# INVESTIGATION OF LIQUID TRAPPING

# FOLLOWING SUPERCRITICAL FLUID EXTRACTION

by

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## ABSTRACT

Supercritical fluid extraction (SFE) is an alternative to traditional extractions with organic solvents. SFE consists of removing the analyte(s) from the matrix, solubilizing them, moving the analyte(s) into the bulk fluid, and sweeping the fluid containing the analyte(s) out of the extraction vessel.

As the fluid leaves the extraction vessel, decompression of the fluid occurs, changing its volume and temperature which can lead to analyte loss.

This work focussed on the trapping process with the restrictor immersed in a liquid after SFE. Experiments compared the effects of trapping parameters on the collection efficiencies of fat-soluble vitamins of similar polarities and structures. The most important variable was the selection of collection solvent and its physical properties, such as viscosity, surface tension and density were found to be important.

Additionally, adding a modifier to the collection solvent in an attempt to change its physical properties and influence collection efficiencies for a polarity test mix was studied. Addition of a modifier can improve collection efficiencies and allow higher collection temperature to be used, but the modifier did not increase trapping recoveries to the extent that collection pressurization did.

The occurrence of a methylation reaction of decanoic acid during the SFE and collection processes, using a methanol modified fluid or collection solvent was investigated. The majority of the reaction occurred during the

collection process and the degree of methylation was found to be dependent on temperature, but not on static or dynamic extraction time. When no additional acidic catalyst other than carbon dioxide in the presence of water was present, conversion was limited to about 2%, but was quantitative with an added acidic catalyst.

The last portion of this work involved the application of the SFE process to the extraction and analysis of extractable material in eight hardwood and softwood pulp samples. Grinding the samples increased extractable fatty acid methyl esters (FAMEs) by ten-fold, and *in-situ* derivatizations resulted in higher FAME recoveries than derivatization after SFE. Liquid trapping enhanced recoveries of lower FAMEs when compared to tandem (solid/liquid) trapping. *In-situ* acetylations sometimes yielded acetylated glucoses. Large differences in FAMEs concentrations were seen for hardwood samples, but lesser differences were seen for the softwood pulp samples.

# DEDICATION

This work is dedicated to the people who have sacrificed their time to make this dissertation a reality. My husband, Carl T. McDaniel, has endured many days and nights of little or no help with the normal tasks of running a household and raising three extremely energetic children. His patience, motivation and encouragement have given me the will to continue during those times that I lacked the confidence and energy necessary. My children, Shannon, Matthew and Eric, have patiently waited for the time that I, along with the "good" computer would be a little more available to them. To these four people I offer a humble thank you and lovingly dedicate this dissertation to you.

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# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# CHAPTER ONE

# TRAPPING FOLLOWING SUPERCRITICAL FLUID EXTRACTION

## 1.0 Introduction

Supercritical fluid extraction (SFE) offers an attractive alternative to liquid-liquid and liquid-solid extractions for many reasons.[1] Carbon dioxide ($CO_2$) is the most commonly used fluid for SFE and presents very little environmental or toxicological hazards, in direct contrast to the most commonly employed liquid solvents. In addition, carbon dioxide is non-reactive with most analytes, and allows successful extraction of light or air sensitive compounds. A fluid passes into the supercritical state as it exceeds its critical temperature and critical pressure, and the resulting supercritical fluid (SF) has both gas-like and liquid-like properties. The densities of SFs are generally high like those of liquids and offer the ability to solubilize many materials. Additionally, the diffusivity of the SF will be higher than the material in the corresponding liquid state. This higher diffusivity allows faster penetration into a matrix and thus the extraction of an analyte with a SF occurs at a much faster rate than a corresponding liquid or gaseous extraction.

For successful SFE, the process can be considered to occur in at least two steps. In the first step, the analyte is extracted from the bulk matrix after being solubilized by the supercritical fluid. Many references exist concerning

the optimization of the extraction step.[2,3,4,5,6,7,8,9,10] After this extraction step is complete, it is necessary to trap the analytes prior to identification and quantitative analysis. It is during this trapping step that the SF decompresses to atmospheric pressure. The resultant expansion offers a great chance for the loss of analytes, since each milliliter of supercritical $CO_2$ expands to about 500 mL gas.

## 1.1 Solid Phase Trapping

Several different trapping methods are used in SFE. One of the most commonly employed methods of trapping is onto an inert solid substrate or solid sorbent, followed by subsequent rinsing of the analyte from the trap with a suitable solvent. These traps can be stainless steel balls, or more frequently a chromatographic packing material, such as octadecyl bonded silica (ODS) or underivatized silica. Mulcahey and Taylor[11] conducted a study with non-modified $CO_2$ to determine the best solid phase trap composition and trapping conditions for a test mixture of analytes representing a wide range of polarities. Mulcahey, Hedrick, and Taylor[12] continued the work with a wider assortment of solid phase sorbent traps. They found that a single trap composition may not effectively trap a wide range of analyte polarities. Furthermore, the addition of a modifier to the extraction fluid caused a decrease in trapping efficiencies.

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[1] S. B. Hawthorne, *Anal. Chem*. **62**, (1990) 633a-642a.
[2] K. Li, C. P. Ong, and S. F. Y. Li, *J. Chromatogr. Sci.*,**32** (1994) 53-56.
[3] M. K. L. Bicking, *J. Chromatogr. Sci*., **30** (1992) 358-360.
[4] A. Meyer and W. Kleiböhmer, *J. Chromatogr. A,* **657** (1993) 327-335.
[5] G. Maio, C. von Holst, B. W. Wenclawiak, and R. Darskus, *Anal. Chem.,* **69** (1997) 601-606.
[6] V. Lopez-Avila, N. S. Dodhiwala, and W. F. Beckert, EPA/600/S4-90/026, 1991.
[7] J. R. Barnabas, J. R. Dean, W. R. Tomlinson, and S. P. Owen, *Anal. Chem*., **67** (1995) 2064-2069.
[8] M. Kane, J. R. Dean, S. M. Hitchen, C. J. Dowle, and R. L. Tranter, *Anal. Chim. Acta*, **271** (1993) 83-90.
[9] M. K. L. Bicking, T. G. Hayes, J. C. Kiley and S. N. Deming, *J. Chromatogr. Sci.*, **31** (1993) 170-176.
[10] I. Fernandez, J Dachs, and J. M. Bayona, *J. Chromatogr. A*, **719** (1996) 77-85.
[11] L. J. Mulcahey and L. T. Taylor, *Anal. Chem*., **64** (1992) 2352-2358.

Eckard and Taylor[13] found that solid phase trapping has an additional drawback in that the sorbent trap has a finite capacity. They found a 50/50 mixture of Porapak Q® and glass beads exhibited the highest sample capacity and was the most effective trap for a wide range of analyte polarities. In another study, Moore and Taylor[14] found that stainless steel ball trap efficiency is greatly affected by the addition of a modifier to the SF. They found that it was necessary to raise the trap temperature in order to achieve quantitative recoveries of digitalis glycosides.

## 1.2 Novel Trapping Methods

In addition to the solid phase trapping described above, there are several other methods for trapping analytes. Vejrosta et al.[15] described a system for trapping triazine herbicides and a mixture of polycyclic aromatic hydrocarbons (PAH) after SFE with non-modified $CO_2$ in which methanol was constantly pumped into a fused silica trapping capillary, which was fitted over the end of the restrictor. They were able to achieve greater than 90% recovery for all analytes studied. The major advantage of their method is that quantitative recoveries can be obtained with smaller solvent volumes than with traditional liquid trapping. Vejrosta et al.[16] also reported a similar trapping scheme using controlled modifier condensation. In this method, 10% methanol modified $CO_2$ was used to extract a urea-based herbicide from inert glass beads. Trapping was accomplished in a manner similar to that discussed above, except a cryofocuser was added to aid in the condensation of

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[12] L. J. Mulcahey, J. L. Hedrick, and L. T. Taylor, *Anal. Chem*., **63** (1991) 2225-2232.
[13] P. R. Eckard and L. T. Taylor, *J. High Resol. Chromatogr*., **19** (1996) 117-120.
[14] W. N. Moore and L. T. Taylor, *Anal. Chem*., **67** (1995) 2030-2036.
[15] J. Vejrosta, J. Plantea, M. Mikesová, A. Ansorgová, P. Karásek, J. Fanta, and V. Janda, *J. Chromatogr. A*, **685** (1994) 113-119.
[16] J. Vejrosta, A. Ansorgová, J. Planeta, D. G. Breen, K. D. Bartle, and A. A. Clifford, *J. Chromatogr. A*, **683** (1994) 407-410.

the methanol modifier after decompression. They found that 75-83% of the analyte recovery occurred in the condensed modifier, with roughly 10% being in the rinse solvent. They also found that lower recoveries resulted when higher flow rates were used for extraction.

Vejrosta et al.[17] have also recently reported another method for analyte collection based on mixing the expanding supercritical fluid with overheated organic vapor. The assumed trapping mechanism involves the supercritical effluent leaving the restrictor and entering a fused silica capillary filled with the solvent vapor. The solvent vapor condenses on the active sites of the capillary resulting in microdrops (solvent only) or microparticles (solvent and analyte). These then recombine to from droplets of liquid, finally resulting in the creation of a moving liquid film at the inner capillary surface. They were able to quantitatively trap n-alkanes, selected PAHs, and a mixture of acetophenone, N,N-dimethylaniline and naphthalene under various conditions. They explored the use of different organic solvents and differing $CO_2$ flow rates. They found the method to use approximately one-tenth the solvent volume generally used, and to reduce the need for preconcentration after SFE.

## 1.3 Liquid Trapping

Various articles in the literature report the use of "liquid trapping." One version of liquid trapping involves immersion of the restrictor into a liquid, as illustrated in **Figure 1-1**, while a second version concerns an inert solid surface in tandem with a liquid trap. In the Dionex 703 model for example, non-volatile analytes are thought to deposit on a hang-down tube (solid surface) while the more volatile analytes partition into the collection liquid after decompression. A schematic of this type of trapping device is shown in **Figure 1-2.**

[17] J. Vejrosta, P. Karasek and J. Paneta, *Anal. Chem*., **71** (1999) 905-909.

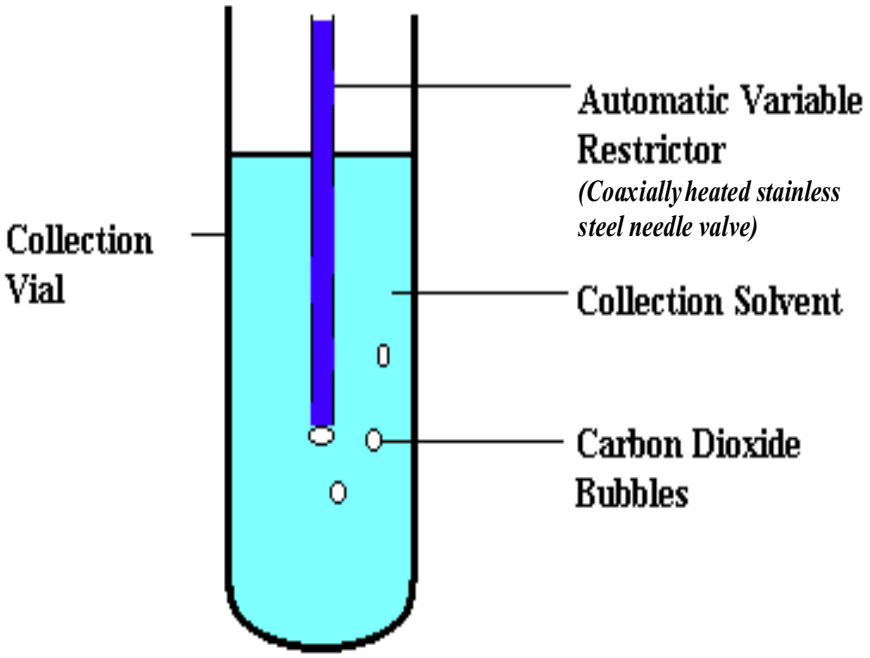


**Figure 1-1:** Schematic Representation of the Liquid Trapping Process

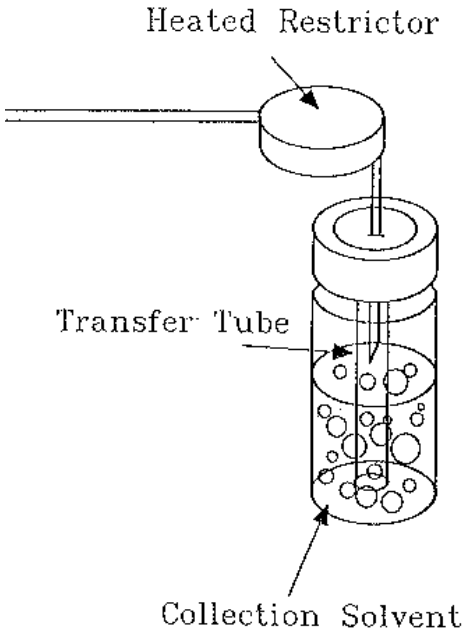


**Figure 1-2:** Schematic Representation of the Collection Systems Used in the Dionex Extractors

Yang et al.[18] compared solvent and sorbent trapping of volatile petroleum hydrocarbons after being extracted from soil. They found that both systems could effectively trap BTEX (benzene, toluene, ethylbenzene and xylenes) at greater than 90% recovery. They also found solid sorbents could quantitatively trap normal hydrocarbons of lower molecular weight (hexane) better than solvent trapping (heptane for pressurized collection, octane for non-pressurized). They used an Isco system with the restrictor immersed in the collection solvent. Approximately half the solvent volume (7 mL) was required with the trap pressurization as was required (15 mL) without trap pressurization for similar recoveries.

Ashraf-Khorassani et al.[19] compared collection efficiencies in: (a) an empty vial, (b) liquid filled collection vessel, and (c) cryogenically cooled adsorbent trap for several PAHs. Recoveries into the empty collection vial were no greater than 23%, while the liquid collection method resulted in recoveries no

greater than 38%. Reduction of the liquid carbon dioxide flow rate to less than 1 mL/min did not improve the recoveries for the empty vial, but resulted in quantitative (>90%) recovery in the liquid trap. Only methylene chloride was investigated for the solvent trap, and only two flow rates, 0.9 and 2.0 mL/min, were used.

Langenfeld et al.[20] performed an extensive study on the effects of collection solvent parameters and extraction cell geometries on SFE efficiencies. A mixture of 66 compounds of a wide range of polarities were extracted and trapped into one of five organic solvents (methylene chloride, chloroform, acetone, methanol, or hexane). Height of the collection solvent, volume of the solvent, and effect of collection temperature on trapping

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[18] Y. Yang, S. B. Hawthorne and D. J. Miller, *J. Chromatogr. A*, **699** (1995) 265-267.
[19] M. Ashraf-Khorassani, R. K. Houck, and J. M. Levy, *J. Chrom. Sci.*, **30** (1992) 361-366.
[20] J. J. Langenfeld, Mark D. Burford, S. B. Hawthorne and D. J. Miller, *J. Chromatogr.*, **594** (1992) 297-307.

efficiencies were all investigated. They found temperature control of the collection vessel at 5°C provided the best recoveries. When addressing the effect of the collection solvents, they concluded that the boiling point of the solvents did not appear to be important, and that methylene chloride was the best solvent while hexane was the worst for these analytes and conditions.

Maio et al.[21] used the Dionex 703M system for the extraction of some chlorinated benzenes and cyclohexanes from soil. They found the behavior of their extracted compounds in the trapping solvent difficult to predict. They recommended that the collection process be thoroughly examined for any new set of analytes prior to optimization of the extraction conditions. Extraction efficiencies were optimized using a factorial design. Porter et al.[22], using a Dionex system, found that the addition of inert bodies such as steel shot, plastic beads or glass wool, and the use of a stirring bar during trapping gave lower recoveries than simply trapping in the liquid.

Wenclawiak et al.[23] using a similar system to that of Porter[22] found that trapping was analyte dependent, and that they were unable to improve the collection efficiency of hexachlorocyclohexane by extending the collection of analyte over a longer period of time, or changing the trapping solvent to different binary mixtures. They found adding glass beads improved recoveries, but the most significant effect was the increase in precision when compared to solvent trapping alone. They also found that by adding a sintered frit and glass fiber filter, along with the glass beads and the solvent, both recoveries and precisions were improved, in direct contrast to Porter's work.

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[21] G. Maio, C. von Holst, B. W. Wenclawiak, and R. Darskus, *Anal. Chem.,* **69** (1997) 601-606.
[22] N. L. Porter, A. F. Rynaski, E. R. Campbell, M. Saunders, B. E. Richter, J. T. Swanson, R. B. Nielsen and B. J. Murphy, *J. Chromatogr. Sci*., **30** (1992) 367-373.
[23] B. W. Wenclawiak, G. Maio, Ch. v. Holst, and R. Darskus, *Anal. Chem*., **66** (1994) 3581-3586.

Thompson et al.[24] found, using the Dionex system and non-modified $CO_2$, that a polarity test mix could not be quantitatively (above 90%) trapped when using a single pure collection solvent. They were only able to quantitatively recover all analytes by using mixed collection solvents. They were unable to correlate any of the solvents' physical properties, such as boiling point, density, viscosity, surface tension, or Hildebrand solubility parameter with collection efficiency. Thompson and Taylor[25] continued this work with acetonitrile-, methanol-, and toulene- modified $CO_2$, and found that the highest recoveries were achieved not with the mixed collection solvents, but instead with pure collection solvents. Hexane proved to be the best overall solvent with poor recoveries seen only when 8% toluene was the extraction fluid modifier.

From this body of work it can be seen that the liquid trapping process, especially in which the restrictor tip is immersed in a liquid, is not well understood, and warrants further study.

## 1.4 Research Objective

It is the objective of this work to attempt to elucidate the process of liquid trapping with direct restrictor immersion. Chapter 2 presents a study of the role of the physical properties of pure collection solvents upon the trapping efficiency of several fat-soluble vitamins of similar solubilities and polarities. Chapter 3 involves a study of the effect of adding modifiers to the collection solvent in order to modify its physical properties. This work uses a test mixture of differing polarities similar to that commonly reported in the literature. The focus of Chapter 4 shifts to the potential reactivity of liquid solvent traps. This phenomenon is illustrated via the formation of fatty acid methyl esters from their

[24] P. G. Thompson, L. T. Taylor, B. E. Richter, N. L. Porter, and J. L. Ezzell, *J. High Resol. Chromatogr.*, **16** (1993) 713-716.
[25] P. G. Thompson and L. T. Taylor, *J. High Resol. Chromatogr.*, **17** (1994) 759-764.

corresponding fatty acids, both during the supercritical fluid extraction and trapping processes. Chapter 5 presents the application of the previous three chapters for the successful SFE and trapping of extractable fatty acids from wood pulp samples. In this Chapter a comparison of solid/liquid (tandem) trapping and liquid trapping alone is presented, as well as a comparison of fatty acid derivatization methods. This work concludes with Chapter 6, which summarizes the findings of the four previous chapters.

# CHAPTER TWO

# THE ROLE OF COLLECTION SOLVENT PHYSICAL PROPERTIES ON LIQUID TRAPPING EFFICIENCIES

## 2.0 Introduction

As a supercritical fluid, carbon dioxide ($CO_2$) is non-reactive with most analytes and allows successful supercritical fluid extraction of light or air sensitive compounds such as fat soluble vitamins. Because of these properties the extraction of these vitamins from a variety of food matrices has recently received a good deal of attention.[26,27,28,29] Traditionally, these vitamins were extracted from pharmaceutical and food matrices with an organic solvent following a saponification step to remove interfering lipids, and then the resulting solution was subjected to a solvent reduction step before subsequent analysis.[26,27] These methods are long, labor intensive, tedious, and afford ample opportunity for oxidation of the analytes to occur. The use of supercritical carbon dioxide to extract these analytes should decrease the likelihood of analyte losses during sample preparation and may prove very advantageous.

