Mitchum et al., 1982, Fernando et al., 2016). The usefulness of this ion cluster for detection and characterization of mixed halogenated dibenzo-p-dioxins has been demonstrated by Fernando et al. (Novotny, 1986); however, its dependence on oxygen levels in the MS source and the possible formation of multiple clusters with varying degrees of halogen loss can negatively affect detection limits when less sensitive mass spectrometers are utilized. Paper II examines positive ion API of halogenated environmental contaminants (PCDDs, PCDFs, and PCBs) for the formation of molecular ion clusters, [M]+, when coupling pSFC to mass spectrometry and also investigates factors affecting pathway promotion and quenching of charge transfer species.

During API it is possible to generate both radical species and protonated molecules that are able to interact with analytes and transfer charge; therefore it is common to observe multiple ionization products which subsequently result in a decrease in overall sensitivity for a target analyte. Conditions need to be determined that maximize the formation of a single ionized species with minimal fragmentation in order to maximize detection limits and limit cross-talk between MS channels. Although branched cycloalkanes (specifically methylcyclopentane and methylcyclohexane) were found to be promising charge transfer reagents
for PCDDs and PCDFs in positive ion APPI and APCI through generation of the $M^+$ cluster during infusion experiments (see Paper II, Figure 1), complete quenching of the reactive cationic species was observed when the protic cosolvent of the pSFC eluent was introduced into the source. Subsequent infusion experiments conducted using cyclohexane and its deuterated analogue, cyclohexane-d$_{12}$, confirmed that the reactive species generated ($C_3H_5^+$ and $C_4H_7^+$) were completely quenched by the pSFC eluent, presumably by electron transfer, rendering them unusable in conjunction with the investigated separation technique. Alternatively aromatic dopants (toluene, trifluorotoluene, and fluorobenzene) were found to be less susceptible to quenching by the pSFC eluent and fluorobenzene was determined to be the most effective charge transfer reagent for both PCDDs and PCDFs. The effectiveness of fluorobenzene as a charge transfer reagent was attributed to the stabilizing ability of the fluorine atom attached to the benzene ring resulting in a $C_6H_5F^+$ cation which is less susceptible to quenching by the pSFC eluent. Using this dopant, it was possible to generate molecular ion clusters of PCDDs and PCDFs with minimal fragmentation when positive ion APPI-MS was coupled to pSFC and the concurrent analysis of thermally labile and legacy halogenated environmental contaminants was also demonstrated. The ability to toggle between positive and negative ion modes allowed for the isomer specific analysis of α-, β-, and γ-HBCDD as well as representative legacy environmental contaminants, PCB-77, PCB-126, PCB-169, 2378-TCDD, and 2378-TCDF (see Paper II, Figure 2).

4.2 Analysis of Emerging Environmental Contaminants

The Stockholm Convention has been amended several times since its inception to include a variety of additional environmental contaminants. In 2009, chlordecone, isomers of hexachlorocyclohexane (α-, β-, and γ-HCH), hexabromobiphenyl, polybrominated diphenyl ethers (tetra-, penta-, hexa-, and hepta-bromodiphenyl ethers), perfluorooctanesulfonic acid and its salts (PFOS), perfluorooctanesulfonyl fluoride (PFOSF), and pentachlorobenzene were incorporated (Xu et al., 2013). Endosulfan was added in 2011 and HBCDD was added in 2013. At the last meeting in 2015, the conference of parties also listed hexachlorobutadiene, pentachlorophenol and its salts and esters, and polychlorinated naphthalenes (Stockholm Convention, 2008). Although the Stockholm Convention identifies POPs of increased concern due to their measured lifetime, global distribution, bioaccumulation potential, and toxicity, the
scope of “emerging environmental contaminants” seems to be ever expanding. With the majority of new POPs being produced intentionally for various commercial and industrial applications (Lohmann et al., 2007), the scientific literature is continually being updated with newly discovered environmental contaminants of concern (Richardson, 2008).

