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Novel Strategies for Rapid Analytical Preparation of Solid Samples

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Novel Strategies for Rapid Analytical Preparation of Solid Samples

by

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Abstract

This thesis describes successful advances in solid-liquid extraction methodology on several fronts from novel applications of existing extraction techniques, to the development of novel extraction methods. These advances aim to reduce the time and the consumption of costly, toxic, and environmentally hazardous solvents often employed in conventional extraction techniques. One such development involves using enhanced fluidity liquid extraction for the first time to extract Fluoxetine Hydrochloride and other polar drugs from various pharmaceutical formulations. Using this approach it is found that it can greatly reduce the organic solvent and the time requirements for pharmaceutical sample preparations. For example, employing an extraction fluid of 50% carbon dioxide / 50% methanol, produced an extraction recovery of Fluoxetine Hydrochloride from Prozac® capsules of 99 ± 2% in just 3 minutes using only about 5 mL of total methanol. Further, the method allows for the incorporation of modest amounts of water as a ternary modifier, which in turn significantly enhances the extractability of hydrophilic analytes. For example, the extraction recovery of Ascorbic Acid from tablets was improved from 10 ± 3% to 63 ± 2% after 10 minutes extraction when 10% water / 90% methanol was employed as a cosolvent instead of pure methanol.

Another advancement in the area of solid-liquid sample preparations that involves developing a novel micro pressurized liquid extraction (μPLE) technique is also demonstrated in this thesis. The method employs rapid heating in a static mode to remove analytes from 5-10 mg samples in only a matter of seconds using only 125 μL of solvent. The required instrumentation and procedure are relatively simple and readily accessible to

most laboratories. The method was explored with different samples and the results compared well to conventional PLE extractions of the same. As applications, the method was used for dried blood spot and polycyclic aromatic hydrocarbons samples. The method proved to be very rapid and solvent efficient compared to conventional extraction methods of such samples.

Finally, the benefits of combining μPLE with an ultra-short GC-FID column apparatus in order to very rapidly monitor thermal degradation of a model pharmaceutical formulation are also demonstrated in this thesis. Using an ultra-short GC column ASA and its degradants were successfully analyzed and results compared well with HPLC for monitoring degradation of the analyte as a function of temperature. Coupling this with μPLE, it was found that a thermally degraded ASA tablet could rapidly be extracted and analyzed for its contents.

Preface

Portions of **Chapter Three** were published as Fadi L. Alkhateeb, Kevin B. Thurbide, Gordon Lambertus, Eric Jensen, Characterization of Sample Preparation of Prozac® Capsules Using Enhanced Fluidity Liquid Extraction. **Anal. Methods. 4 (2012) 3219.**

Portions of **Chapter Four** were published as Fadi L. Alkhateeb, Kevin B. Thurbide, A Novel Micro Pressurized Liquid Extraction Method for Very Rapid Solid Sample Preparation. **Anal. Methods. 7 (2015) 1509.**

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List of Symbols, Abbreviations, Acronyms, and Initialisms

A	Analyte
API	Active pharmaceutical ingredient
ASA	Acetylsalicylic acid
Atm	Atmosphere (1 atm = 101325 Pa)
Ave	Average
CAN	Canada
cm	Centimeter
cm^2/s	Centimeter squared per second
DBS	Dried blood spot
e.g.	Exempli gratia (for example)
EFLE	Enhanced fluidity liquid extraction
FID	Flame ionization detector
g/cm.s	Gram per centimeter per s
g/mL	Gram per milliliter
g/mol	Grams per mole
GC	Gas chromatography
GC-FID	Gas chromatography- Flame ionization detector
HPLC	High performance liquid chromatography
HPLC-UV	High performance liquid chromatography-ultra violet detector
i.e.	id est (in other words)
I.D.	inner diameter