Several published reports describe the extraction of fat-soluble vitamins from various matrices with differing fat contents.[27,28,29] Schneiderman et al.[28] extracted Vitamin K from a powdered infant formula with non-modified $CO_2$ and analyzed the extracts with high performance liquid chromatography with

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[26] AOAC. *Official Methods of the Association of Official Analytical Chemists*, 13th ed., AOAC: Washington, D.C., 1992.
[27] *U. S. Pharmacopeia*, 22nd Revision, U. S. Pharmacopeia Convention: Rockville, MD, 1990.
[28] M. A. Schneiderman, A. K. Sharma, K. R. R. Mahanama and D. C. Locke, *J. Assoc. Off. Anal. Chem.*, **71**, (1988) 815-817.
[29] S. Scalia, G. Ruberto, and F. Bonina, *J. Pharm. Sci.*, **84** (1995) 433-436.

electrochemical detection. They were able to achieve quantitative recoveries in just 15 minutes at an extraction vessel pressure of 8000 psi (545 atm) and temperature of 60°C. Only a static extraction step was used, without a defined dynamic step. The analytes were trapped onto a silica gel solid phase. Scalia, Ruberto, and Bonina[29] extracted Vitamins A and E, along with their esters from tablet preparations using non-modified $CO_2$ at 250 atm and 40°C at a decompressed flow of 190-220 mL/min in 15 minutes. They achieved vitamin recoveries of over 95%. Analytes were trapped into a liquid collection vial containing tetrahydrofuran (THF) at 0°C, wherein the fixed restrictor tip was just above the surface of the THF. Masuda et al.[30] extracted retinol palmitate and tocopherol acetate from a hydrophobic ointment using non-modified $CO_2$ at 200kg/cm$^2$ (194 atm) and 40°C. They used a liquid flow rate of 4 mL/min for 4 minutes and trapped onto a solid support of deactivated silica gel, prior to on-line supercritical fluid chromatography. Quantitative recoveries of 102% were observed for both analytes using these rather gentle extraction conditions. Prior to extraction, it was necessary to mix the ointment with diatomaceous earth powder.

Decompression of a supercritical fluid (SF) in a liquid can be considered to be analogous to the formation of a bubble at a submerged, wetted orifice, under constant flow conditions. Using fluid dynamics models, the factors affecting the size or volume of the bubble formed at the restrictor tip may be considered to be the: (a) restrictor internal diameter, (b) SF superficial velocity through the orifice, (c) gravitational acceleration, as well as the (d) density, viscosity, and surface tension of the trapping liquid.[31] If the analyte molecule is surrounded by extraction fluid molecules, it becomes obvious that smaller bubbles offer greater likelihood for the analyte molecule to pass more quickly

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[30] M. Masuda, S. Koike, M. Handa, K. Sagara, and T. Mizutani, *Analytical Sciences*, **9** (1993) 29-32.
[31] *Encyclopedia of Fluid Mechanics, Volume 3: Gas-Liquid Flows*, Gulf Publishing Co.: Houston, TX, 1986.

and efficiently into the liquid trap, due to the increased relative surface area of the bubble. The restrictor internal diameter is directly related to the velocity of the SF through the restrictor at fixed pressure, and can be adequately represented by flow rate (measured as a liquid at the pump). This allows factors a and b to be combined and leaves the factors affecting bubble volume to be considered the physical properties of the liquid in which the analyte is to be trapped as well as the flow rate through the restrictor.

Though several manuscripts have dealt with the optimization of a liquid trapping step none has suggested a trapping mechanism for predictive use. Since limited data are available concerning the mechanisms involved in liquid trapping, the goal of this study was to investigate the effect of five trapping parameters on the collection efficiencies of several fat-soluble vitamins, shown in **Figure 2-1**, given a fixed set of (non-optimized) extraction conditions. By using these vitamins, differences in polarity and solubility in the collection solvents should not account for much of the variation noted in collection efficiency. This study should allow for the identification of the most important trapping parameters for analytes of similar structure.

## 2.1 Experimental

### 2.1.1 Extraction

All extractions were performed using an Isco SFX 3560 (Lincoln, NE) supercritical fluid extraction system. Carbon dioxide with helium headspace from Air Products and Chemicals, Inc. (Allentown, PA) was used as the extraction fluid, since the instrument was not configured for cooling of the pump

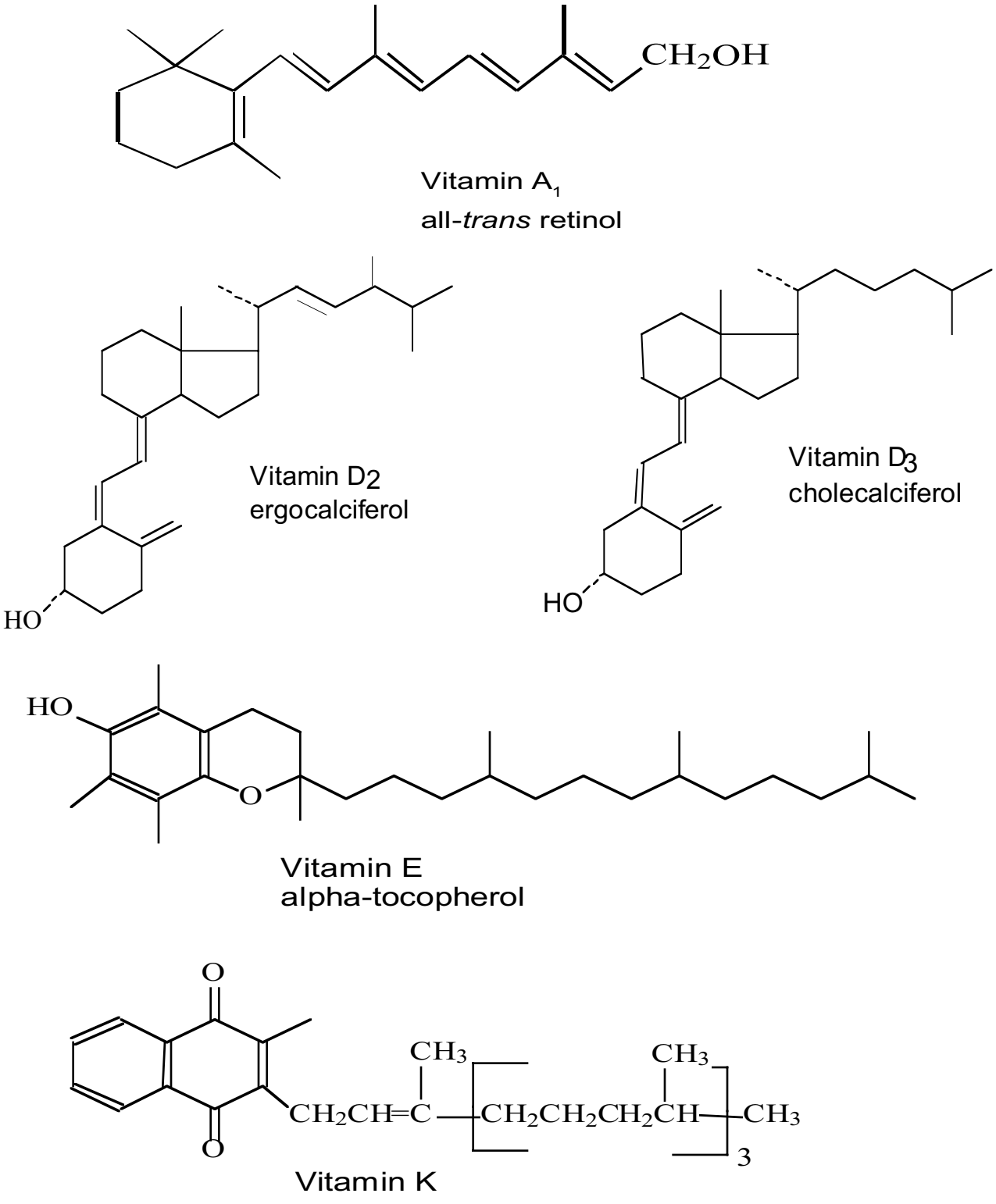



**Figure 2-1**: Structures of the Fat-Soluble Vitamins Used in this Trapping Study

heads, though carbon dioxide without helium headspace is recommended for use in this system with cooled pump heads. Approximately 14 grams of Ottawa Cement Testing Sand (Fisher Scientific, Houston, TX) was placed in a 10 mL Isco PEEK extraction vessel. The sand was used as received, with no clean-up steps or preliminary extractions performed. A spiking solution of Vitamin A alcohol, Vitamin E, Vitamin $D_2$, Vitamin $D_3$, and Vitamin K (Fluka, Ronkonkoma, NY) (300 µg/mL of each) was prepared in ethanol (Aaper, Shelbyville, KY) stabilized with approximately 0.005% butylated hydroxytoluene from Fluka. Fresh spiking solution was prepared each week, and the flask wrapped in aluminum foil, and stored in a 4°C refrigerator to minimize decomposition of the primary standard. A 100 µL aliquot of the spiking solution (primary standard) was then spiked onto the sand to yield around 30 µg of each component. All extractions were performed at an oven temperature of 75°C and a pressure of 340 atmospheres (density ~ 0.80 g/mL). The restrictor temperature, collection temperature, flow rate, collection pressure, and collection solvent were varied. **Table 2-1** represents a full factorial experimental design for a system that contains four variables performed for each of four collection solvents. The volume of the collection solvent was held constant at 10 mL, there was no static extraction time, and the dynamic extraction time was 10 minutes. No solvent replenishment of the trapping solvent was performed to replace losses which occurred during the course of the extraction. The collection solvents were HPLC grade hexane, absolute ethanol, and isopropanol, all from Fisher Scientific, and n-heptanol from Fluka.

### 2.1.2 Extract Analysis

After the extraction was completed, the collection vial was removed from the extractor and the appropriate solvent was added to assure a volume of

**Table 2-1:** Trapping Methods Used in this Study

| Trapping Method | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| Extraction Pressure, atm | 340 | 340 | 340 | 340 | 340 | 340 | 340 | 340 |
| Extraction Temperature, °C | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 |
| Restrictor Temperature, °C | 50 | 50 | 100 | 100 | 50 | 50 | 100 | 100 |
| Collection Temperature, °C | -15 | -15 | -15 | -15 | 15 | 15 | 15 | 15 |
| Extraction Flow Rate, mL/min | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Dynamic Time, min | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Collection Volume, mL | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Collection Solvent | variable | variable | variable | variable | variable | variable | variable | variable |
| Collection Pressure | on | off | on | off | on | off | on | off |

| | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|
| Extraction Pressure, atm | 340 | 340 | 340 | 340 | 340 | 340 | 340 | 340 |
| Extraction Temperature, °C | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 |
| Restrictor Temperature, °C | 50 | 50 | 100 | 100 | 50 | 50 | 100 | 100 |
| Collection Temperature, °C | -15 | -15 | -15 | -15 | 15 | 15 | 15 | 15 |
| Extraction Flow Rate, mL/min | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Dynamic Time, min | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Collection Volume, mL | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Collection Solvent | variable | variable | variable | variable | variable | variable | variable | variable |
| Collection Pressure | on | off | on | off | on | off | on | off |

10 mL. To establish an equivalent 100% recovery, 100 µL of the same spiking solution was added to an empty collection vessel, and the volume was adjusted
to 10 mL. A portion of the solution was transferred to an amber autosampler vial for analysis. Each standard was injected four times and the areas were averaged to calculate recovery.

The extracts were analyzed by high performance liquid chromatography using a Hewlett-Packard (Little Falls, DE) Series 1050 Liquid Chromatograph, Autosampler, and Ultraviolet/Visible detector, and a Hitachi (Middlebury, CT) F-1000 Fluorescence detector. An isocratic mobile phase of 75/25 HPLC grade Acetonitrile/Methanol (Fisher Scientific) flowed at 2.0 mL/min through a 250 x 4.6
mm Lichrosorb(Hewlett Packard) ODS column (5µm particles). The UV/Vis detector was operated at 325 nm for 4 min for the detection of Vitamin A, and 265 nm for the remaining 10 min to detect the D vitamins and Vitamin K. The fluorescence detector was operated with an excitation wavelength of 294 nm and an emission wavelength of 325 nm for the detection of Vitamin E. A sample of the chromatograms is shown in **Figure 2-2**.

### 2.1.3 Data Analysis

Analysis of the generated data consisted of graphing to determine preliminary trends, and analysis of variance (ANOVA) and paired t-tests for differences in means. All graphing, ANOVA, and paired t-tests were performed using Microsoft (Redmond, WA) Excel version 5 software. Additional analysis of the data was performed using the student edition of Minitab (State College, PA) Extra, version 10.

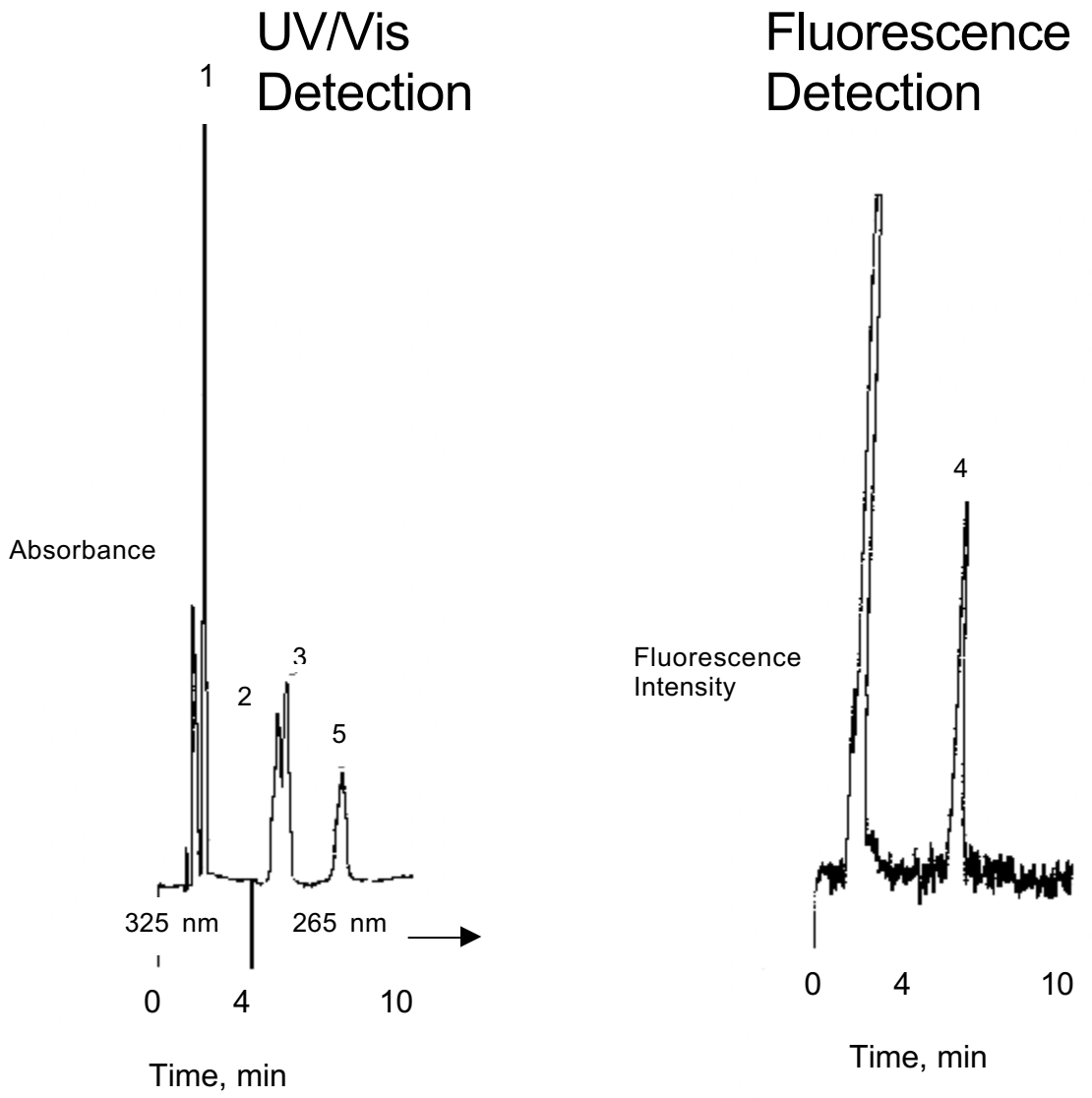


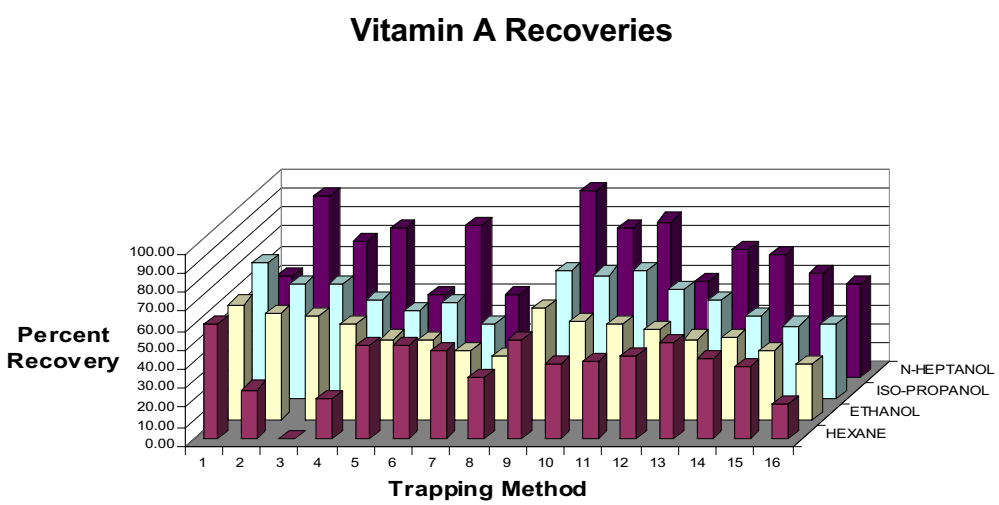

**Figure 2-2**: Sample Chromatograms from the Supercritical Fluid Extraction of Fat Soluble Vitamins, Peak Identification: 1-Vitamin A, 2-Vitamin $D_2$, 3-Vitamin $D_3$, 4-Vitamin E, 5-Vitamin K. Chromatographic conditions in text.

## 2.2 Results And Discussion

Since the objectives of this work were to determine the effect of the collection solvent, liquid $CO_2$ flow rate during the extraction, collection temperature, collection pressurization and restrictor temperature on the collection efficiencies, a statistical analysis of the data was performed after the extractions were completed. This was accomplished by performing either one-way or two-way ANOVA testing and calculating the F statistic. This allowed the separation of the error associated with the measurements into: (a) inherent, random error associated with replication of the measurement and (b) error associated with the change (or changes) in the trapping parameters. The resulting F statistic indicated that either there was or was not a statistically significant difference in the variances between the tested methods. If there was a difference this could indicate either that the treatment had changed the mean value of the measurements, or that the variability of the measurements was statistically different. Since this work used four full factorial experimental designs, paired t-testing was also used to determine if there was any significant change in the mean value.

The first step in the data analysis was to plot the data in order to determine if any trends were apparent. This was accomplished by using the mean values of the percent recovery for the given methods and collection solvents. After this, it was necessary to perform two-way ANOVA (with replication) testing to determine the effect of changing the collection solvent and/or the trapping method. The variances resulting from changing solvents were calculated as were the variances for changing trapping methods. These variances were then compared to the variance (reproducibility) of the measurement at the given trapping method and solvent to give an F value. This statistical testing was performed for each vitamin, and in every case it was found that the choice of trapping solvent played a significant role in the trapping

efficiencies of the analytes at the 95% confidence level. The graphical representation of the data for the five vitamins investigated is shown in **Figure 2-3**, and the results of F-testing from the ANOVA are presented in **Table 2-2**. Error bars have been removed for clarity of presentation, but all data collected were used for ANOVA testing. Relative standard deviations for replicate analyses (e.g. extraction and assay) were in the 14% range, while those for replicate HPLC injections were about 8%. It can be seen from the F statistics (**Table 2-2**) that for every vitamin the choice of trapping solvent makes a significant difference in the trapping efficiency. The average percent recovery for all trapping methods in each collection solvent is shown in **Table 2-3**, and indicates a definite trend for all the vitamins except for Vitamin A. In each case the recovery increased when changing from hexane to ethanol and ethanol to isopropanol. For all vitamins except Vitamin A there was a decrease in average percent recovery when changing from isopropanol to n-heptanol. The D vitamins were not completely resolved by the chromatographic method and recoveries greater than 100% for Vitamin $D_2$ are probably area integration related. Some physical properties of the collection solvents are shown in **Table 2-4**. This increase in percent recovery for all vitamins except A when moving from a lower to a higher viscosity would seem to indicate that an optimum viscosity of collection solvent exists. At this viscosity the bubble is slowed in its upward flow towards the air liquid interface, but not so retarded that coalescence of the bubbles occurs, resulting in larger bubbles with less opportunity for the analyte to pass into the liquid during its upward journey. It can also be seen from **Table 2-2** that in all cases except for Vitamin K the trapping method makes a significant difference in the percent recovered, and that interaction between the collection solvent and collection method occurred. Since this work was composed of four complete factorial experiments, it was possible to look at the effect of each of the trapping parameters varied



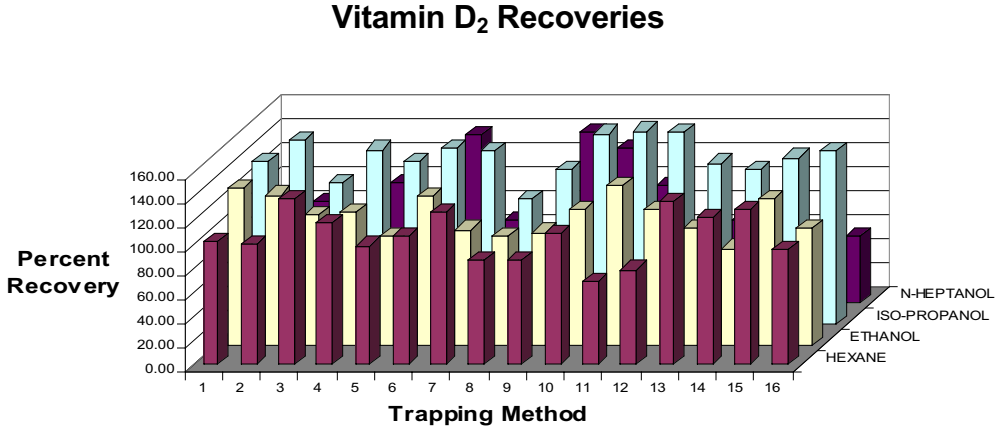




**Figure 2-3:** Percentage Recovery for Fat Soluble Vitamins A and $D_2$ Using Various Trapping Methods (Methods defined in Table 2-1.)