Well studied groups of environmental contaminants not currently listed on the Stockholm Convention include chlorinated paraffins (Campbell and McConnell, 1980, Bayen et al., 2006), pharmaceuticals and personal care products (Caliman and Gavrilescu, 2009, Liu and Wong, 2013), per- and poly-fluorinated substances (Kannan, 2011, Krafft and Riess, 2015), and flame retardants (Wei et al., 2015, Lyche et al., 2015, Wang et al., 2016). For the purpose of demonstrating the potential application of pSFC for the separation of emerging environmental contaminants, three different types of flame retardants (with varying chemical functionality: polychlorinated, polybrominated, and organophosphorus) were selected for investigation.

4.2.1 Achiral pSFC/MS Analysis of the Polychlorinated Flame Retardant Dechlorane Plus

The use of a hybrid MS source in both positive and negative ionization modes highlights the versatility of pSFC when this separation technique is coupled to a sensitive and selective MS detector. In order to develop ionization parameters relevant for the variety of chemical functionalities observed during environmental screening, the optimization of ionization conditions for non-aromatic environmental contaminants such as Dechlorane Plus (DP) was investigated. Reaction mechanisms associated with the commonly observed [M-Cl+O]- ion cluster were examined in an attempt to optimize signal strength (see Paper III).

Dechlorane Plus was chosen for investigation because it is a highly chlorinated additive flame retardant that is produced globally and utilized in a variety of applications such as electrical wire and cable coatings, computer connectors, and roofing materials (Feo et al., 2012). DP exists in two isomeric forms known as syn- and anti-DP (abbreviated as s-DP and a-DP respectively; see Paper III, Figure 1). Since its first detection in air and sediment samples from the Great Lakes region of North America in 2006, DP has received a significant amount of interest from the scientific community (Shen et al., 2011a, Sverko et al., 2011, Wang et al., 2016). It is typically analysed using gas chromatography coupled to ECNCI mass spectrometry (Hoh et al., 2006). This ionization method has been favoured over EI due to the observation of extensive fragmentation and
extremely low intensity of the molecular ion using the latter technique (Hoh et al., 2006). Although the most widely used analytical technique for the detection of DP in environmental samples remains GC with MS detection, other methods of separation and ionization have been reported, such as LC coupled with atmospheric pressure ionization MS (Guerra et al., 2011, Zhou et al., 2011), for the simultaneous analysis of DP with LC amenable analytes. Since pSFC offers the possibility of analysing both LC and GC amenable analytes using a single analytical technique, the optimization of DP for pSFC/MS analysis seemed fitting.

APCI tune parameter optimization for DP was first performed via MS infusion experiments in negative-ion mode. A positive correlation between superoxide formation and the signal response associated with the [M-Cl+O]- cluster was noted leading to an optimization of ionization parameters based on the formation of the reactive superoxide species, O2-. The observation of an [M+O2]- cluster during the ionization process supported the oxidation product mechanism proposed by Lépine et al. (Lepine et al., 1996a) and suggested that the rate-limiting step in the ionization process was the transfer of chlorine to the oxygen atom before dissociation of ClO-. Furthermore, superoxide formation was observed to be temperature dependent (see Paper III, Figure 2) and the higher analyte response resulting from addition of a dopant to the MS make-up solvent, to suppress possible side reactions (see Figure 6), was investigated.

It was determined that the ideal APCI reagent should scavenge nitrogen dioxide (NO2) without introducing a protic source which could result in rapid quenching of the superoxide species. Infusion experiments were conducted with triethylamine (TEA) as the APCI reagent in acetonitrile. It was postulated that if ammonia can scavenge NO2, then TEA might be effective as well since it has been reported that TEA can react with NO/NO2/H2O mixtures to form diethylnitroamine (White, 1992). It is also possible for TEA to act as an electron donor to aid in the formation of O2-. At the optimized level of 0.15% TEA in acetonitrile, a 52% increase in the a-DP signal ([M-Cl+O]- ion cluster) was observed. The separation of DP compounds using pSFC was also established in Paper III and the applicability of this technique was demonstrated by quantifying syn- and anti-DP in Lake Ontario sediment. The calibration was found to be linear for all compounds investigated (see Paper III, Figure S4) with linear regression (R2) values greater than 0.993 and %RSDs of less than 20% obtained. Syn- and anti-DP were the only compounds related to DP detected in the sediment sample and levels were back-calculated to be 8.2
and 10.0 ng/g respectively. These results fall close to the range of DP concentrations reported in the scientific literature for Lake Ontario sediments (Shen et al., 2011b, Sverko et al., 2008).