IP	Intellectual property
K	Partition coefficient
LC-MS	Liquid chromatography-mass spectrometry
m	Number of moles
μFID	Micro flame ionization detector
mg	Milligram
μg	Microgram
mg/mL	Milligram per milliliter
min	Minute
μL	Microliter
mL	Milliliter
mL/min	Milliliter per minute
mm	Millimeter
μm	Micrometer
mol/L	Mole per liter
μPLE	Micro Pressurized Liquid Extraction
mV	Millivolts
ng	nanogram
°C	Degrees Celsius
°C/min	Degrees Celsius per minute
O.D.	Outer diameter
PAH	Polycyclic aromatic hydrocarbons
P_c	Critical pressure

pH	Negative logarithm of the hydronium concentration
PLE	Pressurized liquid extraction
q	Fraction
RSD	Relative standard deviation
SA	Salicylic acid
S	Second
SF	Supercritical fluid
SFE	Supercritical fluid extraction
SS	Stainless steel
SWE	Subcritical water extraction
T	Temperature
T_c	Critical temperature
USA	United States of America
UV-Vis	Ultra violet-visible
V	Volume
V/V	Volume per Volume
wt/wt	Weight per Weight

Chapter One: **INTRODUCTION**

Chemical analysis frequently consists of several steps such as sampling, sample preparation, separation, detection, and data analysis. Aspects of sample preparation are often the bottleneck in chemical analyses and they can take up to 80% of total analysis time. Solids are the largest single category of samples analyzed in laboratories, and procedures for extracting analytes from these samples are particularly challenging. This is because solid samples are normally complex and interactions between analyte molecules and solid surfaces are strong.[1] As such, conventional solid sample preparation methodologies often involve lengthy and solvent-intensive steps that are not always effective at extracting analytes from solid samples for analysis. For instance, a commonly used procedure for extracting analytes from solid samples is the classic shake-filter method. In this method, solvent is added to the sample while it is being agitated to allow analytes to dissolve into the surrounding solvent until they are completely recovered. While this method is quite simple and reasonably effective it still can be slow (e.g. 1 h per sample).[2] Further, since this method requires large amounts of organic solvent for each sample (e.g. 100 mL) it can also accumulate large quantities of toxic organic solvents in routine usage. Therefore, given the demand for solid sample extraction, there exists a growing need for not only faster, but also greener analytical chemistry practices that can reduce solvent usage in these processes. The main theme of my research is to advance the development of novel extraction techniques that promote faster analysis times, lower solvent consumption, and lower overall costs.

1.1 Fundamentals of the Extraction Process

The extraction process is the transfer of an analyte (A) from one phase to another. In the case of liquid-liquid extraction, the analyte is partitioned between two immiscible liquid phases in which it has two different solubilities. Normally, one phase is aqueous and the other phase is organic. The basis of the extraction process is that the more polar analytes prefer the aqueous (polar) phase and the more nonpolar analytes prefer the organic (less polar) phase. The partitioning of the analyte between the two immiscible phases can be represented in the following Equilibrium:

$$A(phase\ 1) \overset{K}{\rightleftharpoons} A\ (phase\ 2)$$

The partition coefficient (K) is an equilibrium constant that describes the distribution of analyte A between the two immiscible phases. This coefficient is represented by the ratio of the activities of A in the two phases.[3] For simplification, K is normally represented in terms of molar concentrations as can be seen in equation 1.1

$$K = \frac{[A]_2}{[A]_1} \quad \text{.....Equation 1.1}$$

It is often useful to describe the fraction of the analyte remaining in phase 1 in terms of the experimental extraction volumes and the partition coefficient. In order to do so we will assume that this fraction is q and the total number of moles of analyte A is m. If we also assume that analyte A, in V_1 mL of solvent 1, is extracted with V_2 mL of solvent 2, then the partition coefficient (K) and the fraction remaining in phase 1 after 1 extraction (q) can be expressed as seen in equations 1.2 and 1.3 respectively.

$$K = \frac{[A]_2}{[A]_1} = \frac{(1-q)m/V_2}{qm/V_1} \quad \text{.....Equation 1.2}$$

$$q = \frac{V_1}{V_1 + KV_2} \quad \text{.....Equation 1.3}$$

The fraction of analyte remaining in phase 1(the original phase) can be used as a measure of the extraction efficiency. The smaller the fraction remaining in phase one (q), the more efficient is the extraction. As can be seen in equation 1.3 the extraction efficiency can be increased by increasing the volume of the extraction solvent (V_2) or changing the solvent itself. Alternatively, for a given amount of the extraction solvent (V_2), the extraction efficiency can be increased by running several extraction cycles with the same portions of the extraction solvent. This approach is even more efficient than using a single large extraction volume. This is because the portion of the analyte remaining in phase one after n extraction cycles is an exponential power of n as follows:

$$q^n = \left(\frac{V_1}{V_1 + KV_2}\right)^n \quad \text{.....Equation 1.4}$$

1.1.1 Solid-Liquid Extraction

In the case of solid-liquid extraction, one phase is solid (the sample) and the other phase is liquid (the extraction solvent). The efficiency of extracting an analyte from a solid sample is influenced by three main factors; solubility, mass transfer, and matrix effects. The solubility of an analyte, similar to liquid-liquid extraction, depends largely on the type of the solvent, and for a selected solvent, it is affected by temperature and pressure. Mass transfer refers to the removal of the analyte from the interior of the matrix into the solvent. While facile in liquid-liquid extraction, mass transfer is a challenge in solid-liquid extractions since it involves solvent penetration into the matrix and removal of analytes from the adsorbed sites. Mass transfer is dependent on the diffusivity of the analyte in the extraction fluid as well as on the particle size and structure of the matrix. High diffusivity

of analytes, low solvent viscosity, and small particle size facilitate mass transfer. Matrix effects can be defined as the influence of a property of the matrix on the extraction efficiency of an analyte from the matrix. For example, an analyte that is highly soluble in an extraction fluid may not be recovered because it is locked in the matrix pores (e.g. inaccessible by solvent), or is strongly bound to its surface.[4]

1.2 Soxhlet Extraction

The extraction of organic analytes from numerous solid matrices has historically been carried out by using the method of Soxhlet extraction.[5-13] Alternate approaches also exist, such as ultrasound extraction,[5,8,14-16] microwave-assisted extraction,[17-19] and various other techniques.[2,20,21] However, some of the most effective techniques that will be discussed later in this chapter, are supercritical fluid extraction,[22-27] enhanced fluidity liquid extraction[28] and pressurized liquid extraction.[29-32] While these alternate approaches have advantages over Soxhlet extraction,[33-37] the latter is still often used and can be regarded as a benchmark against which all other approaches could be compared. As such, a closer examination of this method is useful.

1.2.1 Description of Soxhlet Extraction

Soxhlet extraction is a well-established technique used for the isolation of organic analytes from solid matrices. A schematic diagram of a typical Soxhlet apparatus is shown in Figure 1.1. Generally, the apparatus consists of three main parts, a reflux condenser at the top, a glass thimble holder at the middle, and a round-bottomed flask at the bottom. The solid sample is ground into fine particles and placed in a porous cellulose thimble. The extraction thimble is then placed inside the glass thimble holder and the round-bottomed

flask containing the extraction solvent is gently heated on a heating mantle. Solvent vapor passes through a side tube (at the middle part) and goes to the reflux condenser, where it condenses and drips back onto the sample in the cellulose thimble. When the analyte-laden solvent reaches the top of the thimble, it overflows and trickles back down into the flask through the siphon arm. This cycle repeats many times for a predetermined period of time. Since the extracted analytes have higher boiling points than the extraction solvent, they accumulate in the flask while the solvent recirculates. Consequently, the sample is always extracted with fresh solvents in each cycle. In this regard, as the sample is always extracted with refluxed pure solvent, extraction efficiency can be great even when target analytes have limited solubility in the extraction solvent.

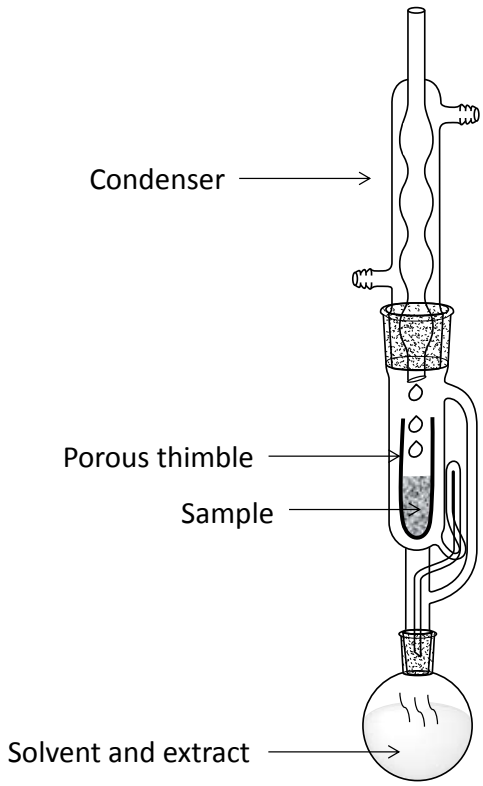

Figure 1.1: A schematic diagram of a Soxhlet apparatus.