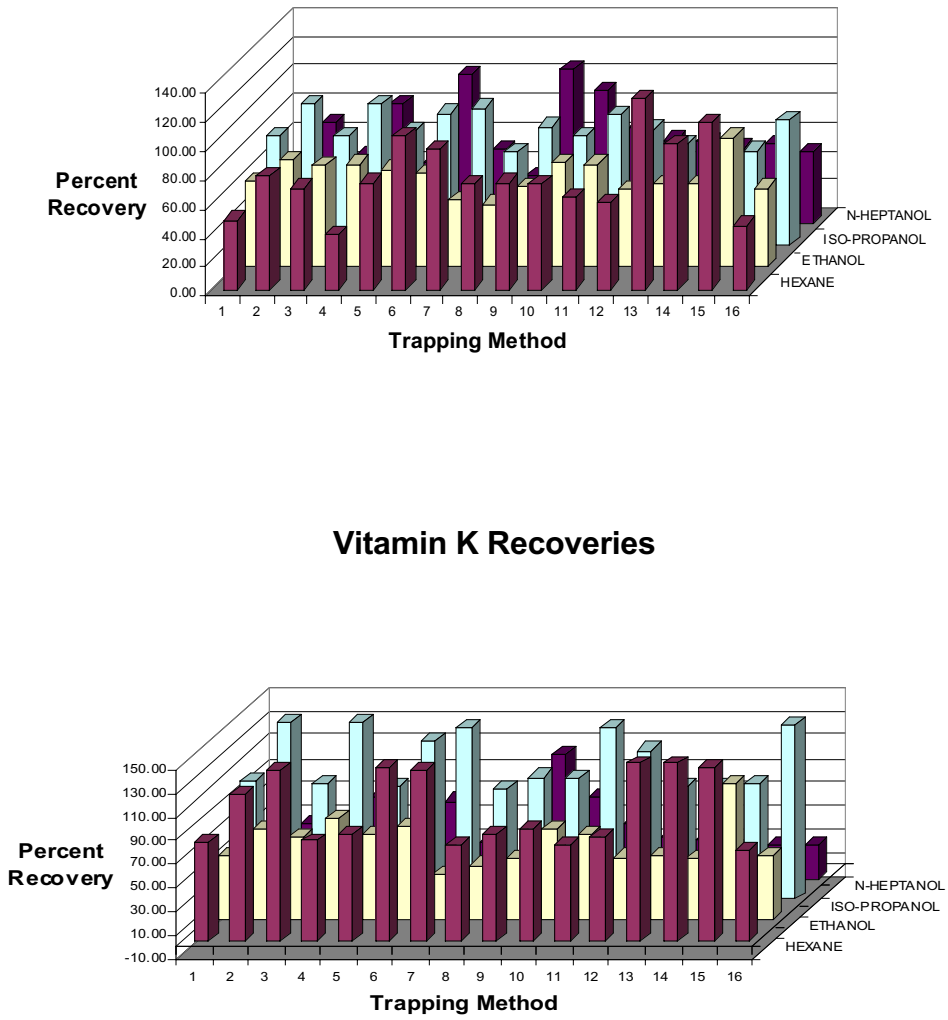



**Figure 2-3:** Percentage Recovery for Fat Soluble Vitamins $D_3$ and K Using Various Trapping Methods (Methods defined in Table 2-1.)

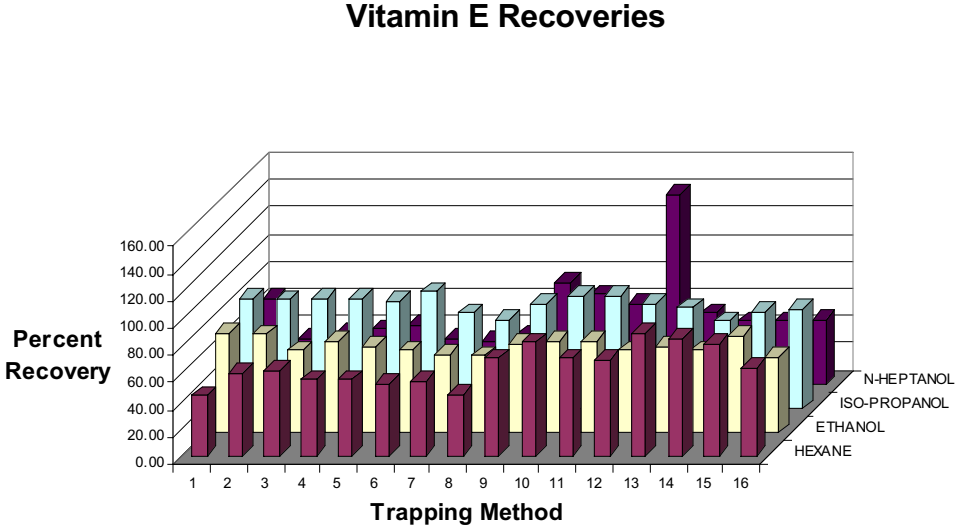



**Figure 2-3**: Percentage Recovery for Fat Soluble Vitamin E Using Various Trapping Methods (Methods defined in Table 2-1.)

**Table 2-2:** Results of Overall ANOVA Testing (Two-Way with Replication)

| F Statistic | | | | | | |
|---|---|---|---|---|---|---|
| **Source of Variation** | **Vitamin A** | **Vitamin $D_2$** | **Vitamin $D_3$** | **Vitamin K** | **Vitamin E** | **$F_{critical}$** |
| Trapping Method | 7.49 | 2.27 | 1.78 | 1.23 | 2.02 | 1.70 |
| Trapping Solvent | 37.82 | 38.19 | 5.93 | 35.59 | 9.16 | 2.63 |
| Interaction | 2.66 | 1.88 | 1.41 | 1.30 | 1.46 | 1.41 |

The null hypothesis was that there was no difference in average recoveries or reproducibilities when changing trapping method or solvent. The alternate hypothesis was that changing trapping method or solvent changed recoveries and/or reproducibilities.

**Table 2-3:** Average Percent Recoveries for all Trapping Methods in Each Solvent

| Trapping Solvent | | | | |
|---|---|---|---|---|
| **Analyte** | **Hexane** | **Ethanol** | **Isopropanol** | **n-Heptanol** |
| Vitamin A | 38.0 | 44.9 | 51.2 | 64.1 |
| Vitamin $D_2$ | 106.5 | 106.6 | 138.0 | 78.0 |
| Vitamin $D_3$ | 78.3 | 63.6 | 81.3 | 63.0 |
| Vitamin K | 116.1 | 66.3 | 118.4 | 41.9 |
| Vitamin E | 33.7 | 64.5 | 77.6 | 54.7 |

**Table 2-4:** Selected Physical Properties of the Collection Solvents

| Solvent | Boiling Point, °C | Density, g/mL (4°C) | Viscosity, cp (15°C) | Surface Tension, dynes/cm (20°C) |
|---|---|---|---|---|
| Hexane | 69 | 0.659 | 0.39 | 18.43 |
| Ethanol | 78 | 0.789 | 1.33 | 24.05 |
| Isopropanol | 82 | 0.786 | 2.86 | 21.70 |
| n-Heptanol | 176 | 0.822 | 8.53 | - |

individually by performing paired t-testing. Paired t-testing compares the values obtained for each method varying only by the change of a specific parameter. For instance, all even numbered methods differed only from the
previous odd numbered method by pressurization of the collection vial. A paired t-test was performed by comparing recoveries for the odd numbered methods to recoveries from the corresponding even numbered methods to test for the significance of the four studied factors. A positive value for the calculated t value indicated that the first value for the parameter (pressurization off or 50°C restrictor temperature or -15°C collection temperature or 0.5 mL/min flow rate) resulted in higher recoveries. A negative value indicated that the other level of parameter yielded the higher recoveries.

### 2.2.1 Effect of Collection Pressure

The SFX 3560 has the ability to pressurize the collection vial with an additional approximately 30 psi of carbon dioxide. It is theorized that this additional pressure would decrease trapping loss due to analyte volatility. In this work, all the analytes investigated were relatively non-volatile, and the results of testing, shown in **Table 2-5**, indicated that pressurization of the collection vial had little effect for these analytes. Only in the case of Vitamin A was any significant difference noted when the collection vial was pressurized, and then only for the solvents ethanol and isopropanol. Though not statistically significant, a generally similar trend was evident for the other two collection solvents. Since Vitamin A was the most volatile analyte of this group, it would appear that pressurization did, indeed, increase percent recoveries. For the other less volatile analytes there appears to be no advantage for pressurizing the collection vessel.

**Table 2-5:** Effect of Collection Pressurization on Percent Recovery Using Paired t-testing

| t Statistic | | | | | |
|---|---|---|---|---|---|
| **Analyte** | **All Solvents** | **Hexane** | **Ethanol** | **i-Propanol** | **n-Heptanol** |
| Vitamin A | -1.32 | -1.37 | -3.55 | -2.76 | 0.29 |
| Vitamin $D_2$ | 0.28 | -1.11 | -0.37 | 0.74 | 0.77 |
| Vitamin $D_3$ | -0.19 | -1.04 | -0.52 | 0.44 | 0.90 |
| Vitamin K | 0.08 | -1.04 | -0.05 | 1.14 | 0.56 |
| Vitamin E | -0.11 | -0.49 | -1.11 | -0.55 | 0.37 |
| $[t_{critical}]$ | 2.04 | 2.36 | 2.36 | 2.36 | 2.36 |

The null hypothesis was that pressurization of the collection vial did not result in a change in average percent recovery, while the alternate hypothesis was that pressurization affects recoveries. A positive t value indicates non-pressurized collection resulted in higher recoveries, while a negative t value indicates higher recoveries with pressurized collection.

### 2.2.2 Effect of Collection Temperature

There are at least two reasons why the temperature of the collection solvent may play a role in trapping efficiency. The first is that an increase in solvent temperature generally leads to an increase in the solvating power, which could lead to more efficient transfer of the analyte molecules into the collection solvent. The second reason is that a decrease in collection temperature may increase recoveries if there were losses due to either solvent or analyte volatility. Thus, selection of a collection temperature becomes a complex issue. For the trapping methods studied here, collection temperature was maintained at either +15°C or –15°C. The results of this testing (**Table 2-6**) indicated that for Vitamins A and E there was a significant increase in the percent recovered at a temperature of –15°C as compared to +15°C for all of the collection solvents taken together. For both of these vitamins (A and E) there was no difference when hexane was the collection solvent, but Vitamin $D_3$ actually showed higher recoveries at the higher collection temperature with hexane as the collection solvent. This increase for Vitamin $D_3$ could be a solubility factor, since it had the lowest solubility of any of the vitamins studied in supercritical $CO_2$ under a given set of conditions.[32] Solubility in hexane is often taken as an indication of solubility in non-modified supercritical $CO_2$. In ethanol, Vitamins A, $D_2$, and E all showed a significant increase in recoveries at the lower temperatures. This could be related to either of two phenomena, or a combination of the two. The first involves a decrease in the volatility of the solvent resulting in less collection solvent loss during the course of the extraction (higher level in the collection vial), and therefore a greater residence time in the solvent, with the resultant greater chance for analyte transfer. The

**Table 2-6:** Effect of Collection Temperature on Percent Recovery Using Paired t-testing

| t Statistic | | | | | |
|---|---|---|---|---|---|
| **Analyte** | **All Solvents** | **Hexane** | **Ethanol** | **i-Propanol** | **n-Heptanol** |
| Vitamin A | -4.54 | 0.70 | -13.61 | -9.93 | -4.43 |
| Vitamin $D_2$ | -1.49 | 1.14 | -3.21 | -1.41 | -1.12 |
| Vitamin $D_3$ | 0.11 | 3.83 | -1.09 | -1.20 | -1.35 |
| Vitamin K | -0.02 | 1.92 | -0.34 | -0.83 | -1.77 |
| Vitamin E | -2.66 | 0.41 | -3.13 | -2.59 | -2.13 |
| $[t_{critical}]$ | 2.04 | 2.36 | 2.36 | 2.36 | 2.36 |

The null hypothesis was that there was no difference in average percent recovery between trapping at +15°C or –15°C. The alternate hypothesis was that there was a temperature effect on recoveries. A negative *t* value indicates higher recoveries at –15°C.

[32] Johannsen and G, Brunner, *J. Chem. Eng. Data*, **42** (1997) 106-111.

second involves an increase in the viscosity of the solvents at lower temperatures. Again, this would presumably result in a longer residence time in the collection solvent, unless coalescence of the bubbles occurs. Moving toward the more viscous solvents of isopropanol and n-heptanol, a decrease in the t values for these vitamins is noted, so that for n-heptanol, only Vitamin A recoveries are significantly improved by the lower temperature. Vitamin K was not effected by the collection temperature, which was consistent with the results of the overall ANOVA testing.

### 2.2.3 Effect of Extraction Flow Rate

The liquid $CO_2$ flow rate for these experiments was either 0.5 mL/min or 1.5 mL/min. The time of extraction remained constant at 10 minutes, so it is obvious that a greater mass of $CO_2$ would be used for the extractions at the higher flow rates. Therefore, if incomplete extraction was occurring, since the extraction conditions were not optimized, use of the higher flow rates should result in increased recoveries. Since the SFX 3560 has a linear variable restrictor, an increase in flow rate will be directly proportional to the increase in the restrictor opening. As discussed previously, this should increase bubble size[6] resulting in less chance for analyte transfer to the liquid phase. However, when looking at the results shown in **Table 2-7**, the sign of the t statistics for all of the solvents combined (first column), the trend was in the opposite direction, suggesting that the extraction may be incomplete with the lesser mass of $CO_2$. The negative value of the t statistic indicated that the higher flow rate resulted in higher recoveries. In the case of Vitamin A, there was a significant difference favoring the low flow rate for ethanol alone. This may be explained by the higher surface tension of ethanol along with the greater volatility of Vitamin A than the other vitamins. Applying the LaPlace equation, the higher surface

**Table 2-7:** Effect of Extraction Flow Rate on Percent Recovery Using Paired t-testing

| t Statistic | | | | | |
|---|---|---|---|---|---|
| **Analyte** | **All Solvents** | **Hexane** | **Ethanol** | **i-Propanol** | **n-Heptanol** |
| Vitamin A | -1.04 | -0.76 | 2.72 | -1.15 | -0.65 |
| Vitamin $D_2$ | -0.36 | 0.54 | 0.39 | -1.17 | -0.50 |
| Vitamin $D_3$ | -0.70 | -1.01 | -0.28 | 1.10 | -0.70 |
| Vitamin K | -0.32 | -0.36 | -0.07 | 0.59 | -0.69 |
| Vitamin E | -3.18 | -7.33 | -0.03 | 0.92 | -2.46 |
| $[t_{critical}]$ | 2.04 | 2.36 | 2.36 | 2.36 | 2.36 |

The null hypothesis was that changing the liquid flow rate from 0.5 mL/min to 1.5 mL/min would not affect average percent recoveries. The alternate hypothesis was that change in flow rate would affect recoveries. A negative t value indicates higher recoveries at 1.5 mL/min.

tension of ethanol would cause the bubbles produced by the same flow rate (from the same amount of pressure) to be larger than in the case of the other solvents. This would mean that bubbles produced in ethanol would be larger at every flow rate, and that increasing the flow from 0.5 to 1.5 mL/min could make the bubble so large that analyte efficient transfer is not favored and the most volatile of the analytes would be swept out of the collection vessel with excess $CO_2$.

### 2.2.4 Effect of Restrictor Temperature

The temperature of the restrictor was varied by only 50 degrees, from 50°C to 100°C. Since a variable restrictor was employed for the extractions, temperature control is not as much of an issue as with a fixed linear restrictor where a relatively high temperature is used to ensure that the analytes or co-extractives will not precipitate from the solution and clog the restrictor. It is observed from **Table 2-8** that the lower restrictor temperature significantly improved recoveries of Vitamin A in ethanol and isopropanol. Though not significant at the 95% confidence level, improvement in recovery was noted for the other two solvents also. Restrictor temperature did not significantly affect the recoveries of any of the other vitamins. An explanation for this behavior could be that Vitamin A is known to degrade at higher temperatures and in acidic environments. Since any residual moisture present in the $CO_2$ would lead to an acidic fluid, it is highly plausible that this degradation could take place within the restrictor itself, prior to decompression into the liquid trap.

**Table 2-8:** Effect of Restrictor Temperature on Percent Recovery Using Paired t-testing

| t Statistic | | | | | |
|---|---|---|---|---|---|
| **Analyte** | **All Solvents** | **Hexane** | **Ethanol** | **i-Propanol** | **n-Heptanol** |
| Vitamin A | 4.86 | 2.29 | 7.79 | 4.33 | 2.27 |
| Vitamin $D_2$ | 1.24 | 0.30 | -0.16 | 0.04 | 1.70 |
| Vitamin $D_3$ | 1.67 | 1.55 | 0.11 | -0.45 | 1.37 |
| Vitamin K | 0.48 | 1.06 | -0.17 | -1.58 | 1.04 |
| Vitamin E | 0.67 | 1.17 | 1.59 | 0.80 | -0.29 |
| $[t_{critical}]$ | 2.04 | 2.36 | 2.36 | 2.36 | 2.36 |

The null hypothesis was that changing the restrictor temperature from 50°C to 100°C would not affect average percent recoveries. The alternate hypothesis was that changing the restrictor temperature influenced recoveries. A positive t value indicates higher recoveries at the lower restrictor temperature (50°C).

## 2.3 Conclusions

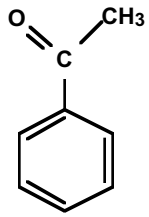
The major conclusion to be drawn from this work is that the physical properties of a collection solvent used after supercritical fluid extraction greatly influence trapping efficiency. In the case of these fat soluble vitamins of similar polarities and solubilities changing the trapping parameters of extraction flow rate, restrictor temperature, collection temperature and collection pressurization had less effect on the trapping efficiencies than did changing the solvent. The major physical properties that influence trapping efficiencies appeared to be the viscosity and surface tension as predicted by gas-fluid dynamics.

# CHAPTER THREE

# MODIFICATION OF THE COLLECTION SOLVENT TO ENHANCE LIQUID TRAPPING EFFICIENCIES

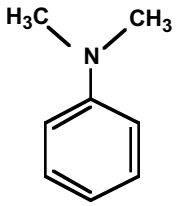
## 3.0 Introduction

As seen in the previous chapter, the choice of pure collection solvent had a greater impact on collection efficiencies than the collection temperature, collection pressurization, restrictor temperature or extraction flow rate when trapping fat-soluble vitamins. By choosing relatively similar analytes any differences due to polarity or volatility were minimized. In this work the goal was to investigate the effect of the addition of a modifier to the collection solvent on the collection efficiencies of compounds of varying polarities and volatilities, given a fixed set of (non-optimized) extraction conditions. The selected polarity test mixture is shown in **Figure 3-1**. Addition of a modifier to the collection solvent induces small changes in the physical properties of the solvent, and allows prediction of trapping differences that would result from the use of non-modified $CO_2$ versus modified $CO_2$. It was hoped that the addition of the collection solvent modifier could also help to overcome some common instrumental constraints, such as lack of collection pressurization or collection temperature control.