4.2.2 Chiral pSFC/MS Analysis of the Polybrominated Flame Retardant HBCDD

In order to establish the wide applicability of this technique, the utilization of preparative scale pSFC for the chiral separation and isolation of 1,2,5,6,9,10-hexabromocyclododecane (HBCDD) stereoisomers was investigated (see Paper IV). Characterization of the isolated enantiomers was completed using published chiral LC methods/elution profiles, as well as x-ray crystallography. Additionally, the resolution of the enantiomers (i.e. the (+) and (-) enantiomers of α-, β-, and γ-HBCDD), along with two minor components of the technical product (δ- and ε-HBCDD), was investigated on an analytical scale using both LC and pSFC separation.
techniques and changes in elution order were highlighted. The development of preparative scale separations emphasized the potential associated with pSFC as a green method of isolating and analyzing environmental contaminants of concern.

The additive brominated flame retardant HBCDD was selected for investigation due to the analytical challenges associated with this particular class of compound. It is utilized at percent levels in extruded and high-impact polystyrene foams and to a lesser extent in electrical equipment housings (Alaee et al., 2003), and since it is not chemically bound to the products into which it is incorporated, movement into environmental matrices is possible. The technical mixture is comprised mainly of 3 pairs of enantiomers (75-89% \(\gamma\)-HBCDD, 10-13% \(\alpha\)-HBCDD, and 1-12% \(\beta\)-HBCDD) with two additional meso forms having been reported to be present at very low levels (\(\delta\)- and \(\varepsilon\)-HBCDD) (Covaci et al., 2006). High temperatures are known to induce thermal isomerization of the \(\alpha\)-, \(\beta\)-, and \(\gamma\)-HBCDD diastereomers (Koeppen et al., 2008, Heeb et al., 2008, Peled et al., 1995) resulting in the detection of a single broad peak during GC separations, therefore isomer specific analyses are currently conducted by LC with MS detection. Enantiomer specific analysis of HBCDD has generated significant interest since enzymes involved in the degradation of this environmental contaminant may be enantioselective as with other chiral POPs (Borga and Bidleman, 2005).

Baseline separation of all HBCDD enantiomers was accomplished using an analytical CEL2 [cellulose tris-(3-chloro-4-methylphenylcarbamate)] column and a 2-propanol modified carbon dioxide mobile phase. HBCDD isomers can be grouped by their most stable conformations (determined by x-ray structure determination), with \(\alpha\) being classified as “square”, \(\beta\) “quadrilateral”, and \(\gamma\) “irregular” (with major distortion) (see Figure 7). The orientation of the bromine atoms in these stable conformations and their subsequent availability for hydrogen bonding (presumably to the N-H of the chiral stationary phase carbamate) may then be a major factor in dictating the retention of each isomer.
All of the isolated HBCDD enantiomer fractions, as well as a technical HBCDD sample, were analyzed by LC/MS to confirm enantiomeric purity using a Nucleodex β-PM chiral analytical column which is commonly cited in enantioselective liquid chromatography separations of HBCDD (Koeppen et al., 2007, Janak et al., 2005, Heeb et al., 2007, Heeb et al., 2008). Figure 8 illustrates the LC and pSFC separations achieved. Of interest is the change in elution order from the traditional chiral LC separation. That is, (-)-α-HBCDD elutes first during the chiral LC separation followed by (-)-β-, (+)-α-, (+)-β-, δ-, (+)-γ-, ε-, and finally (-)-γ-HBCDD, but the elution order changes to (-)-α-, (+)-α-, δ-, (-)-γ-, (+)-γ-, ε-, (-)-β-, and finally (+)-β-HBCDD by pSFC. Although the overall elution order is very different, if the enantiomer pairs are examined specifically, only the (+)/(-)-γ-HBCDD enantiomer pair changes order. Also, baseline separation of all enantiomers was achieved and both δ- and ε-HBCDD demonstrate adequate resolution from their closest neighbour (10% valley between δ-HBCDD and (-)-γ-HBCDD and baseline resolution between ε-HBCDD and (+)-β-HBCDD). The successful application of a green enantioselective separation technique (chiral pSFC) to an environmental contam-
inant of concern emphasizes the potential associated with this method for analyzing and isolating similar compounds.