Overall, the use of Soxhlet for extracting analytes from solid samples offers great advantages in terms of the cost, simplicity and the ruggedness of the instrument as well as the quantitative extractions that can be achieved by this technique. However, it still has major drawbacks represented by the long extraction times (e.g. 12-24 h) and the large consumption of toxic organic solvents (e.g. 300 mL per 10 g of sample).[1,2,8,9,38-44] Additionally, since Soxhlet extracts are usually collected in few hundreds of millilitres of solvent, a preconcentration step of removing solvent by rotary evaporation prior to analysis is normally required which in turn makes the overall sample preparation procedure even slower.

1.3 Supercritical Fluid Extraction (SFE)

One approach that has been developed over the years in order to make sample preparation faster and less solvent intensive is SFE. SFE is an extraction technique that uses supercritical fluids to selectively remove an analyte from a liquid, semi-solid or solid matrix.[45] As can be seen in Figure 1-2 a supercritical fluid resides on the phase diagram above its critical pressure (P_c) and critical temperature (T_c). The critical pressure is the pressure beyond which any increase in temperature will no longer evaporate the fluid into a gas, while the critical temperature is the temperature beyond which any increase in pressure will no longer condense the fluid into a liquid.

The critical region (i.e. above T_c and P_c) contains only a single phase, termed the supercritical fluid (SF) phase, having properties similar to both a liquid and a gas. For example, as can be seen in Table 1.1 SFs can have liquid-like densities while retaining gas-like viscosities and diffusion. The solvating power of the SF is proportional to its density,

which can easily be adjusted by changing the fluid pressure or temperature. In the SF state, at constant pressure, the density of the fluid can be decreased by increasing temperature. Similarly, at constant temperature, the density of the fluid increases with increasing pressure. As a result, the density can be optimized by changing temperature or pressure, potentially allowing the selective dissolution of a target analyte by adjusting the density.