Acetophenone

MW=120.15
m.p.=20.5°C
b.p.=202°C



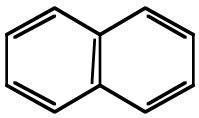

N,N-Dimethylaniline

MW=121.18
m.p.=2°C
b.p.=193°C

$CH_3(CH_2)_{22}CH_3$

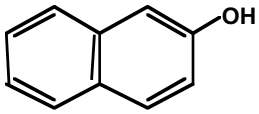
Tetracosane

MW=338.66
m.p.=51.1°C
b.p.=391°C



Naphthalene

MW=128.16
m.p.=80.2°C
b.p.=218°C




2-Naphthol

MW=144.16
m.p.=122°C
b.p.=285°C

**Figure 3-1:** Structures of the Analytes Used in This Trapping Study

## 3.1 Experimental

### 3.1.1 Extraction

All extractions were performed using an Isco SFX 3560 (Lincoln, NE) supercritical fluid extraction system. Carbon dioxide with helium headspace (2000 psi) from Air Products and Chemicals, Inc. (Allentown, PA) was used as the extraction fluid. Approximately 14 grams of Ottawa Cement Testing Sand (Fisher Scientific, Houston, TX) was placed in a 10 mL Isco high crystalline polymer extraction vessel. The sand was used as received, with no clean-up steps or preliminary extractions performed. A spiking solution of acetophenone, N,N-dimethylaniline, tetracosane, naphthalene, and 2-naphthol (Fisher Scientific, Fairlawn, NJ) (~10 mg/mL of each) was prepared in HPLC grade methylene chloride from Fisher Scientific. A fresh spiking solution was prepared each week, wrapped in aluminum foil, and stored in a 4$^{o}$C refrigerator to minimize decomposition of the primary standard. A 100 µL aliquot of the spiking solution was then spiked onto the sand to yield around 1 mg of each component. The methylene chloride was allowed to evaporate under ambient conditions prior to SFE. An internal standard solution of pyrene (~10 mg/mL) from Fisher Scientific was also prepared in methylene chloride.

All extractions were performed at an extraction chamber temperature of 80$^{o}$C and a pressure of 340 atmospheres, corresponding to a density of 0.88 g/mL. The restrictor temperature was held at 80°C and the liquid flow rate at 1.5 mL/min. The collection temperature, collection pressure, and collection solvent composition were varied. The volume of the collection solvent was held constant at 10 mL, there was no static extraction time, and the dynamic extraction time was 15 minutes. No solvent replenishment of the trapping solvent was performed to replace losses which occurred during the course of

the extraction. The collection solvents were made by volume percent with HPLC grade methylene chloride, hexane, methanol and n-propanol, all from Fisher Scientific.

### 3.1.2 Extract Analysis

After the extraction was completed, the collection vial was removed from the extractor and collection solvent was added to approximate a volume of 10 mL. A 100 µL aliquot of the internal standard solution was added and the solution was thoroughly mixed. To establish an equivalent 100% recovery, 100 µL of the same spiking solution was added to an empty collection vessel, the volume was adjusted to 10 mL with the specific collection solvent being studied, and 100 µL of the internal standard solution was added. A portion of the solution was transferred to an amber autosampler vial for analysis. Each standard was injected four times to determine response factors. The extracts were injected and response factors compared to that for the standard to calculate recoveries.

All extracts were analyzed using a Hewlett Packard (Little Falls, DE) HP 5890 gas chromatograph equipped with a split/splitless capillary column inlet system which was maintained at 275°C. A 30 m, 0.25 mm i.d., 0.25 µm $d_f$ DB-5 (J & W Scientific, Folsom, CA) fused silica capillary column was used for the separation. Ultra High Purity helium (Air Products and Chemicals, Inc., Allentown, PA) was used as the carrier gas at a flow rate of ~2.5 mL/min (22 psi head pressure) and the flame ionization detector was maintained at 325°C. The temperature program employed contained a 1 min initial temperature of 50°C, followed by a ramp of 15°C/min to a temperature of 140°C, which was maintained for 1 min. A second ramp of 30°C/min was then used to raise the temperature from 140 to 300°C. The final temperature (300°C) was maintained for 3 min. For all of the extracts, 1 µL was injected using a HP 7673 (Hewlett

Packard) automatic injector in the splitless mode. A representative chromatogram of the extracted polarity mix components is presented in **Figure 3-2**.

## 3.2 Results and Discussion

This work was conducted in two phases, using methylene chloride and hexane as the collection solvents. The modifier added was either methanol or n-propanol. Some physical properties of both the collection solvents and modifiers
are shown in **Table 3-1**. The first phase of this work focussed on determining whether the addition of a modifier to the collection solvent could overcome the effect of lowered collection temperature or collection pressurization. Though the Isco SFX 3560 system used for these extractions is capable of pressurizing the collection vessel and of collection temperature control down to –20$^{o}$C through the use of an auxiliary coolant, it was recognized that many instruments available, especially the more basic models, do not present such advantages to the user.

### 3.2.1 Effect of Collection Temperature

For volatile and semi-volatile analytes, lowering the temperature lowers the volatility of the analyte directly leading to less loss of the compound[33]. For the more non-volatile analytes, a decrease in the collection temperature will usually result in a decrease in the solubility of the analyte in the fluid. Since the

[33] M. Ashraf-Khorassani, R. K. Houk, and J. M. Levy, *J. Chromatogr. Sci.*, **30** (1992) 361-366.

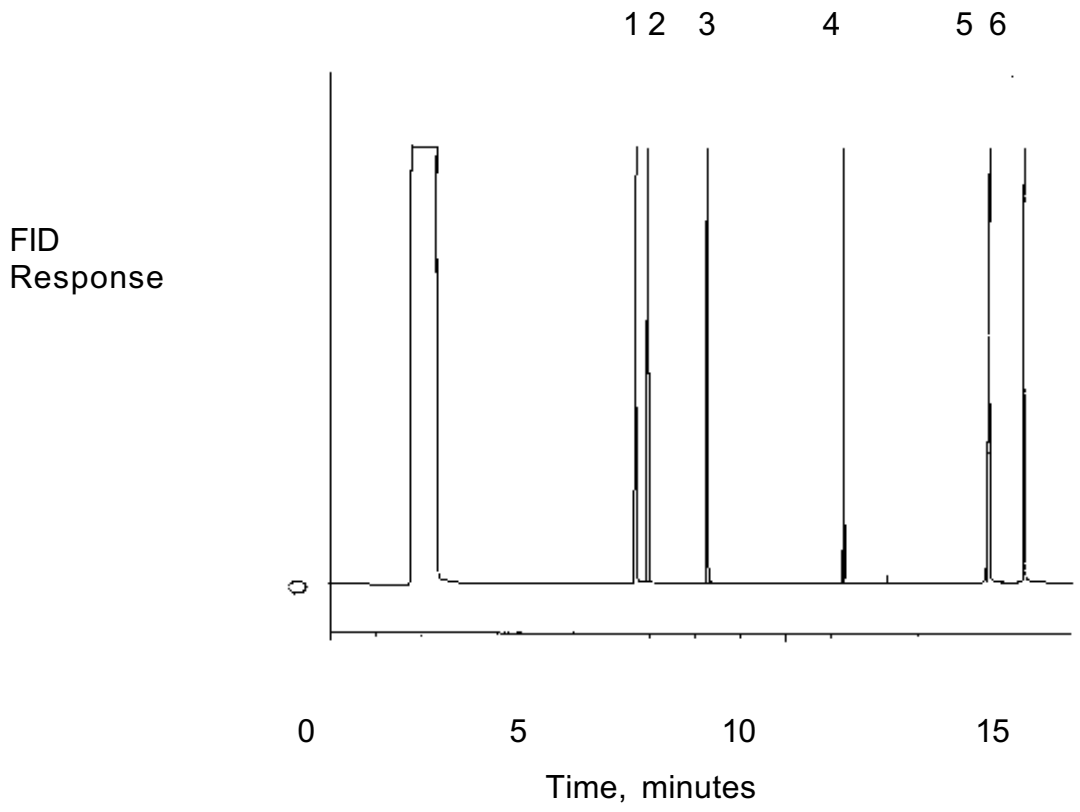



**Figure 3-2:** Representative Gas Chromatogram of the Extracted Polarity Mix. Peak Identification: 1. Acetophenone, 2. N,N-Dimethylaniline, 3. Naphthalene, 4. 2-Naphthol, 5. Pyrene (internal standard), 6. Tetracosane. Chromatographic conditions in text.

**Table 3-1:** Selected Physical Properties of the Collection Solvents and Collection Solvent Modifiers

| Identity | Boiling Point, °C | Density, g/mL | Viscosity, cp @ 20°C | Surface Tension, dynes/cm |
|---|---|---|---|---|
| Methylene Chloride | 40 | 1.335 | 0.449 | 26.52 |
| Methanol | 65 | 0.792 | 0.754 | 22.60 |
| Hexane | 69 | 0.659 | 0.393 | 18.43 |
| n-Propanol | 97 | 0.802 | 2.256 | 23.78 |

collection conditions in most extractions do not approach the solubility limit for the analytes, temperature is normally reduced to as low as instrumentally possible. In this work we chose to look at 25°C, relatively close to room temperature, and 0°C, easily obtainable with an ice bath. In both cases, the collection vial was not pressurized. The target compounds were arbitrarily divided into semi-volatile (acetophenone and N,N-dimethylaniline) and non-volatile (naphthalene, 2-naphthol and tetracosane) groups. **Figures 3-3a and 3-3b** compare the recoveries of the analytes at 0° and 25°C, when methylene chloride was the collection solvent and methanol was the modifier. As seen in both of the figures, the addition of higher volumes of methanol to the methylene chloride allows the collection efficiencies obtained at 25°C to approach those obtained at 0°C. Upon initial addition of the methanol (5%), the recoveries decrease at 25°, versus change very little for the 0° collection. This can be attributed to two competing phenomena: a) the solubility of the $CO_2$ in the collection fluid, and b) the volatility of the collection solvent. Higher recoveries are obtained at the lower temperature because the $CO_2$, carrying the analyte, is more soluble in the collection solvent than at higher temperatures. The lower temperature also minimizes collection solvent (and analyte) loss due to simple volatilization. Once higher levels of methanol (20%) are present in the collection solvent, the losses due to volatility are comparable to those seen at lower temperature. Simply decreasing the volatility of the collection solvent alone will not account for the observed behavior, since recoveries for both the volatile and non-volatile compounds in the test mix are comparable.

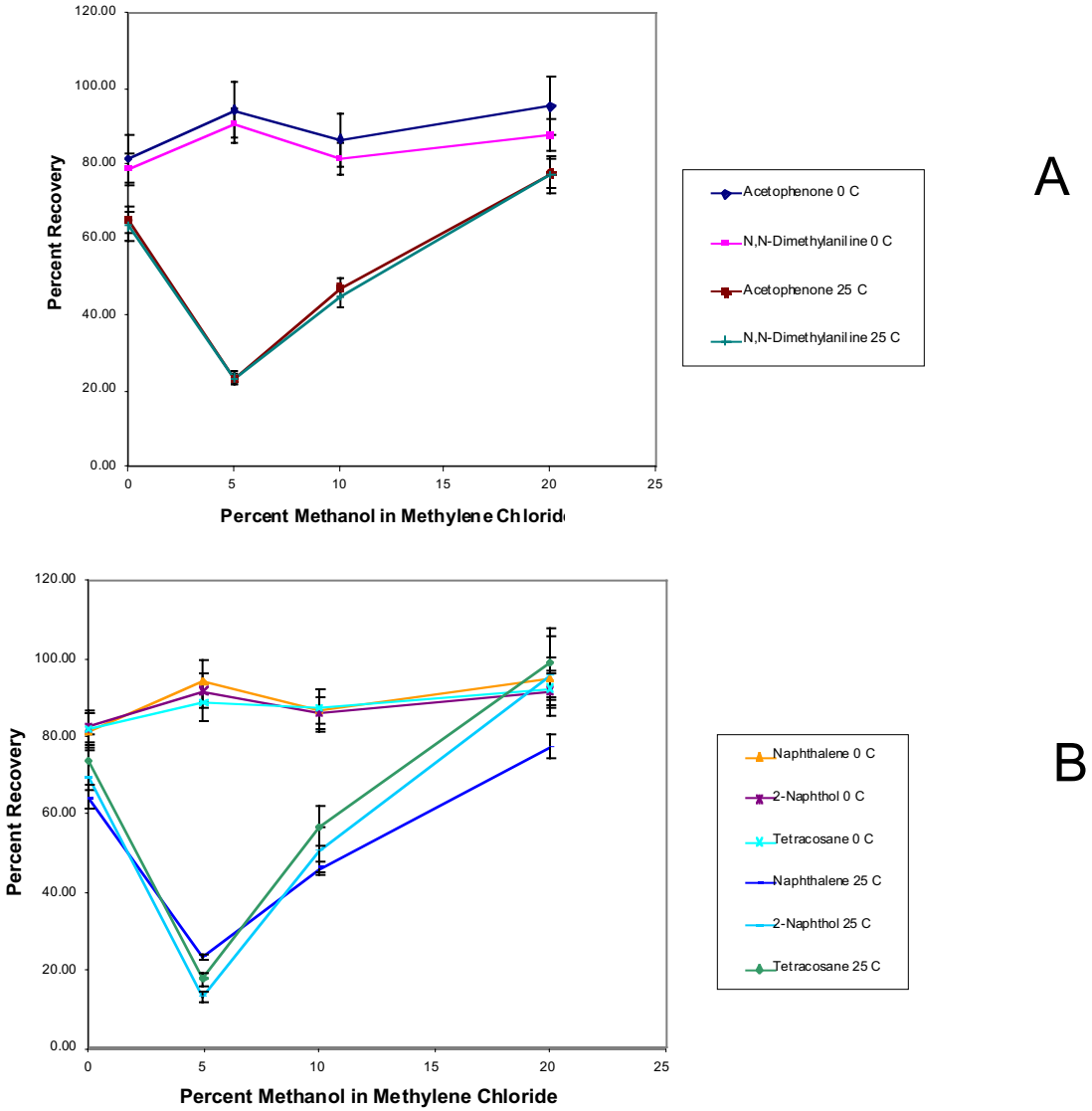



**Figure 3-3:** Effect of Collection Temperature on the Recoveries of the Polarity Mix Components. SFE conditions in text. Non-pressurized collection, Methylene chloride collection solvent, Methanol modifier. A. Semi-volatile Analytes, B. Non-Volatile Analytes.

The same effect is seen at 25°C for hexane with n-propanol modifier, but no increase in collection efficiencies is seen when using hexane with the methanol modifier or methylene chloride with n-propanol modifier. When hexane is modified with methanol there is very little change in the overall volatility of the solution due to the similarity of the boiling points of the two liquids, and it affords no advantages during the trapping process. The case of methylene chloride modified with n-propanol is not explicable in terms of the decreased volatility of the collection solvent.

It can be seen that in some cases increased collection temperatures can be used, if the collection solvent is modified to decrease its overall volatility, and enhance collection efficiencies.

### 3.2.2 Effect of Collection Pressurization

In order to determine the effect of pressurizing the collection vessel, the collection temperature was fixed at 25°C, which would actually favor analyte loss. For the cases in which hexane was the collection solvent, the reproducibility of the data for non-pressurized collection was much worse than when pressurized. This makes direct statistical comparison of the mean values difficult, due to the unequal variances, but in both cases (methanol and n-propanol as modifier) pressurization of the collection vial results in higher recoveries for each analyte in the test mix. When n-propanol is the modifier, collection efficiencies are improved as the modifier is added. This increase is seen for both the pressurized and non-pressurized collection, as shown in **Figures 3-4a and 3-4b**.

For the cases in which methylene chloride was the collection solvent, again lower reproducibility was seen for the non-pressurized collection. The pressurized collection proved to be superior with both methanol and n-propanol

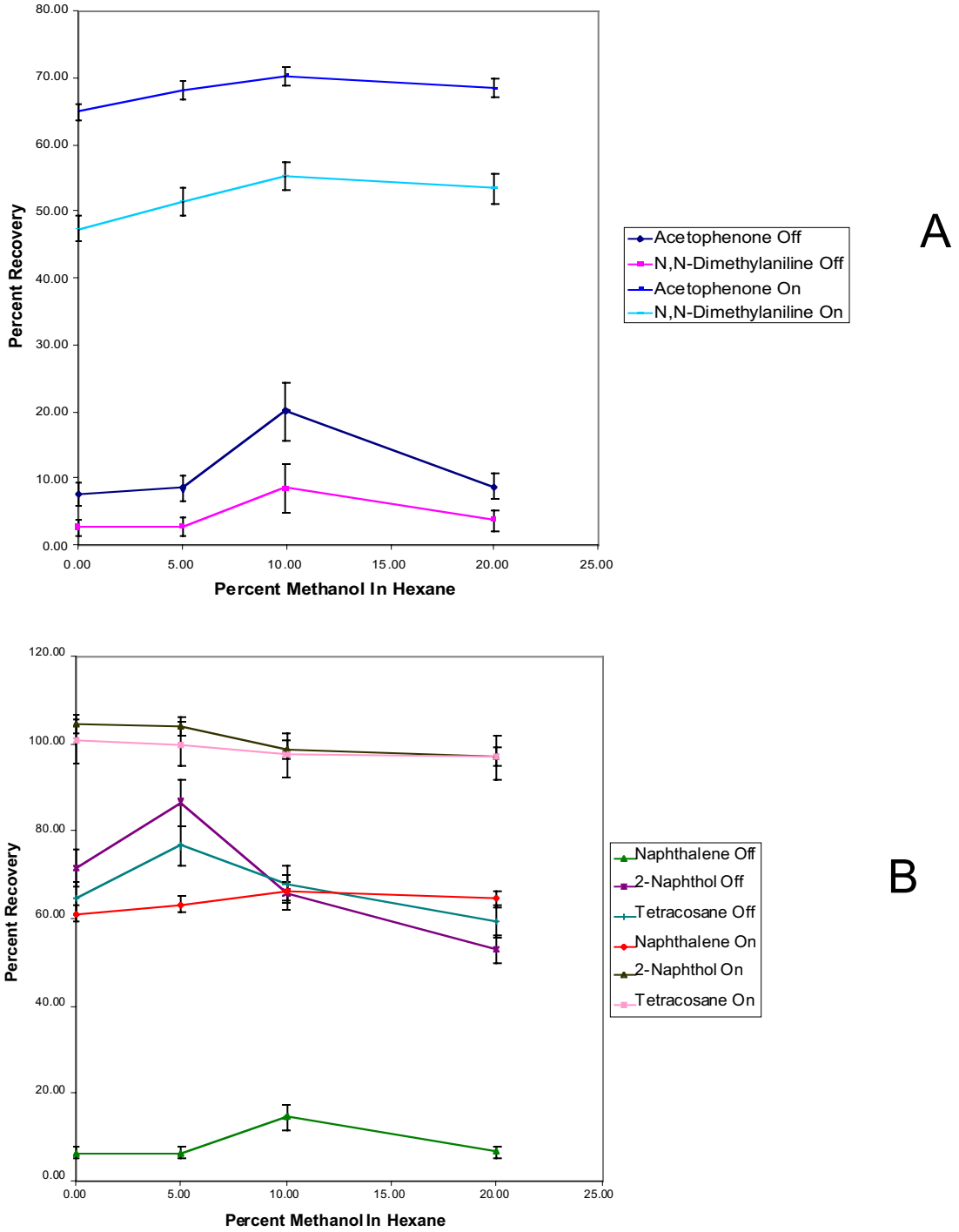


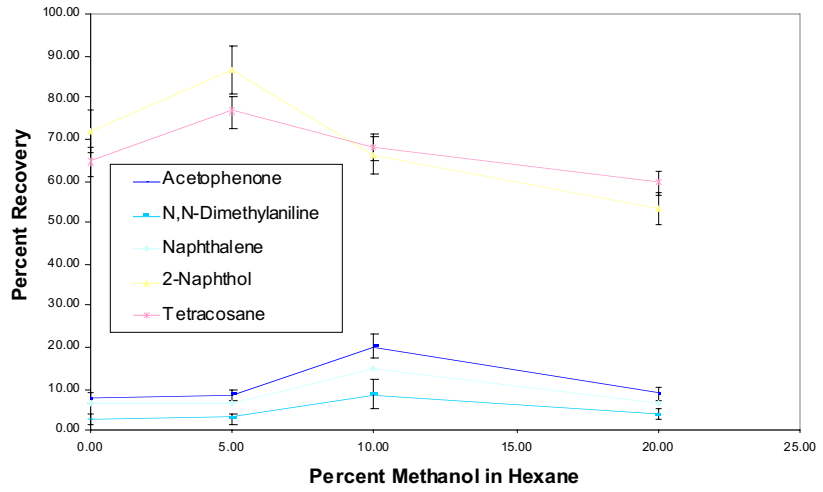

**Figure 3-4:** Effect of Collection Pressurization on the Recoveries of the Polarity Mix Components. SFE conditions in text. Collection Temperature = 25°C, Hexane collection solvent, Methanol modifier. A. Semi-volatile Analytes, B. Non-Volatile Analytes.

modifiers. In the case of n-propanol, addition of the modifier had no significant effect on the recoveries of any of the analytes. With methanol, however, though the pressurized collection gave higher recoveries, addition of the methanol to the methylene chloride, at 20 volume percent, resulted in higher recoveries than without modifier. These data are shown in the 25°C recoveries in **Figures 3-3a and 3-3b**.