Figure 8: Chiral analytical scale (A) pSFC/MS and (B) LC/MS separation of a technical HBCDD mixture

4.2.3 pSFC/MS Analysis of Halogen-Free Phosphorus Flame Retardants

Finally, following the determination of the constituents of three technical mixtures of halogen free organophosphate flame retardants [resorcinol bis(diphenyl phosphate) (RBDPP), bisphenol A bis(diphenyl phosphate) (BPA-BDPP), and 9,10-dihydro-9-oxa-10-phosphaphenanthrene-10-oxide (DOPO)], multiple analytical separation techniques (GC, LC, and pSFC) were investigated for the analysis of these products. The advantages and disadvantages of each separation technique were highlighted and ionization products were compared (see Paper V).

The additive phosphorus flame retardants RBDPP and BPA-BDPP were chosen for investigation along with the reactive DOPO due to their emergence as alternative flame retardants as well as the challenges associated with analyzing these OPFRs using a single analytical technique (see Figure 9 for structures). Existing and emerging regulations are forcing the chemical industry to develop halogen-free flame retardants (HFFRs) for use in a growing number of polymer based electronic consumer products (van der Veen and de Boer, 2012, Brandsma et al., 2013) and as such, phosphorus-based flame retardants are receiving notable attention (Levchik and Weil, 2006). RBDPP and BPA-BDPP are currently being used as replacements
for BDE-209 primarily in electronic plastics (Ballesteros-Gomez et al., 2014, Roth et al., 2012, Ballesteros-Gomez et al., 2016) such as television housings composed of acrylonitrile-butadiene–styrene (ABS), polyphenylene oxide (PPO)/high impact polystyrene (HIPS), or polycarbonate (PC)/ABS) (Brandsma et al., 2013, Ballesteros-Gomez et al., 2014). DOPO, and its derivatives, have demonstrated significant improvements in fire suppression at decreased loadings (as low as 2-3% in epoxy resins (Schartel et al., 2007)), but are known to cause esthetic issues such as blooming and discoloration of polymers processed at high temperatures (Lee et al., 2014).

Figure 9: Chemical structures of RBDPP \((n = 1 – 4)\) and BPA-BDPP \((n = 1 – 2)\) as well as the closed and open forms of DOPO.

The compositions of the RBDPP and BPA-BDPP technical mixtures were first determined by isolation of their components using preparative thin-layer chromatography (prep-TLC) and characterization using proton \((^1\text{H})\) and phosphorus \((^{31}\text{P})\) nuclear magnetic resonance spectroscopy (NMR). The technical RBDPP and BPA-BDPP samples were analyzed by LC/MS and pSFC/MS and the percentages of each component were determined assuming equal response factors (results are presented in Paper V, Table 1). Although a certain degree of variation is expected to be present between the analytical and gravimetric techniques utilized to characterize these samples, the overall variation in relative percentages was found to be quite low (percentages determined by LC/MS and pSFC/MS were within \(\pm 9\%\) of the molar percentages that were determined by \(^{31}\text{P}\) NMR).
DOPO can exist as multiple chemical forms (Paper V, Figure 3), but through multiple NMR experiments it was possible to definitively assign $^1$H NMR signals to the open and closed forms. A sample of technical DOPO was dissolved in deuterated methanol and integration suggested a ratio of 65% of the closed form and 35% of the open form (Figure 10). LC/MS conditions could not be determined which resulted in the resolution of the two main chemical forms of DOPO and GC/MS resulted in the detection of only a single species indicating that the thermally sensitive open form of DOPO closes when exposed to elevated injector port temperatures. Indeed, attempted optimizations of both LC and GC methods for DOPO resulted in shifts in the chemical equilibrium resulting in percentages of the open and closed forms of this compound that were not representative of that present in the actual sample.