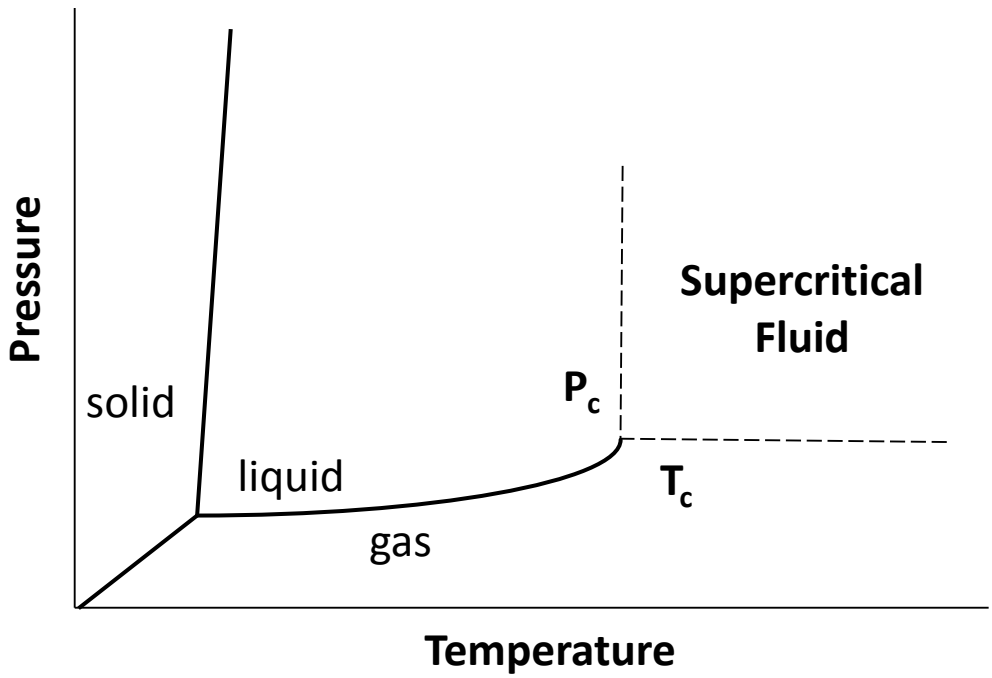

Figure 1.2: Pressure-temperature phase diagram for a single substance

Further, the density of an SF is most dramatically adjusted with small changes in pressure and temperature just above the critical point, while large changes are needed to adjust the density further above the critical point.[4,45-50] Due to its gas-like diffusion properties and liquid–like solvating power, supercritical fluids make effective extraction solvents. Compared to normal liquids, supercritical fluids show higher diffusion

coefficients of solutes, lower fluid viscosities, and near zero surface tension.[4] These gas-like properties allow rapid analyte mass transfer out of matrices, resulting in faster extractions. Therefore SFE has the power to efficiently and economically improve extraction recovery. As outlined later, it can also decrease the use of toxic and expensive organic solvents.

Table 1.1: Typical physical properties of liquids, gases, and supercritical fluids[20]

Property	Liquid [a]	Supercritical fluid [b]	Gas [a]
Density (g/mL)	**0.6-2.0**	**0.2-0.5**	**10^{-3}**
Viscosity (g/cm.s)	**10^{-2}**	**10^{-4}**	**10^{-4}**
Diffusion coefficient (cm^2/s)	**10^{-5}**	**10^{-3}-10^{-4}**	**10^{-1}**

a. Standard temperature and pressure (0°C, 1 atm)
b. T_c, P_c

1.3.1 SFE Operation and Apparatus

SFE instrumentation is displayed schematically in Figure 1.3. It is mainly composed of a fluid source (usually CO_2), a high pressure pump, and an oven to heat the extraction vessel. The SFE system also contains a valve to control the flow of the fluid into the heated extraction vessel and an outlet valve leading to a restrictor that maintains high pressure in the system and transfers the extracted analyte into a collection vessel.

SFE can be operated in two mode: static extraction and dynamic extraction. In a static extraction, a fixed amount of extracting fluid soaks/saturates the analyte/matrix in the extraction vessel for a predetermined period of time until the extraction is completed. At this point, the outlet valve is opened and the extraction fluids are allowed to depressurize, trapping the analyte in a collection vessel. Frequently, a static extraction is followed by several minutes of dynamic extraction to enhance removal of the extracted analytes from the extraction vessel. A dynamic extraction on the other hand employs a continuous flow of fresh extracting fluid over the sample matrix until the analyte is completely recovered from the sample.[4,51] Static, dynamic or often a combination of both are required for extractions because the extractability of a target compound can be affected by many factors, such as analyte solubility, the interactions between analyte and matrix, and the location of the analyte within the matrix.