In all instances investigated in this work, collection pressurization improves not only collection efficiencies for both semi-volatile and non-volatile analytes, but the reproducibilities for replicate trials, and it appears that addition of a modifier to the collection solvent is unable to overcome these advantages.

### 3.2.3 Effect of Modifier Identity

For this determination, we used the worst case scenarios with a collection temperature of 25°C and no collection pressurization. For the hexane based collection solvents, addition of methanol to the solvent did not statistically enhance recoveries for any of the analytes. However, when n-propanol was the collection solvent modifier recoveries were greatly enhanced as seen in **Figure 3-5**. Though neither methanol or n-propanol is soluble in hexane at the 20 volume percent level, a two phase collection system did not diminish analyte recoveries, and even increased them in the case of n-propanol. (Recoveries were determined based on a spiked standard treated in the same manner as the extracted samples. In these cases only the hexane or methylene chloride phase was injected into the chromatograph, but any partitioning effect between the two liquids would be the same for the standard and samples.) The excess alcohol after the hexane was saturated would tend to act in much the same manner as an inert solid particle, in that it would increase residence time in the collection solvent. Enhancement of the recoveries when n-propanol is the modifier is



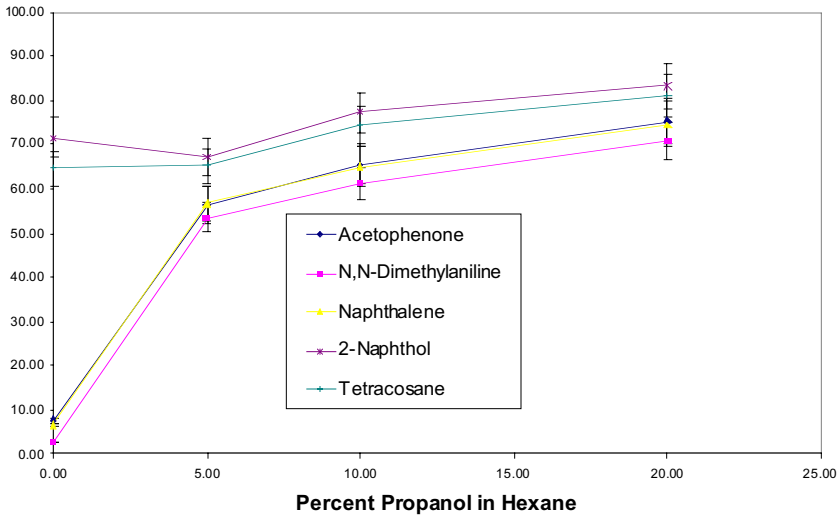




**Figure 3-5:** Effect of Collection Solvent Modifier on the Recoveries of the Polarity Mix Components. SFE conditions in text. Non-pressurized collection, Collection Temperature = 25°C, Hexane collection solvent.

thought to be due to the increase viscosity of the solution ( in comparison to hexane alone, or hexane with methanol modifier) and the inclusion of the additional (immiscible) n-propanol in the collection vial. Both of these situations cause an increase in the time the $CO_2$-analyte bubbles spend in the collection solvent.

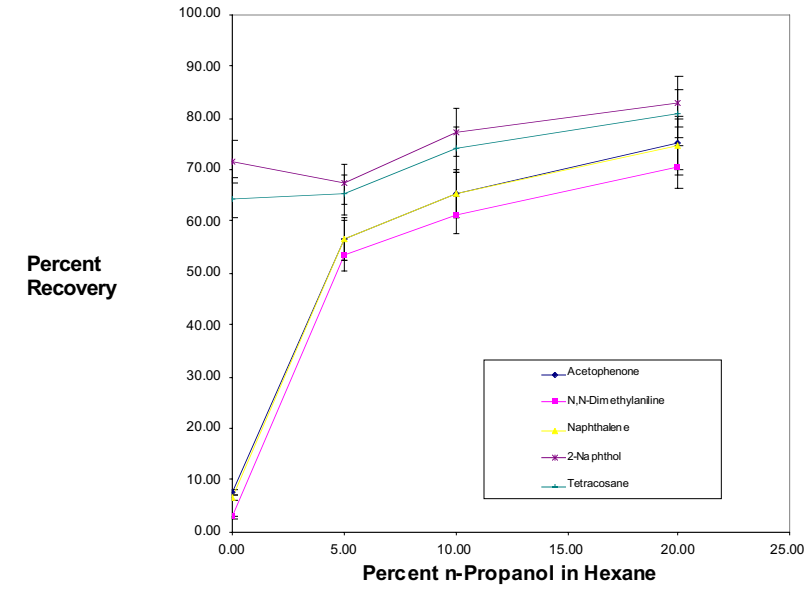
For the methylene chloride based solvents the addition of n-propanol resulted in no significant change (increase or decrease) in the collection efficiencies for any of the analytes. The addition of methanol resulted in significantly improved recoveries only for tetracosane and 2-naphthol, and only at the 20% level. The reason(s) for this occurrence are unclear at this time.

### 3.2.4 Effect of Collection Solvent Identity

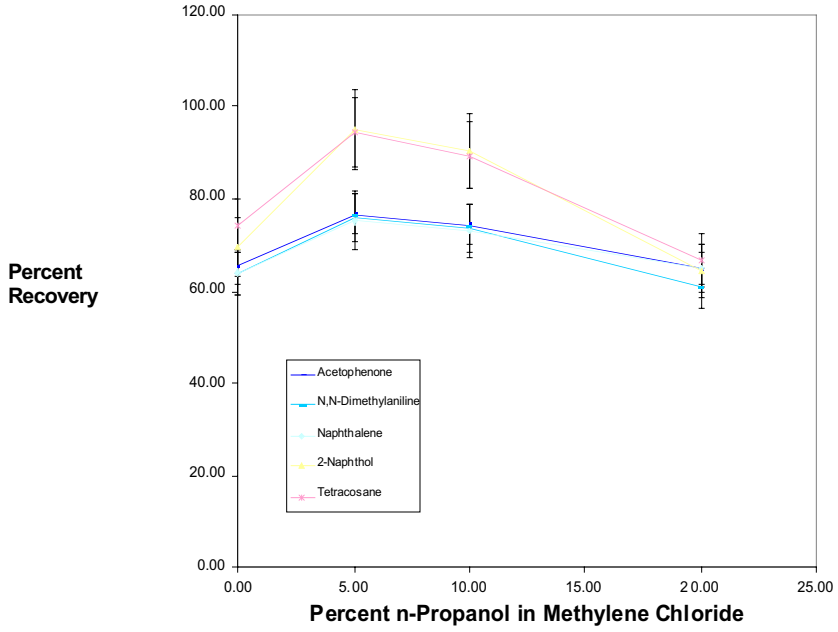
Finally if the collection solvent itself is considered, when the modifier is the same, we see a much more pronounced effect with hexane than with methylene chloride when n-propanol is the modifier. These data are illustrated is **Figure 3-6**, and the results can be attributed to the ability of the n-propanol to function in several ways to modify the collection fluid. The volatility of the collection fluid is decreased, the viscosity is increased, and the immiscible n-propanol droplets act to change the flow path, increasing residence time of the analytes. Apparently an increase in fluid polarity is not important since the same general trend is seen for all of the analytes, regardless of their polarity.

## 3.3 Conclusions

In agreement with the previous work from Chapter 2, we have found the choice of the collection solvent is immensely important in achieving effective liquid trapping (with direct restrictor immersion) of analytes. The addition of a




Hexane Collection Solvent




Methylene Chloride Collection Solvent

**Figure 3-6:** Effect of Collection Solvent on the Recoveries of the Polarity Mix Components. SFE conditions in text. Non-pressurized collection, Collection Temperature = 25°C, n-Propanol modifier.

modifier to the collection solvent allowed for the use of higher trapping temperatures to trap semi-volatile and non-volatile analytes. This should allow trapping systems to be operated under ambient conditions instead of sub-ambient temperatures. Collection pressurization makes a significant difference at higher collection temperatures, which is most profound when trapping more volatile analytes in a more volatile solvent. This effect unfortunately cannot be overcome by the addition of a collection solvent modifier, and thus pressurization is highly recommended.

Changing the viscosity and surface tension of the collection solvent can also change trapping efficiencies. This is an important point that must be considered when trapping in a pure collection solvent after extraction has been performed with a modified fluid. The collection efficiency will change with the addition of the modifier, which can either be beneficial or detrimental to the collection process. This indicates that the choice of SFE trapping parameters can be as important as the choice of extraction parameters. The previous two chapters have described the importance of the choice of collection solvent with emphasis on the ability of the solvent to trap the analytes. In the next chapter, the role of the collection solvent in chemical reactions occurring during the trapping process will be explored.

# CHAPTER FOUR

# ESTERIFICATION OF DECANOIC ACID DURING SUPERCRITICAL FLUID EXTRACTION EMPLOYING EITHER METHANOL-MODIFIED CARBON DIOXIDE OR A METHANOL TRAP

## 4.0 Introduction

Fatty acids are an important class of compounds found in a wide variety of matrices. Because of their appreciable polarity, it is quite common for analysis of the free fatty acids by gas chromatography (GC) to be troublesome, due to the interaction between the polar carboxylic acid functionality and the chromatographic stationary phase. Though analysis of free fatty acids is possible with highly deactivated stationary phases[34], the most common method of GC analysis involves forming a more volatile, less polar derivative of the acid. One of the more popular methods involves the formation of the methyl ester of the fatty acid. This is generally accomplished by reaction of the acid(s) with methanol in the presence of an acid catalyst, such as boron trifluoride, at elevated temperature. This reaction results in the methyl ester formation, but the ester must be separated from the acid catalyst to avoid column degradation during chromatographic analysis. This is usually done via a liquid-liquid extraction with a chromatographically amenable solvent such as hexane[35].

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[34] A. Nomurs, J. Yasmada, K. Tasunoda, K. Sakaki, and T. Yokochi, *Anal. Chem.*, **61** (1989) 2076-2078.
[35] AOAC, Official Methods of the Association of Official Analytical Chemists, 13th ed., AOAC: Washington, D.C., 1992.

Supercritical fluid extraction (SFE) is one method of removing fatty acids from a matrix. SFE with carbon dioxide offers the advantages of using a non-toxic extraction fluid, in direct contrast to many liquid-solid or liquid-liquid extraction fluids, and the ability to control the temperature, pressure, and density of the fluid, thus allowing selection of its solvating power. Carbon dioxide is generally regarded as a non-reactive fluid when used for SFE. An additional advantage of SFE involves the ability to perform derivatization reactions during the extraction. Various reports appear in the literature discussing the derivatization of polar compounds during SFE.

Field[36] has published an excellent review of derivatization reactions coupled with SFE, which includes a discussion of the theories and approaches to derivatization with SFE, as well as many applications. In addition to organics, organometals have also been determined using coupled techniques[37,38]. Hawthorne et al.[39] used trimethylphenylammonium hydroxide and boron trifluoride to enhance the extraction of polar species from solid samples. They reported immediate GC analysis and a large time savings. However, they did find that multiple derivatization/extraction steps were necessary to achieve quantitative recoveries (>90%) for some samples which contained high levels of reactive matrix components. The samples they investigated included microbial phospholipid fatty acids from whole cells and wastewater phenolics from water, both as their methyl esters.

Rochette, Harsh, and Hill[40] investigated a variety of sample preparation methods to improve extraction recoveries of 2,4-dichlorophenoxy acetic acid (2,4-D) from soils. They used silylation, ion-pairing, methyl esterification and ionic displacement, finding the methyl esterification and ionic displacement to be the most promising for quantitative SFE work. Though they abandoned *in-*

[36] J. Field, *J. Chromatogr. A,* **785** (1997) 239-249.
[37] Y. Cai, R. Alzaga and J. Bayona, *Anal. Chem.*, **66** (1994) 1161-1167.
[38] B. Wenclawiak and M. Krah, *Fresnius J. Anal. Chem.*, **351** (1995) 134-138.
[39] S. Hawthorne, D. Miller, D. Nivens, and D. White, *Anal. Chem.*, **64** (1992) 405-412.

*situ* methylation (using $BF_3$/methanol) because of the appearance of holes at unions made of PEEK material, they reported a 90% recovery of 2,4-D as its methyl ester for a single (non-optimized) extraction. They also found it necessary to destroy the $BF_3$ with aqueous sodium chloride, and to partition the methyl ester into a non-polar solvent such as benzene for gas chromatographic analysis. Prior to initiating destruction of the $BF_3$, they found chromatographic deterioration leading to substantially broadened chromatographic peaks.

Meyer and Kleiböhmer[41] developed a rapid efficient method for the extraction of pentachlorophenol (PCP) from wood and leather products based on an *in-situ* derivatization method (acetylation with triethylamine and acetic anhydride). They found comparable results with conventional methods and SFE reduced analysis times from about 2 days to less than 3 hours. They did find a large matrix effect when the extraction of PCP from leather and wood was compared to that from soils[42]. They also found that sample clean-up conditions sometimes needed to be adjusted because of problems with co-extracted material[43]. Lee, Peart, and Hong-You[44] also developed an *in-situ* derivatization method for determining PCP in soils. They acetylated with acetic anhydride and triethylamine, and were able to achieve quantitative recoveries of the di-, tri-, tetra- and penta- chlorophenols.

Hills, Hill and Maeda[45] used a silylation reagent, tri-sil concentrate, to derivatize samples of roasted coffee beans, roasted Japanese tea and marine sediment. They stated that the extracts were GC-ready, resulting in not only time savings, but also improved extraction yields of both the derivatized and underivatized species. They postulated that the derivatizing reagent not only made the compounds more soluble in the supercritical extraction fluid, but that

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[40] E. Rochette, J. Harsh and H. Hill, Jr., *Talanta*, **40** (1993) 147-155.
[41] A. Meyer and W. Kleibohmer, *J. Chromatogr. Sci*., **35** (1997) 165-168.
[42] A. Meyer and W. Kleibohmer, *J. Chromatogr. A*, **718** (1995) 131-139.
[43] A. Meyer and W. Kleibohmer, *J. High Resol. Chromatogr.*, **19** (1996) 267-271.
[44] H. Lee, T. Peart and R. Hong-You, *J. Chromatogr.*, **605** (1992) 109-113.
[45] J. Hills, H. Hill, Jr. and T. Maeda, *Anal. Chem*., **63** (1991) 2152-2155.

it was involved in a competition for active matrix sites, resulting in displacement of the analyte from the matrix. King, France and Snyder[46] have reported the on-line derivatization of triglycerides under supercritical conditions. They used a solid catalyst, alumina pretreated with methanol, for the *in-situ trans*-esterification of the triglycerides. The methyl esters were then preferentailly eluted via ASFC from the alumina catalyst. They applied this technique to the analysis of a single soybean, evening primrose and peanut seeds, and found results to be comparable with the much more time consuming and labor intensive conventional method.

All of these papers have involved the derivatization reaction as a result of the addition of materials to the extraction chamber. Kawakura and Hiata[47] have recently reported the methylation of carboxylic acids in methanol-modified supercritical fluid carbon dioxide using a flow through system. They investigated the methylation of substitiuted benzoic acids and the use of a cation exchanger as a catalyst for the reaction. They also looked at the uncatalyzed reaction and found conversion to the methyl esters in the uncatalyzed reaction increased with increasing temperature and decreasing pressure. They were able to achieve a maximum conversion of just under 60% for p-nitrobenzoic acid. However, when using the cation exchanger as catalyst, quantitative conversions were obtained for all the investigated substituted benzoic acids. They also reported the transesterification of myristic acid, originally present as the ethyl ester, to its corresponding methyl ester. They were able to apply this technique to phenoxy acids retained on a solid phase extraction (SPE) disk, although under the investigated conditions, recoveries were not quantitative (less than 50%). All of this work used methanol-modified carbon dioxide and a liquid trap of methylene chloride.

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[46] J. King, J. France and J. Snyder, *Fresnius J. Anal. Chem.*, **344** (1992) 474-478.
[47] H. Kawakura and Y. Hirata, *J. Chromatogr. A*, **815** (1998) 225-230.

The objective of this work was to investigate the methylation (with no derivatization agent) of decanoic acid that occurs during the supercritical fluid extraction process, and to determine the effects of both extraction and collection parameters on the reaction. Factors such as the chemical nature of the extraction fluid, collection fluid, collection temperature, and the presence of a catalyst in the collection vessel were all considered.

## 4.1 Experimental

### 4.1.1 Extraction

All extractions were performed using an Isco SFX 3560 (Lincoln, NE) supercritical fluid extraction system. Carbon dioxide with helium headspace (2000 psi) from Air Products and Chemicals (Allentown, PA) was used as the extraction fluid, and HPLC grade methanol (Fisher Scientific, Fairlawn, NJ) was used as the extraction fluid modifier.

Approximately 14 grams of Ottawa Cement Testing Sand (Fisher Scientific, Houston, TX) was placed in a 10 mL Isco special high crystalline polymer extraction vessel. The sand was used as-received, with no clean-up steps or preliminary extractions performed. A solution of approximately 10 mg/mL decanoic acid (Fisher Scientific) in HPLC grade methylene chloride (Fisher Scientific) was made, and 100 µL of the solution was spiked onto the Ottawa sand. The sand was allowed to air dry prior to extraction. The volume of the collection solvent was held constant at 10 mL, there was no static extraction time, and the dynamic extraction time was 10 minutes. No solvent replenishment of the trapping solvent was performed to replace losses which occurred during the course of the extraction. The collection solvents were HPLC grade hexane, methanol, and methylene chloride, all from Fisher Scientific.

The SFE conditions used for this study were a fluid pressure of 340 atm, an extraction temperature of 40°C, a restrictor temperature of 80°C, a liquid flow rate of 1.0 mL/min, along with variable dynamic and static extraction times and collection temperatures. A pressurized (~30 psi) collection mode was used for all experiments.

### 4.1.2 Extract Analysis

After the extraction was completed, the collection vial was removed from the extractor, solvent was added to a volume of 10 mL. Then 100 µL of an internal standard solution (5 mg/mL tetradecane in methylene chloride (Fisher Scientific)) was added. To establish an equivalent 100% recovery, 100 µL of the same spiking solution and 100 µL of the internal standard solution were added to an empty collection vessel, and the volume was adjusted to 10 mL. A portion of the solution was transferred to an amber autosampler vial for analysis. Each standard was injected four times and the response factors were averaged in order to calculate recoveries by the internal standard method.

In those cases where actual SFE was not performed, 100 µL of the spiking solution was deposited into a collection vial containing 10 mL of the collection solvent. The SFE program was then run on an empty extraction thimble, and the decompression of the fluid occurred in the spiked collection solvent.

All extracts were analyzed using a Hewlett Packard (Little Falls, DE) HP 5890 GC equipped with a split/splitless capillary column inlet system which was maintained at 275°C. A 30 m, 0.25 mm i.d., 0.25 µm $d_f$ DB-5 (J & W Scientific, Folsom, CA) fused silica capillary column was used for the separation. Grade

5.0 helium (BOC, Murray Hill, NJ) was used as the carrier gas and the flame ionization detector was maintained at 325°C. The GC temperature program contained a 2 min initial temperature of 80°C, followed by a ramp of 10°C/min to a final temperature of 180°C which was maintained for 3 min. For all of the samples, 1 µL was injected using a HP 7673 (Hewlett Packard) automatic injector in the splitless mode.

Identification of the eluted chromatographic peaks was also performed for several samples using GC with mass spectrometric detection. These samples were analyzed using a Hewlett Packard (Little Falls, DE) HP 5890 Series II GC equipped with a split/splitless capillary column inlet system which was maintained at 280°C. A 30 m, 0.25 mm i.d., 0.25 µm $d_f$ DB-5-MS (J & W Scientific, Folsom, CA) fused silica capillary column was used for the separation. Mass spectra were obtained by directly interfacing the GC with a HP 5972 (Hewlett Packard) mass selective detector (MSD). Grade 5.0 helium (BOC, Murray Hill, NJ) was used as the carrier gas and the transfer line was maintained at 280°C. The GC temperature program employed a 3 min initial temperature of 60°C, followed by a ramp of 10°C/min to a final temperature of 300°C which was maintained for 10 min. For all of the extract solutions, 1 µL was injected using a HP 7673 (Hewlett Packard) automatic injector in the splitless mode. The mass spectra were obtained by electron impact (EI) ionization at 70 eV. The ion source temperature was set at 280°C and the masses scanned were from 30 to 550 amu. All data were analyzed using a Hewlett Packard ChemStation equipped with the Wiley library of mass spectral data.