![Figure 10: $^1$H NMR spectrum of technical DOPO dissolved in deuterated methanol.](image)

Of the analytical methods examined, the concurrent analysis of all components of the three technical samples was only accomplished using pSFC (see Figure 11). During ESI, both RBDPP and BPA-BDPP produced the expected ionized species ([M+H]$^+$ and [M+Na]$^+$) in positive ion mode, but multiple ionized forms of DOPO were detected in both positive and
negative ion mode depending on the separation method utilized (see Paper V, Figure 5 and Table S1). Although the open form of DOPO was separable from the predominant closed form using pSFC, the open phosphinic acid underwent a ring closure during negative ESI resulting in a ion corresponding to \([M-3H]^-\) (see Paper V, Figure 5). Multiple ionized species were also detected during LC/MS analysis and chemical forms are discussed in Paper V. This result further indicates that DOPO can exist in multiple chemical forms depending on its chemical environment.

**Figure 11:** pSFC/MS chromatogram illustrating the concurrent analysis of the components of technical RBDPP, BPA-BDPP, and DOPO.
5.0 Conclusions and Future Work

The main objectives of this thesis were achieved by demonstrating the applicability of pSFC/MS to well-known classes of environmental contaminants. Analytical methods were developed for legacy POPs (PCDDs, PCDFs, and PCBs) as well as the emerging environmental contaminant DP, and issues relating to the ionization of target analytes when pSFC was coupled to MS were explored. The use of fluoro benzene as an APPI dopant for halogenated aromatic compounds (PCDDs, PCDFs, and PCBs) was introduced and the relationship between APCI probe temperature and superoxide formation was highlighted for optimal formation of the [M-Cl+O]\(^-\) ion cluster of the non-aromatic polychlorinated DP analytes. The applicability of the developed methods to environmental samples was also demonstrated through the extraction and analysis of real samples.

The possibility of both chiral and preparative scale pSFC separations was demonstrated through the isolation and characterization of thermally labile HBCDD stereoisomers. The analytical separation of the \(\alpha\), \(\beta\), and \(\gamma\)-HBCDD enantiomers as well as the \(\delta\) and \(\epsilon\) meso forms was shown to be superior to results obtained using a published LC method without the need for column coupling. Possible retention mechanisms associated with the chiral stationary phases investigated were also discussed with respect to the observed separations.

The usefulness of pSFC as a separation technique for a group of related compounds which are challenging to analyze concurrently by other means was also demonstrated. Technical mixtures of phosphorus flame retardants RBDPP, BPA-BDPP, and DOPO were analyzed using multiple analytical techniques and pSFC was found to be the only method which facilitated the accurate determination of the components of all 3 mixtures. Issues relating to the handling and analysis of DOPO were specifically highlighted since the structure of this particular compound proved to be highly dependant on its chemical environment.

The possibility of simultaneous analysis of multiple compound classes as well as the inclusion of thermally labile compounds in a single targeted analysis exists with pSFC technology if suitable ionization and detection methods are available. Indeed, the use of this alternative separation technique may provide a fast, green, and cost effective means of analyzing environmental samples and, at the very least, serve as a complementary technique to GC and LC. Based on the results obtained in this doctoral
work, the application of pSFC/MS to other emerging compound classes should continue to be investigated. The development of comprehensive pSFC/MS methods for the screening of environmental samples could reduce operating costs, increase through-put, and drastically reduce solvent consumption.

The separation of complex technical mixtures such as polychlorinated paraffins and toxaphene should also be investigated since pSFC may provide a capacity for separation that differs from GC. Also, a significant amount of work has been published on the pSFC separation of pharmaceutical compounds (Perrenoud et al., 2014, Desfontaine et al., 2015) such as doping agents (Novakova et al., 2015, Novakova et al., 2016, Parr et al., 2016, Desfontaine et al., 2016), cannabinoids (Berg et al., 2016), and androgenic steroids (Quanson et al., 2016). These works were not highlighted in this thesis because environmental samples were not targeted, but the application of this technology to the detection of pharmaceutical compounds in environmental matrices is very promising and should also be explored further.
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