## 4.2 Results and Discussion

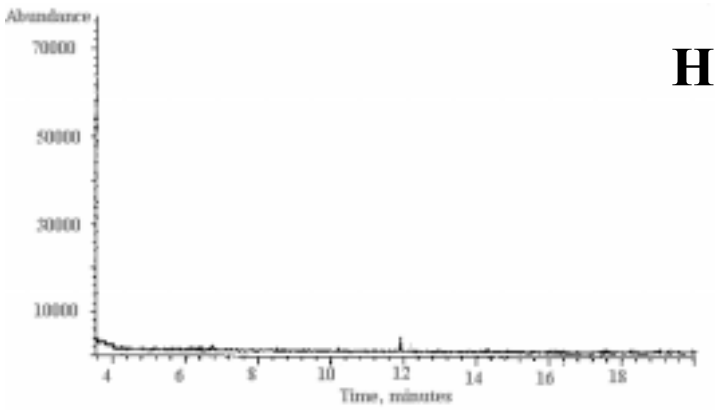
This work was divided into preliminary scouting experiments to determine whether the majority of the methylation reaction was occurring

during the supercritical fluid extraction, collection, or the chromatographic process, and specific experiments to determine the effects of various parameters on the methylation. During the scouting experiments no internal standard was added to the collection vials, as only qualitative data were desired.

### 4.2.1 Effect of Collection Solvent

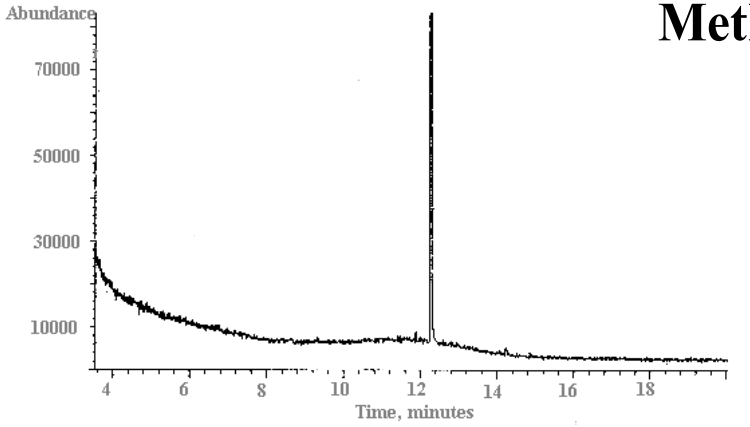
The first experiment involved the formation of the methyl ester of decanoic acid during SFE with non-modified carbon dioxide. After spiking the extraction vessel with decanoic acid, the SFE was performed and collection occurred in either methanol or hexane, during a 12 min extraction period. Obviously, hexane lacks the ability to provide the methylating agent necessary to from the methyl ester, and the presence of the fatty acid methyl ester (FAME) was not seen in the chromatograms resulting from these extractions, as seen in **Figure 4-1a**. It should be noted that there is no evidence of a chromatographic peak for decanoic acid either, due to the activity of the chromatographic system itself. However, when methanol was used as the collection solvent a well shaped peak appeared in the chromatogram, as seen in **Figure 4-1b**, indicating the formation of the methyl ester of decanoic acid, as confirmed by GC with mass spectrometric detection. This indicated that the FAME formation took place during the collection step, since no methylating agent was available during the extraction step.

A




**Hexane Trap**

B




**Methanol Trap**

**Figure 4-1:** Effect of Extraction Fluid on Methyl Decanoate Formation, non-modified carbon dioxide a: Hexane trap, b. Methanol trap.

### 4.2.2 Effect of Extraction Fluid

The next preliminary experiment was designed to investigate if the methylation occurred more readily under favorable extraction or collection conditions. The same experiment as above was repeated using 20% methanol-modified $CO_2$ as the extraction fluid, and the resultant chromatograms are shown
in **Figures 4-1c and 4-1d**. These chromatograms indicate that some formation of the methyl ester has occurred even when using hexane as a collection solvent, but it must be remembered that methanol was continually being added to the liquid trap during the course of the extraction, so that formation of the FAME could have occurred during either the extraction or collection step. **Figure 4-1d** indicates that the majority of the methylation took place during the collection step. Though this work was done at a qualitative level it was obvious that a higher amount of the FAME was obtained when using methanol instead of hexane as a collection solvent, although it was not obvious if there was a greater amount of the FAME formed when using methanol-modified $CO_2$.

### 4.2.3 Effect of Static Extraction Time when Using Methanol-Modified Carbon Dioxide

If the FAME was being formed during the SFE, in addition to the collection step, it would follow that an increase in static extraction time; the period that decanoic acid and methanol are in contact with one another under supercritical conditions, should increase the amount of FAME formed. (For this experiment, a 3 min dynamic extraction was followed by the static extraction time period. This initial short dynamic extraction was performed to assure a reproducible extraction fluid composition. The static extraction time was always followed by a 30 min dynamic extraction.) Results of these experiments,

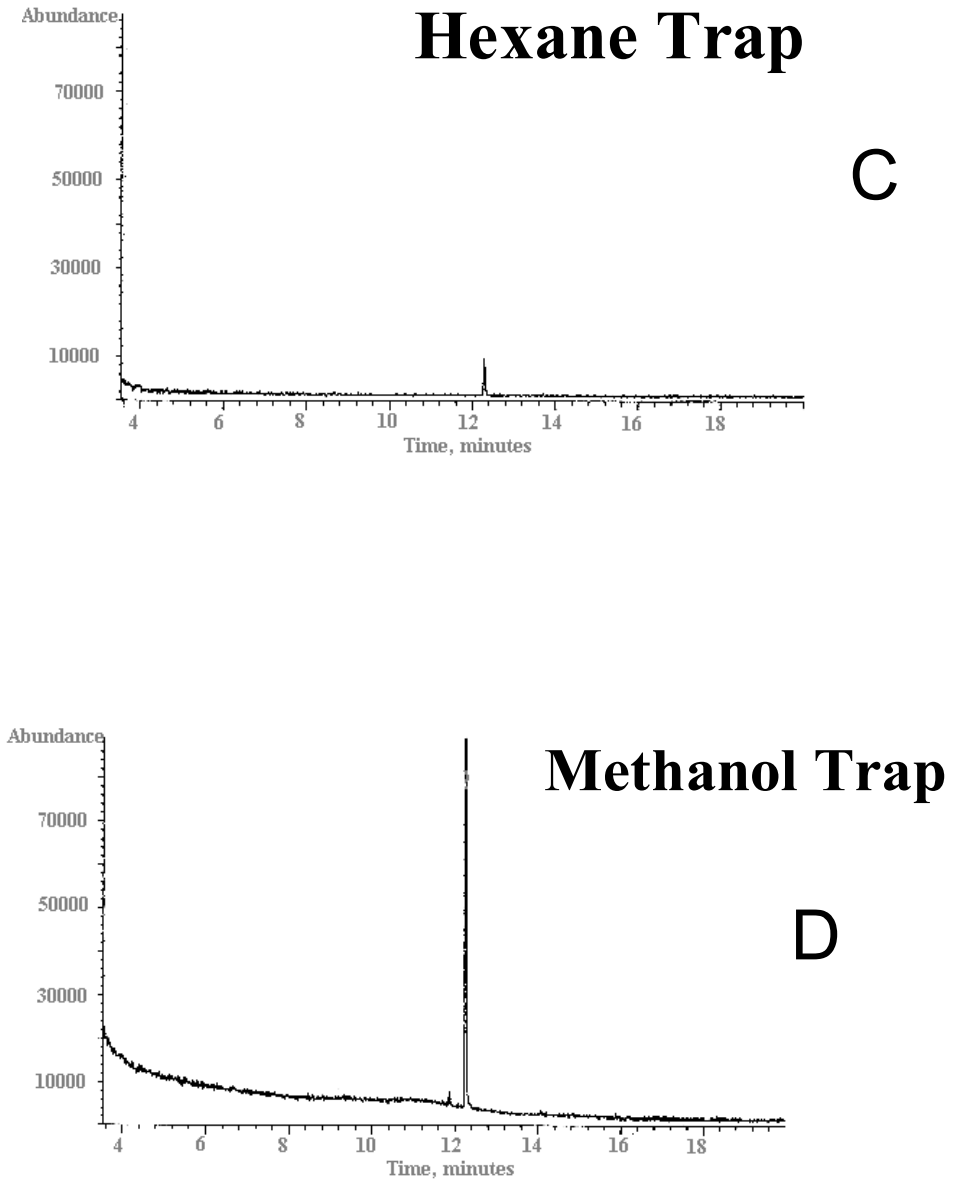



**Figure 4-1:** Effect of Extraction Fluid on Methyl Decanoate Formation, 20% methanol-modified carbon dioxide c: Hexane trap, d. Methanol trap.

shown in **Table 4-1**, indicated that there was no statistical increase or decrease (at the 95% confidence level) in FAME formation with increasing static time.

The clear indication at this point was that the majority of the reaction occurred during the collection step. To accomplish the separation of FAME formation during extraction from FAME formation during collection, subsequent experiments involved spiking the decanoic acid into the collection vessel, thus bypassing the extraction vessel completely. The $CO_2$ passed into the collection solvent after depressurization, at near atmospheric conditions.

### 4.2.4 Effect of Dynamic Extraction Time

In order to determine the effect of dynamic extraction time on the formation of methyl decanoate, dynamic extraction time was varied from 0 to 60 minutes, in 15 minute increments. A small amount of methyl decanoate was seen in the 0 minute samples, probably formed during the injection of the trapping solvent into the gas chromatograph. The length of the blank extraction could affect the methyl ester formation in two ways. First, the reaction could be ongoing, and the increase in time would result in an increase in product formed. The second effect could be based on the fact that the addition of the carbon dioxide to the collection vessel results in a more acidic solution in the presence of residual water, serving to catalyze the reaction. There were no special precautions taken to remove water from either the SF carbon dioxide or the methanol prior to use. The results of these experiments are shown in **Table 4-2** and **Figure 4-2** and indicate that over the course of a 60 minute extraction, the amount of methyl ester present is about twice that present at the beginning of the experiment. A plot of the natural logarithm of the methyl decanoate concentration versus time (**Figure 4-2**) for the suspected pseudo-first order reversible reaction yielded a reaction rate of 0.011 $min^{-1}$ or 0.69 $sec^{-1}$.

**Table 4-I:** Effect of Dynamic Extraction Time on Methyl Decanoate Formation During SFE

| Dynamic Time, min | Methyl Decanoate Concentration (moles/L) | Percent Conversion to Methyl Decanoate |
|---|---|---|
| 0 | $1.16 \times 10^{-3}$ | 2.0 (4) |
| 15 | $1.53 \times 10^{-3}$ | 2.6 (8) |
| 30 | $1.71 \times 10^{-3}$ | 3.0 (7) |
| 60 | $2.37 \times 10^{-3}$ | 4.1 (5) |

Numbers in parentheses are % relative standard deviation, n=3.

**Table 4-2:** Effect of Dynamic Extraction Time on Methyl Decanoate Formation During SFE

| Dynamic Time, min | Methyl Decanoate Concentration (moles/L) | Percent Conversion to Methyl Decanoate |
|---|---|---|
| 0 | $1.16 \times 10^{-3}$ | 2.0 (4) |
| 15 | $1.53 \times 10^{-3}$ | 2.6 (8) |
| 30 | $1.71 \times 10^{-3}$ | 3.0 (7) |
| 60 | $2.37 \times 10^{-3}$ | 4.1 (5) |

Numbers in parentheses are % relative standard deviation, n=3.

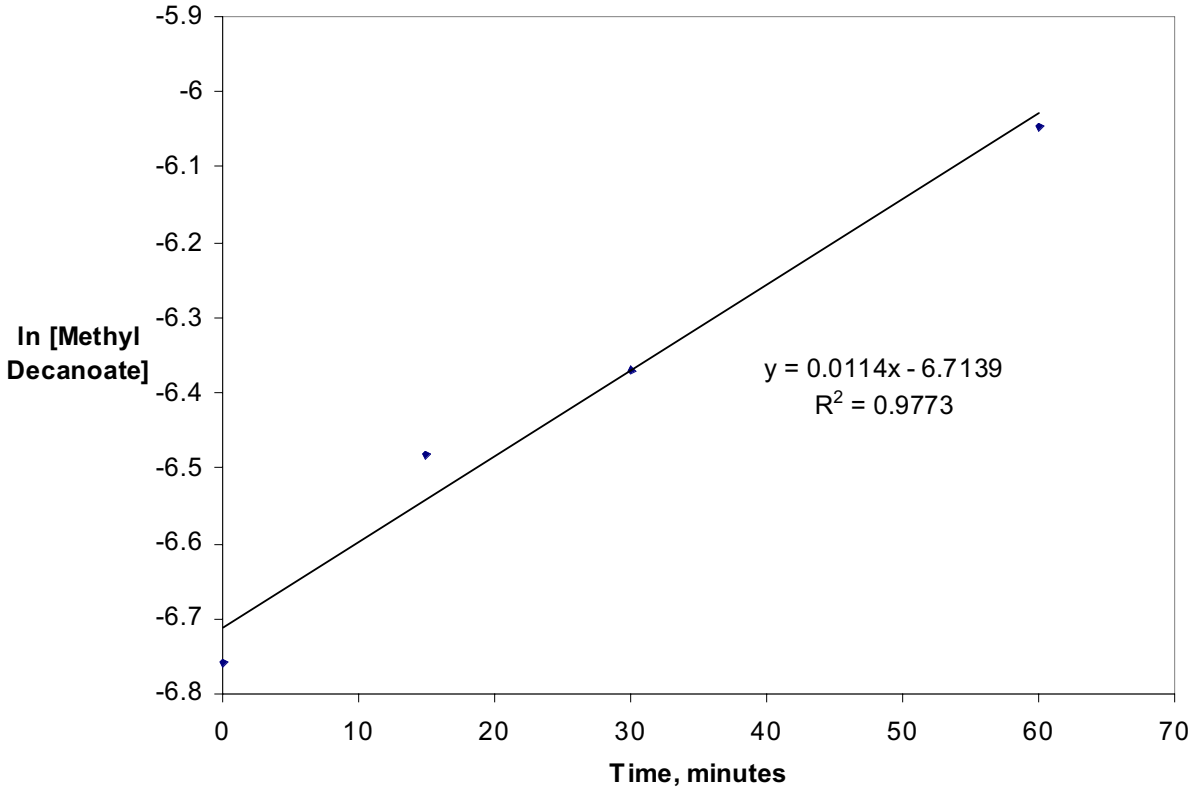



**Figure 4-2:** Formation of Methyl Decanoate During the Uncatalyzed Reaction

Determination of the methyl ester concentrations indicated that there was 2% conversion (from acid to ester) at 0 minutes, which increased to 4.1% at 60 minutes.

### 4.2.5 Effect of Adding Hydrochloric Acid (HCl) to the Collection Vessel

The objectives of this experiment were to determine if it was possible to add small amounts of the mineral acid, HCl, to the collection vessel to catalyze the methylation reaction, and determine if the acid catalysis was more important than a longer reaction time with the weaker acid, resulting from the $CO_2$ and water present. Varying amounts of HCl were added to the collection vessel, with the dynamic extraction time being held constant at 30 minutes. The results, shown in **Table 4-3** and **Figure 4-3**, indicate that addition of the HCl greatly enhanced the amount of methyl ester formed and that larger changes in HCl concentration do not appreciably change the amount of ester formation. In **Table 4-3**, the second column indicates the amount of methyl ester present compared to a standard prepared in methanol (100 µL of 10 mg/mL decanoic acid in methylene chloride). The third column indicates the amount of methyl ester formed when compared with a standard prepared in a similar manner, except that the prescribed amount of HCl was added to the standard prior to chromatography. These columns indicate that the presence of HCl greatly enhanced methyl ester formation, but that the dynamic extraction time had little effect in comparison. **Figure 4-3** shows the comparison of the conversion to the methyl ester for the uncatalyzed versus catalyzed reaction, and indicates that the reaction rate (when calculated for 0 and 30 minute data) increases from 0.013 $min^{-1}$ to 0.126 $min^{-1}$, or roughly a ten-fold increase. Conversion of the acid to the ester went from 3% for the uncatalyzed to 88% for the HCl catalyzed reaction. It should be noted that there was no evident deterioration in

**Table 4-3:** Effect of Adding Hydrochloric Acid to the Collection Solvent

| Amount HCl Added, mg | Amount Methyl Decanoate (Relative to Standard Containing No HCl) | Amount Methyl Decanoate (Relative to Standard Containing HCl) |
|---|---|---|
| 0 | 1.14 | 1.14 |
| 8 | 41.91 | 1.15 |
| 22 | 43.36 | 1.11 |
| 44 | 44.11 | 1.07 |

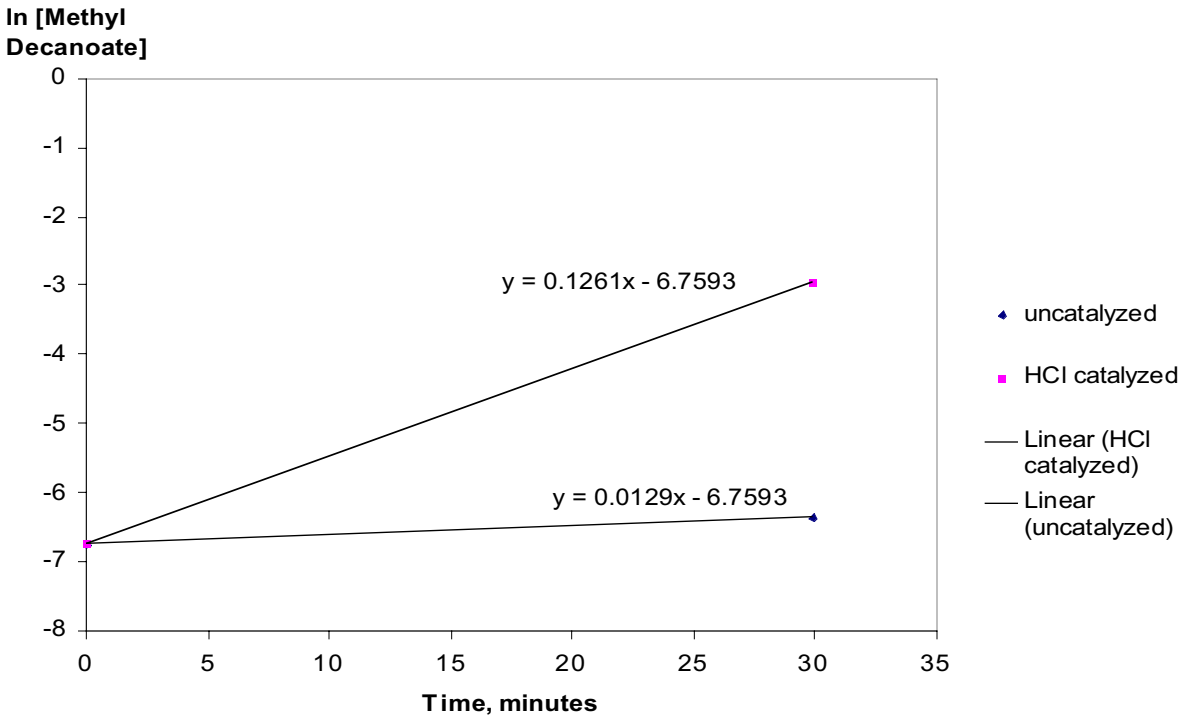



**Figure 4-3:** Comparison of the HCl-catalyzed and Uncatalyzed Formation of Methyl Decanoate

the chromatographic column when injecting the acid containing samples, though only a limited number of injections were made.

### 4.2.6 Effect of Collection Temperature

The last collection variable to be investigated was the collection temperature. The objective of this experiment was to determine if an increase in collection temperature would result in an increase in conversion to the methyl ester. **Table 4-4** shows the results for this experiment, which indicate that the effect of collection temperature is difficult to discern. Though it appears that the amount of methyl ester decreased with increasing temperature ($2^{nd}$ column), the amount of fatty acid also decreased ($3^{rd}$ column), which indicated that the fatty acid was somehow being lost in the reaction. The methyl ester/acid ratio ($4^{th}$ column) indicated a greater amount of methyl ester was present for the amount of acid present. Though these ratios are based in part on the fatty acid determinations, which have a large amount of error due to quantitative chromatographic problems, they do show a clear trend toward increasing conversion at higher collection temperature. It is quite possible that the methyl ester was being preferentially lost at the higher collection temperatures, due to its increased volatility.

## 4.3 Conclusions

The objective of this work was to investigate the methylation of decanoic acid occurring during the supercritical fluid extraction process. The methylation was found to occur primarily during the collection process and was greatly enhanced (reaction rate increased almost ten-fold) by the presence of an acid catalyst (i.e. additional to any carbonic acid formed from the $CO_2$ and

**Table 4-4:** Effect of Collection Temperature on Methyl Decanoate Formation

| **Collection Temperature, °C** | **Methyl Decanoate** ***Amount Relative to Standard*** | **Decanoic Acid** ***Amount Relative to Standard*** | **Methyl Decanoate/Decanoic Acid Ratio** |
|---|---|---|---|
| +40 | 0.75 (4) | 0.03 (98) | 20.22 (5) |
| +20 | 0.64 (8) | 0.11 (22) | 5.99 (8) |
| 0 | 0.83 (7) | 0.29 (15) | 2.93 (26) |
| -20 | 1.26 (3) | 0.37 (16) | 3.45 (19) |

Numbers in parentheses are % relative standard deviation, n=6.

residual water.) Increasing reaction time and collection temperature also increased the conversion rate to the methyl ester, but very little in comparison to the catalyzed reaction. This work indicates that extraction and derivatization can be performed simultaneously for subsequent chromatographic analysis.

In the following Chapter the knowledge acquired from the previous three chapters will be used to develop a method for the determination of extractable material in wood pulp samples. The collection solvent will be chosen not only for its ability to enhance collection efficiencies, but to allow methylation to occur during collection. Additionally, two *in-situ* (within the SFE cell) derivatization reactions will be investigated.

# CHAPTER FIVE

# SUPERCRITICAL FLUID EXTRACTION OF WOOD PULP WITH ANALYSIS BY CAPILLARY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

## 5.0 Introduction

Wood pulp is an important raw material used in the manufacture of many economically important products, including paper and paper products, cellulose nitrate and cellulose acetate. The amount of extractive material remaining after the pulping and bleaching processes may influence additional processing of the pulp, or be of health or environmental concern. Extractives are commonly defined as the material that can be removed from the pulp or paper sample by a particular solvent system during Soxhlet extraction.

The purpose of most of the extractives produced by a tree is to provide protection from predators that wish to consume the structural components of the cell wall[48]. The amounts and types of extractives produced are quite variable within individual species, and are also site specific. Because they appear to have no metabolic function in a tree, they are classified as secondary metabolites, and were, until recently, considered to be waste products of the plants' metabolism. Extractives are most concentrated in the bark of the tree, which is removed prior to pulping, and fall into the categories of volatile oils,

[48] *Wood Chemistry: Fundamentals and Applications*; E. Sjostrom; Academic Press; New York; (1993) 2nd Edition.

wood resins, fats and waxes, tannins, lignins, and carbohydrates. The recovery of wood extractives for further use is, therefore, in itself a lucrative industry[49].

However, extractive material present in the debarked, soaked and chipped wood can be detrimental during the pulping process. It can cause excessive chemical consumption, pitch problems, and pulp yellowing[50]. Additionally, after pulping, these extractives may influence the processing of the cellulose or cellulose derivatives in a subsequent manufacturing process.

Because of the ability of the extractives to influence cellulose or cellulose derivative processing, extractive content is commonly determined. The current TAPPI (Technical Association of the Pulp and Paper Industry) method involves Soxhlet extraction of the pulp sample with either methylene chloride, 2:1 benzene/ethanol, or 2:1 toluene/ethanol. The sample is air-dried prior to extraction, extracted for not less than 24 solvent cycles over a four to five hour period, transferred to a tared weighing dish, and the solvent removed by heating. The weighing dish is dried in a 110$^{o}$C oven and the extractive matter is taken as the weight gain of the dish after correction for the blank[51].

Supercritical fluid extraction (SFE) presents an attractive alternative to Soxhlet extractions for many reasons[52]. SFE is commonly performed with carbon dioxide, a relatively inert and nontoxic solvent. Since the solvating power (density) of the fluid can be changed as a function of pressure and temperature, the extraction can be fine-tuned to selectively extract certain analytes while leaving others behind. The solvating power of the fluid can then be increased to remove the remaining analytes. Because of the higher diffusivity (faster mass transfer) of a supercritical fluid compared to a liquid, extraction rates are generally much higher than with corresponding liquid/solid

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[49] *Handbook for Pulp and Paper Technologists*; G. A. Smook; Angus Wilde Publications; Vancouver; (1992); 2nd Edition.
[50] *Handbook of Pulping and Papermaking*; C. J. Biermann; Academic Press; New York; (1996).
[51] *Tappi Test Methods*, Volume 1 (1989) Method T204 om-88.
[52] S. B. Hawthorne, *Anal. Chem.,* **62** (1990) 633a-642a.

extractions. One of the more important advantages to using carbon dioxide for SFE is that removal of extraction solvent and waste disposal are neither a major problem or expense.

Various articles can be found in the literature in which supercritical fluids were used in the liquefaction of wood[53,54,55] and of the use of SFE to determine extractives in wood, pulp, paper and pitch[56-60]. McDonald et al.[56] used supercritical acetone and methanol to convert wood to liquid fuels, and found a maximum yield of 74%. They also were able to extract resin and fatty acids from southern pine and waxes from Douglas Fir bark using either supercritical carbon dioxide, nitrous oxide, propane, or ethylene. They found that propane and nitrous oxide produced higher yields than the other fluids studied. Demirbas[57] found that extraction of beech wood samples with supercritical acetone gave approximately four times greater extracted masses than Soxhlet extraction with the same solvent. Fatty acids were analyzed and linoleic acid was found to be the major constituent in both extracts.

Lee et al.[58] investigated the extraction of chlorinated phenolics in sediments collected downstream of a chlorine-bleaching mill using carbon dioxide with an *in-situ* derivatization process. They found that under moderate extraction conditions the SFE-derivatization method gave comparable results to conventional techniques, with great time and solvent savings. Lee and Peart[59] developed an SFE method using supercritical $CO_2$ for the extraction of resin and fatty acids from sediments downstream from pulp mills that provided recoveries equal to or greater than Soxhlet extraction, again with great savings in time and solvent consumption. Sequeira and Taylor[60] also found SFE to be a

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[53] J. Jezko and J. Howard, *Can. Bioenergy R&D Semin.* 398 (1984).
[54] C. McDonald, B. Bennett, and J. Howard, *Can. Bioenergy R&D Semin.*, 393 (1984).
[55] T. Reyes, S. Bandyopadhyay and B. McCoy, *J. of Supercritical Fluids*, **2** (1989) 80.
[56] C. McDonald, J. Howard, and B. Bennett, *Fluid Phase Equilibria*, **10** (1983) 337.
[57] A. Demirbas, *Wood Sci. Technol.*, **25** (1991) 365.
[58] H. Lee, T. E. Peart, and R. L. Hong-You, *J. Chromatogr.*, **636** (1993) 263.
[59] H. Lee and T. Peart, *J. Chromatogr.,* **594** (1992) 309.
[60] A. Sequeira and L. T. Taylor, *J. Chromatogr. Sci.*, **30** (1992) 405.

comparable sample preparation technique for the analysis of several bleached wood pulps that had been treated by different methods.

The objectives of this work were to compare the amount and type of materials extracted via SFE with those obtained from a standard Soxhlet extraction with methylene chloride. In addition, the extracted material was to be separated (chromatographed) and as many extracted components as possible identified by capillary gas chromatography/mass spectrometry. A comparison of off-line and *in-situ* methylation reactions and determination of acetylation products was to be performed. Certain identified components were then to be quantified by an internal standard method.

## 5.1 Experimental

### 5.1.1 Sample Preparation

The wood pulp samples were received from Buckeye Cellulose Corporation (Memphis, TN) as sheets wrapped in aluminum foil inside a kraft envelope. (Earlier extractions had determined that storing the samples in plastic bags resulted in contamination of the extracts with plasticizers (esters of adipic and phthalic acids) from the plastic.) Two methods of sample preparation were used. In the first method, sheets of wood pulp were cut into strips approximately 0.5 x 3 cm using scissors previously washed in HPLC grade methanol and methylene chloride (EM Science, Gibbstown, NJ). In the second method, the wood pulp was cut into small pieces (~1 $cm^2$) which were then ground to the consistency of a cotton ball in a Mr. Coffee® (Deerfield, IL) Model IDS-50 coffee mill. In either case, care was taken to avoid contamination of the pulp sample with any skin oils by wearing gloves. The strips or ground samples were then thoroughly mixed.

### 5.1.2 Standards Preparation

For quantitative determinations, a stock caffeine (Sigma Chemical Co., St. Louis, MO) internal standard solution containing 320 mg in 100 mL methylene chloride was prepared. Fatty acid, fatty acid methyl ester (FAME), and alkane standards were obtained from PolyScience (Niles, IL). Multicomponent standard mixtures were dissolved in 50/50 methylene chloride/methanol.

### 5.1.3 Soxhlet Extraction

A sample containing approximately 10 g of wood pulp was placed in a glass thimble equipped with a fritted disk, after which the thimble was placed in the Soxhlet extractor. The round bottom extraction flask was filled with 200 mL of methylene chloride, and extraction at the boiling point was allowed to proceed for 24 hours. The extraction flask was then fitted on a Kuderna-Danish concentrator (Supelco, Bellefonte, PA). The extractor was then rinsed into the concentrator with an additional 25 mL of solvent. The concentrator was kept in a water bath until the solvent volume was less than 1 mL. For quantitative determinations, 100 µL of a stock 320 µg/mL caffeine internal standard was added to the concentrated extract solution and the total volume subsequently was adjusted to approximately 1 mL. Blank determinations were performed using an empty extraction thimble and identical extraction procedures.

### 5.1.4 Supercritical Fluid Extraction (Liquid Trapping)

Approximately 3.5 g of wood pulp were placed into each of three 10 mL high temperature crystalline polymer extraction thimbles (ISCO, Lincoln, NE). All extractions were performed with an ISCO SFX 3560 dual syringe pump

system. Extraction fluids were composed of SFE Grade Carbon Dioxide (Air Products and Chemicals Inc., Allentown, PA) and $CO_2$ modified with HPLC grade methanol. The modified $CO_2$ was prepared by addition of methanol to liquid $CO_2$ by volume percentage, with mixing and equilibration occurring downstream, prior to the extraction thimble. All extractions were performed at 80°C and 516 bar (density = ~0.89 g/mL for 100% $CO_2$; ~0.90 g/mL for 10% methanol modified $CO_2$; ~0.90 g/mL for 20% methanol modified $CO_2$), with an electronically controlled automatic variable restrictor temperature at 80°C. The flow rate was 1.5 mL/min (liquid flow measured at the pump) and the time of extraction was 20 min, unless otherwise stated. The extracted components were collected in 10 mL of 50/50 HPLC grade methylene chloride/methanol contained in an ISCO pressure resistant collection vial fitted with a cap and Teflon septa. The vial was pressurized with an additional approximately 30 psi of $CO_2$. In addition, a solvent replenishment routine to replace collection solvent lost during trapping of the extract was available and used as noted. The solvent volume was reduced in a warm water bath to approximately 1 mL and 100 µL of the internal standard solution was added prior to analysis by GC/MS.

### 5.1.5 Supercritical Fluid Extraction (Tandem Trapping)

Approximately 10 g of wood pulp were placed into a 50 mL stainless steel extraction vessel. All extractions were performed with an ISCO/Suprex Prepmaster supercritical fluid extractor (ISCO, Lincoln, NE). The extraction fluid was composed of SFE Grade Carbon Dioxide (Air Products and Chemicals Inc., Allentown, PA) modified with HPLC grade methanol. The 95/5 methanol modified $CO_2$ was prepared by addition of methanol by volume percentage (of liquid), with mixing and equilibration occurring downstream, prior to the extraction vessel. All extractions were performed at 80°C and 450

atm with an electronically controlled automatic variable restrictor temperature of 80°C. The flow rate was 2.0 mL/min (liquid flow measured at the pumps) and the static time was 30 min, followed by a 45 min dynamic extraction. The extracted components were collected on an ODS solid phase trap, held at 40°C during the course of the extraction. The trap temperature was held at 25°C as 8 mL of 50/50 HPLC grade methylene chloride/methanol was used to rinse the trap. A second (tandem) trap containing 5 mL of methanol at room temperature was also used. The trap rinse and tandem trap liquid were combined prior to concentrating the extract. The solvent volume was reduced in a warm water bath to approximately 5 mL for the hardwood samples and 2 mL for the softwood samples. Internal standard solution (100 µL) was added prior to each analysis by GC/MS.

### 5.1.6 Derivatizations

Wood pulp extracts containing free fatty acids were derivatized using 14% $BF_3$ in methanol obtained from Alltech Associates (Naperville, IL) to form methyl esters. Acetic anhydride and pyridine, both obtained from Alltech, were used for acetylation of hydroxy acids. For *in-situ* methylations when liquid trapping was performed, 60 µL of the $BF_3$/methanol solution was added directly to each extraction thimble immediately prior to extraction. Approximately 20 µL was placed at the top, bottom, and middle of the wood pulp sample in each extraction thimble. For *in-situ* methylations when tandem trapping was performed, 1-1.5 mL of the $BF_3$/methanol solution was added directly to each extraction thimble immediately prior to extraction, again distributed evenly throughout the sample. For *in-situ* acetylations 60 µL of acetic anhydride and 120 µL of pyridine were added to each extraction thimble, in the same fashion as the $BF_3$/methanol, except where noted. Acetylations were only conducted

when using liquid trapping. For off-line methylations, the esters were formed using 5 mL of the $BF_3$/methanol reagent, with the products then being extracted into OPTIMA grade hexane (Fisher Scientific, Fairlawn, NJ) for GC/MS analysis. The off-line acetylations were carried out upon the previously esterified samples using 10 mL of pyridine followed by 5 mL of acetic anhydride. The products of these reactions were also extracted into hexane prior to GC/MS.

### 5.1.7 Gas Chromatography

All extracts were analyzed using a Hewlett Packard (Little Falls, DE) HP 5890 Series II GC equipped with a split/splitless capillary column inlet system, which was maintained at 280$^{o}$C. A 30 m, 0.25 mm i.d., 0.25 μm $d_f$ DB-5-MS (J & W Scientific, Folsom, CA) fused silica capillary column was used for the separation. Mass spectra were obtained by directly interfacing the GC with a HP 5972 (Hewlett Packard) mass selective detector (MSD). Grade 5.0 helium (BOC, Murray Hill, NJ) was used as the carrier gas and the transfer line was maintained at 280$^{o}$C. The temperature program employed contained a 3 min initial temperature of 60$^{o}$C, followed by a ramp of 10$^{o}$C/min to a final temperature of 300$^{o}$C, which was maintained for 10 min. For all of the extracts, 1 μL was injected using a HP 7673 (Hewlett Packard) automatic injector in the splitless mode. The mass spectra were obtained by electron impact (EI) ionization at 70 eV. The ion source temperature was set at 280$^{o}$C and the masses scanned were from 30 to 550 amu. All data were analyzed using a Hewlett Packard ChemStation equipped with the Wiley library of mass spectral data.

### 5.1.8 Moisture Determination

Moisture content of the wood pulp samples was determined by placing approximately 10 g (accurately weighed) of the sample, either as strips or ground, into a weighing dish in a 100°C oven for 24 hours. The sample was allowed to cool to room temperature in a desiccator after being removed from the oven. The loss in weight divided by the starting weight was taken as the fraction moisture of the sample. All subsequent quantitations of extracted components were corrected for moisture content.

### 5.1.9 Infrared (IR) Spectroscopy

The extracts were dried with gentle heating under a stream of nitrogen and the solids reconstituted in a small amount of methylene chloride. The solution was dripped onto a salt plate (sodium chloride) using a disposable pipette. The $CH_2Cl_2$ was allowed to evaporate prior to IR analysis. A Perkin Elmer Model PE 1615 Fourier Transform Infrared Spectrometer was used to obtain spectra from 4000 to 800 $cm^{-1}$. Four scans were co-added to obtain the resultant spectra.

### 5.1.10 Supercritical Fluid Extraction (Extraction Profile Determination)

Approximately 3 g of dried ground wood pulp was placed in a 7 mL stainless steel extraction thimble and extracted with non-modified $CO_2$ using a LECO (St. Joseph, MO) model FA-100 supercritical fluid extractor. The extraction temperature was 80°C and the pressure was held at 450 atm. Decompressed $CO_2$ flow was 1 L/min (approximately 2 mL/min measured as a

liquid) and dynamic extraction time was 15 minutes. The restrictor temperature was 110°C, and the extracted material was trapped onto glass wool in a preweighed collection vial. This instrument was designed for gravimetric fat determinations, and results were automatically reported as percent extracted based on the weight gain of the collection vial.

## 5.2 Results and Discussion

### 5.2.1 Determination Of Optimum Extraction Conditions

In the initial phase of this study the objective was to qualitatively determine if SFE presented a viable alternative to Soxhlet extraction with $CH_2Cl_2$, and if so, what extraction parameters would allow for maximum removal of extractives. A 48 hour Soxhlet extraction was used as a basis for comparison, as shown in **Figure 5-1a**. **Figure 5-1b** and **Figure 5-2b** present the results from a 30 mL extraction (20 min at 1.5 mL/min) with pure $CO_2$, and **Figure 5-2c** shows a second 30 mL extraction of the same (previously extracted by SFE with 100% $CO_2$) sample with 10% methanol modified $CO_2$. **Figure 5-2d** is a subsequent extraction of the same sample (extracted with 30 mL 100% $CO_2$ and 30 mL 10% methanol modified $CO_2$) with an additional 30 mL of 20% methanol modified $CO_2$. The purpose of these series of extractions was to determine what differences exist in the nature of the analytes extracted by Soxhlet extraction with methylene chloride and those extracted by modified or non-modified $CO_2$. From examination of the total ion chromatograms, it can be seen that the Soxhlet extraction appeared to extract more earlier eluting material than the SFE and that some late eluting peaks (around 23-26 min) appeared in the Soxhlet extract, but not in any of the SFEs. It is also obvious that increasing the methanol modifier concentration does not allow the

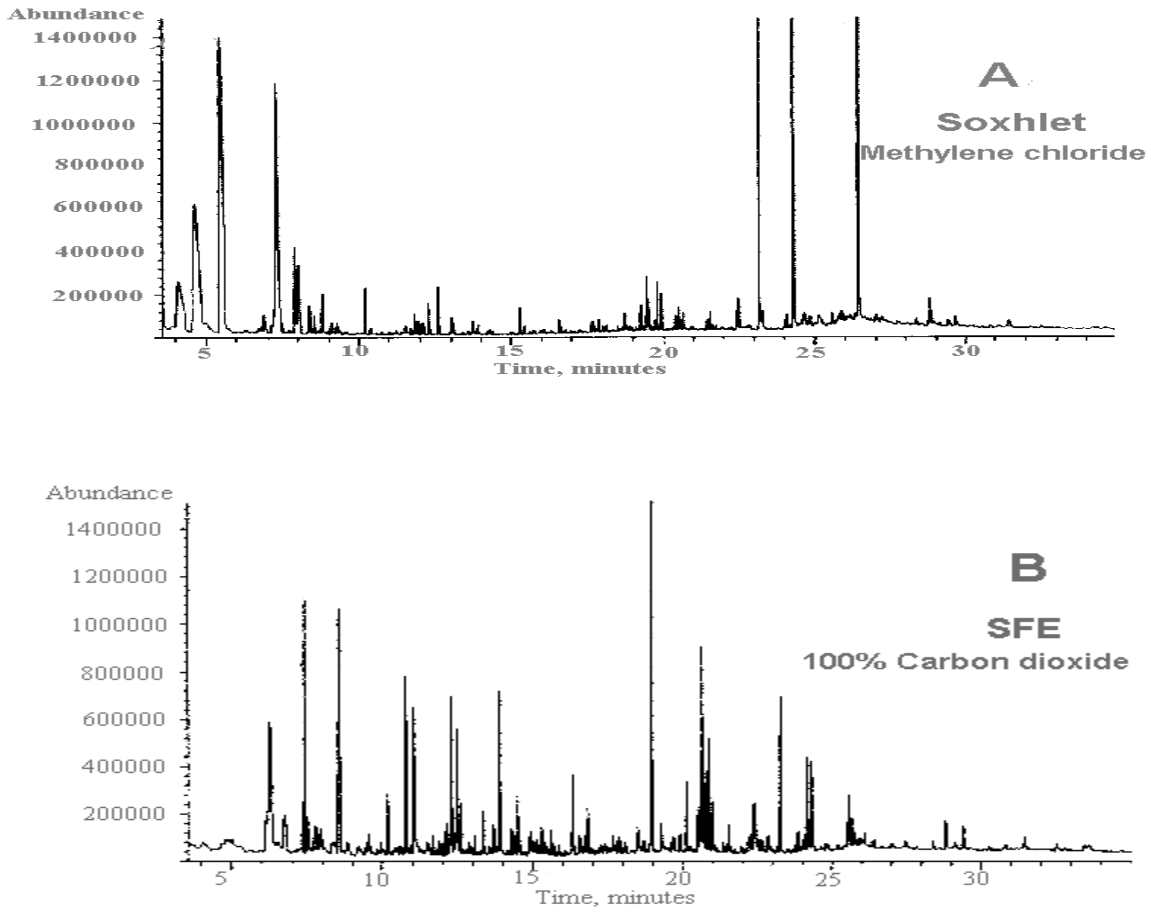


**Figure 5-1:** Total ion chromatograms of bleached softwood pulp extract. A; Soxhlet extraction with methylene chloride, B; supercritical fluid extraction with 100% carbon dioxide. Sample size 10g. Chromatographic conditions in text. Peak identities correspond to retention times in Table 5-2.

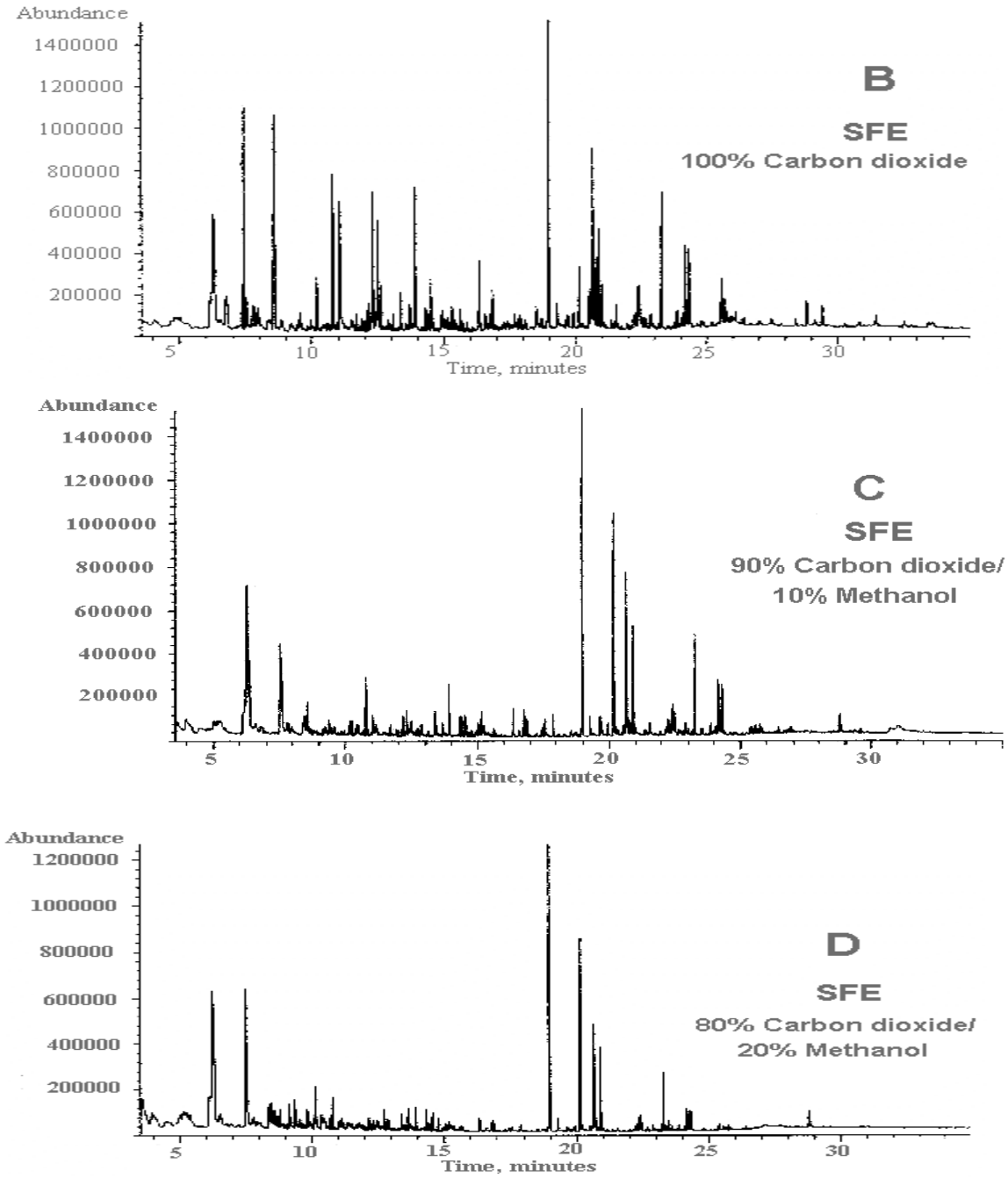


**Figure 5-2:** Total ion chromatograms of bleached softwood pulp extract. B; supercritical fluid extraction with 100% carbon dioxide, C; SFE of raffeinate with 90/10 carbon dioxide/methanol, D; SFE of second raffeinate with 80/20 carbon dioxide/methanol. Sample size 10g. Chromatographic conditions in text. Peak identities correspond to retention times in Table 5-2.

extraction of any new analytes, but continues to extract additional amounts of the same analytes that were being extracted with non-modified $CO_2$. The methylene chloride Soxhlet extraction shows via GC/MS the presence of alcohols, alkanes, alkenes, aldehydes, ketones, sterols and fatty acids. Interestingly, SFE shows the presence of the same classes of compounds, with the exception of alkenes and the presence of methyl esters of fatty acids. **Table 5-1** reports for both extracts the identities of the major peaks identified by the GC/MS library software with a matching quality of 90 or greater. Identification was also accomplished by retention time matching for members of homologous series. It is interesting to note that if either SFE or Soxhlet extracts were allowed to go to dryness during the concentration process, a total ion chromatogram similar to the one shown in **Figure 5-3** resulted. This chromatogram indicated the appearance of only caffeine, the internal standard, and tetracosane. Since no special precautions were taken to deactivate the chromatographic system, it was believed initially that the extracts contained free fatty acids, which were not chromatographable under the chosen conditions. **Figure 5-4a** contains an infrared spectrum of the dried and reconstituted (in $CH_2Cl_2$) Soxhlet, that is consistent with the presence of free fatty acids in the extract. Since it is known that methylation of free decanoic acid can occur during the SFE collection process (see Chapter 4) and that the SFE extract, when not allowed to go to dryness, showed the presence of methyl esters of fatty acids, a chromatogram of the dried extract should show the presence of the methyl esters, unless the methylation reaction was reversible. (The samples were taken to dryness in a water bath, but the temperature did not exceed ~100$^{o}$C, therefore simple volatilization was highly unlikely.) As the methanol in the solvent was evaporated, the reaction equilibrium should shift towards formation of the free fatty acids. As the last of the methanol was driven off, practically complete conversion to the FFAs had occurred. Inspection of a

**Table 5-1:** Identification of Extracted Components from Bleached Softwood Pulp Sample. Quality of match is greater than 90 percent.

| Compound | Soxhlet | SFE |
|---|---|---|
| 2-Cyclohexen-1-one | X | |
| Phenol | | X |
| 1,2-Cyclohexanediol | X | |
| Nonanal | X | X |
| Decanal | X | X |
| Dodecanal | | X |
| Benzoic acid, 4-hydroxy-methyl ester | | X |
| 1-Dodecanol | | X |
| Phenol, 2,6-bis (1,1-dimethylethyl) | | X |
| Dodecanoic acid, methyl ester | | X |
| Tetradecanoic acid, methyl ester | | X |
| Hexadecanoic acid, methyl ester | | X |
| Octadecenoic acid, methyl ester | | X |
| Octadecanoic acid, methyl ester | | X |
| Docosane | X | X |
| Tetracosane | X | X |
| Cholest-5-en-3-ol (3.beta.) | X | X |

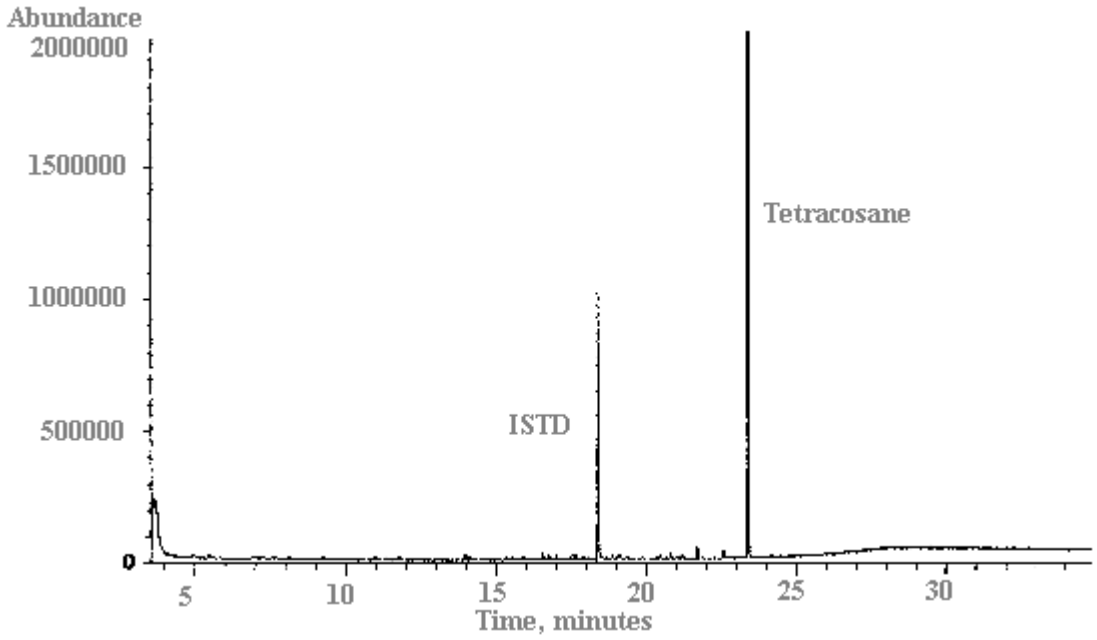


**Figure 5-3**: Total ion chromatogram of reconstituted bleached softwood pulp extract after extract was allowed to go to dryness. Sample size 10g. Chromatographic conditions in text.

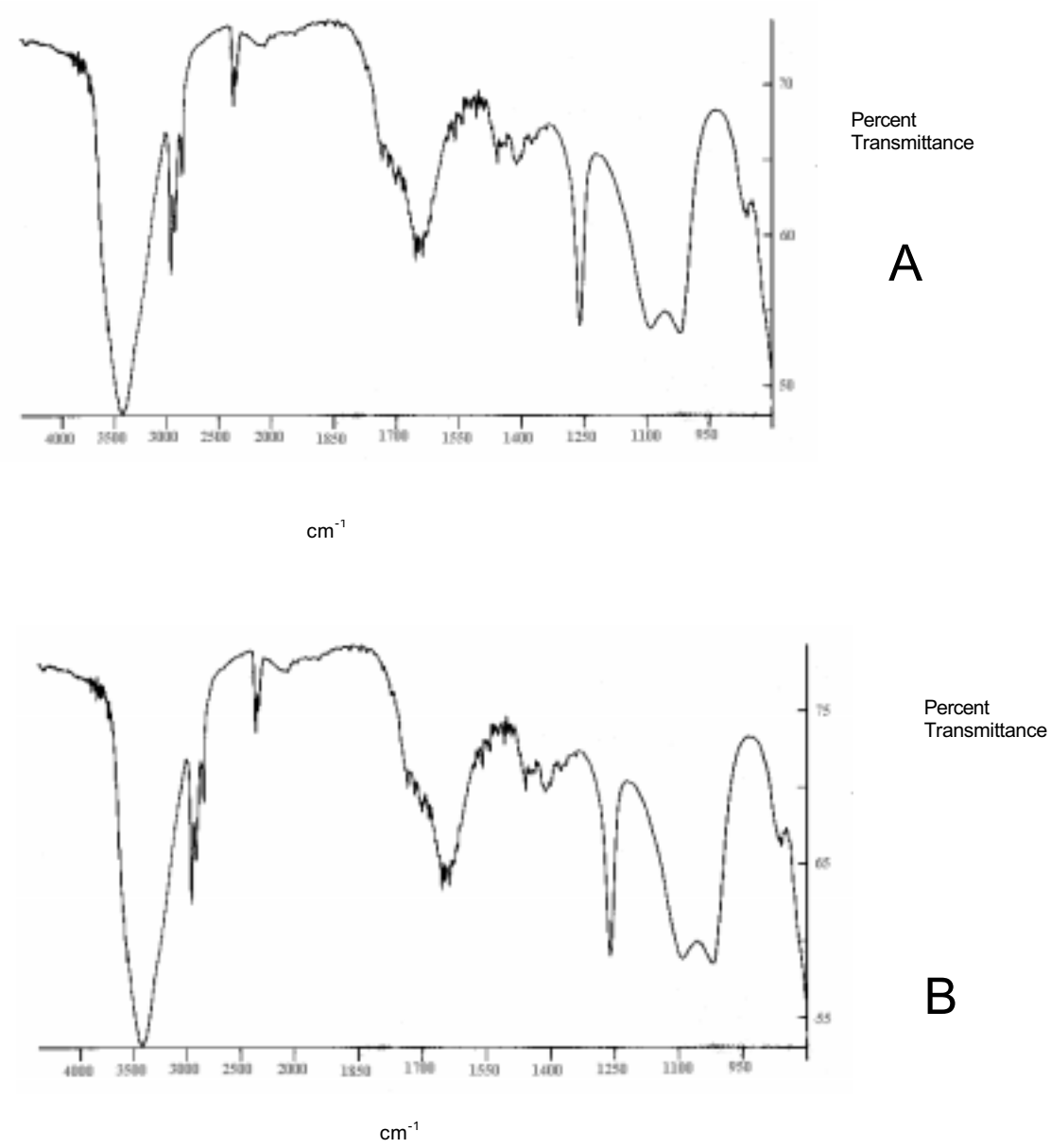


**Figure 5-4:** Infrared spectra of dried bleached softwood pulp extracts. Extracts were reconstituted in methylene chloride. A: Soxhlet extract; B: Supercritical fluid extract.

resultant IR spectrum, shown in **Figure 5-4b**, revealed consistency with fatty acids. Though quantification was not performed on the amount of fatty acids present before and after drying of the sample, this lends credence to the theory that methylation following SFE, under the gentle acidic conditions resulting from $CO_2$ and water is a reversible reaction. Conversion of the FFAs back to the FAMEs upon the addition of a methanol containing solvent does not occur to any measurable degree, since the $CO_2$, which provided the acid catalyst for the reaction, is no longer present in the sample.

### 5.2.2 Determination Of Percent Extractives

Several attempts were made to determine conditions that would lead to exhaustive extraction of the wood pulp samples. Using non-modified $CO_2$ and ground wood pulp samples, a series of extractions was performed as described in Section 5.1.10. The results of these experiments are presented in **Figure 5-5**, and indicate that the extraction is diffusion controlled (from the practically linear rise in percent extractives), and exhaustive extraction has not been achieved (the graph has not leveled off).

A second method, using ground samples and 20% methanol-modified $CO_2$, with analysis by GC/MS (simply looking for analyte peaks in the extract) revealed that even after 4 hours (240 mL extraction fluid), the analytes were still being extracted from the sample.

At this point, it was learned that the Soxhlet extraction (current TAPPI method) also does not exhaustively extract the sample.[61] The method does give reproducible results however, giving it utility in the pulp and paper industry. The decision was made to determine the reproducibility of a fixed set of extraction

[61] Personal communication with David McWilliams, Hoechest Celanese Corporation.

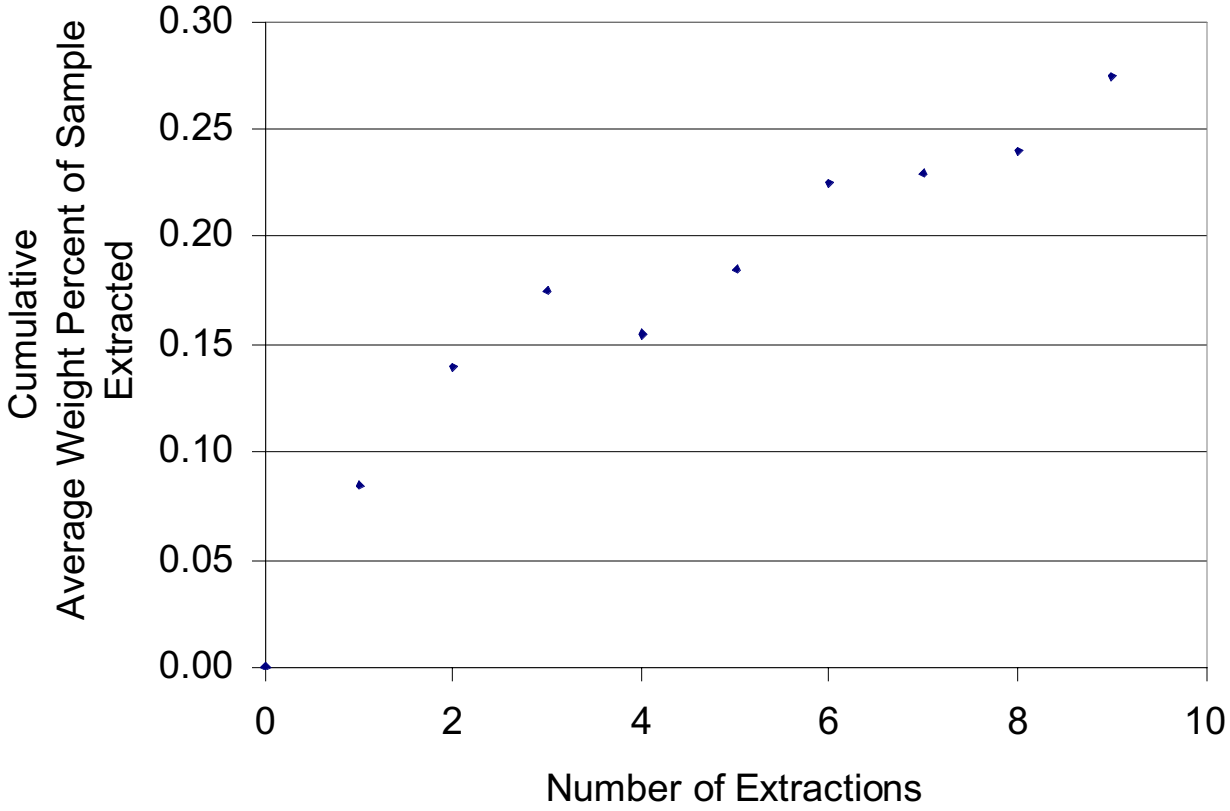


**Figure 5-5:** Gravimetric extraction profile of a bleached softwood pulp sample. Each extraction was 15 minutes. Decompressed $CO_2$ flow was 1 L/min. Percent extracted was based on the weight gain of the collection vial compared to sample weight.

conditions, to determine if SFE would be a viable alternative to the Soxhlet method. This was accomplished by determining the weight lost during extraction of a previously dried sample. The results of these analyses indicate for 6 determinations, an average of 1.26% by weight on a dry basis (3.2% RSD) was extracted from the softwood sample used for method development. Though the data obtained were reproducible, it was approximately 100 times higher than the
values reported when using the TAPPI method (0.01%). The task at this point then became to identify the extracted components and quantitate as much as possible in an attempt to determine a mass balance with the weight lost upon extraction.

### 5.2.3 Identification of Extracted Components

After the extractions described in Section 5.2.1 were performed, only a limited number of components (**Table 5-1**) were identified with a 90% or greater quality of match by the GC/MS identification algorithm. However, many more compounds eluted from the chromatographic column, as was evident after inspection of the chromatograms in **Figures 5-1 and 5-2**. Extensive searching of the mass spectral library tentatively identified many more components, with lesser qualities of match. (The mass spectrometer software used a standard tuning file, which rates all mass ranges fairly equally when comparing to standard spectra.[62]) The results of these tentative identifications, when the quality of the library match was greater than 40, are presented in **Table 5-2**. Though the actual identities of the extracted compounds may be incorrect, the chemical class (aldehyde, ketone, etc.) is usually reliable.

[62] Hewlett Packard 5971 MS ChemStation G1034 Version C.01.05, Hewlett Packard Co., 1989-93.