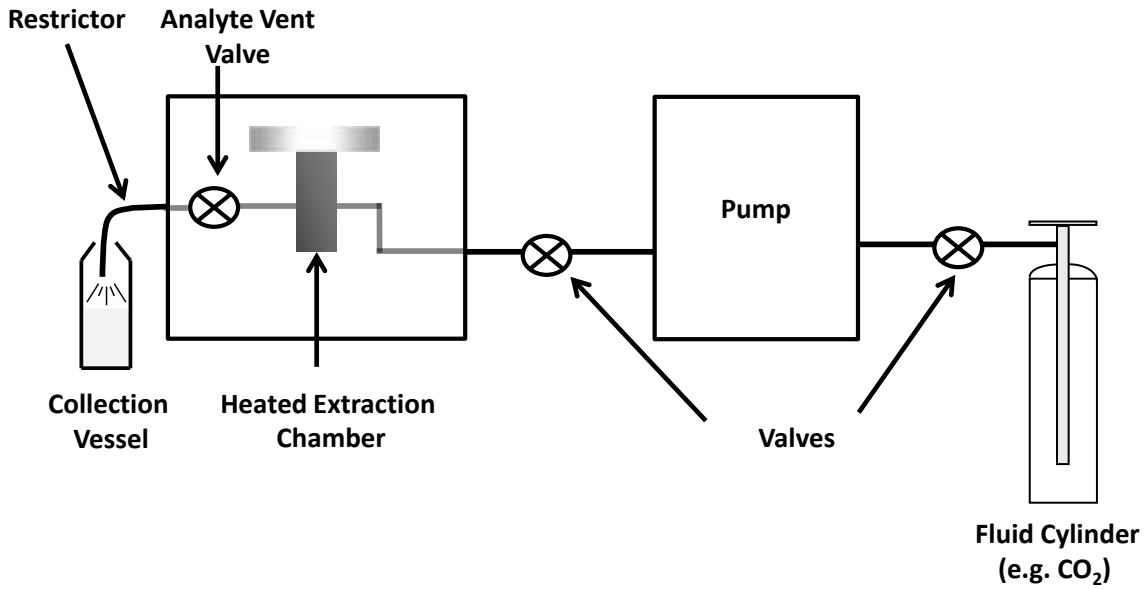

Figure 1.3: A schematic diagram of a conventional SFE instrument.

1.3.2 Supercritical Carbon Dioxide

Fluids that have been employed as SFs include ammonia,[46] sulfur hexafluoride,[52] xenon,[53] methanol,[22] and nitrous oxide.[22,54] But, since most of these options are unsafe, corrosive and/or expensive they have not garnered much interest in the field. Comparatively, CO_2 is the most commonly used supercritical fluid for extraction due to its inertness, non-flammability, non-toxicity, high purity, environmental compatibility and its easily achievable critical parameters (P_c = 72.9 atm, T_c = 31°C).[55] The use of CO_2 as an extraction solvent has many advantages, one of which is the elimination of the preconcentration step which follows conventional solvent extraction. This is because supercritical carbon dioxide (SC-CO_2) depressurizes into a gas at room temperature, and thus the analyte can be collected in a very small volume of solvent, as the main fluid is removed inherently.

Carbon dioxide is a non-polar fluid with a solvating power largely comparable to liquid hexane,[56,57] even though adjusting both pressure and temperature can slightly alter this.[48] This allows CO_2 to be effectively used for the extraction of nonpolar to slightly polar compounds such as alkanes, aldehydes, esters, and polycyclic aromatic hydrocarbons.[48,58-65] As such, SFE with CO_2 is a desirable approach for the extraction of such organic compounds from various solid matrices.[4,46,47,49,50,66,67]

Overall, the use of SF-CO_2 in sample preparation protocols successfully reduces the solvent consumption in chemical analyses with improvements in the speed of analysis. However pure CO_2 does not always succeed in extracting polar organic compounds since they are not soluble in it.[68] On the other hand, sometimes analytes that are CO_2 soluble also

cannot be efficiently extracted by SFE because CO_2 is unable to disrupt their strong analyte-matrix interaction and release them for extraction.[69,70]

1.3.3 Modified SFE

In order to enhance the extraction of organic compounds with CO_2, different approaches utilizing modified SFE have been studied.[4,34,71-74] In order to use CO_2 for extracting more polar compounds, usually 1-10% v/v of a polar modifier, such as methanol, is introduced to the extraction fluid. The main functions of the modifier are to increase the solvating power of the SF and to facilitate the disruption of analyte-matrix interactions. Methanol is the most common modifier used with CO_2 due to its good solubility over a wide range of temperatures and pressures. The solubility of methanol in supercritical carbon dioxide increases dramatically when operating at a pressure above 95 atm and as temperatures increase.[75] A ternary modifier (i.e. additive) can also be added to the primary modifier or directly to the matrix to achieve successful analyte extraction. These are often organic acids, bases, and ion-pairing reagents added in 1-3% v/v amounts.[76]

Modifiers can be introduced by premixing them with the extraction fluid or adding them directly to the matrix. Premixing with the fluid can be achieved either by using premixed cylinders (if a single concentration of modifier is desired) or by using a secondary pump to introduce the modifier.[4,77-80]

Modifiers can significantly improve SFE, due to the increased solubility of a target analyte in the extraction fluid, and the strong modifier interactions with the matrix active sites. The modifier helps the extraction of a target analyte by covering the active sites on the matrix and preventing the analyte from repartitioning into, or readsorbing onto, the matrix.[81]

In general, modified SFE efficiencies are improved compared to extraction using pure CO_2 for polar analytes. However, while useful, this approach is not always fully effective at polar/ionic analyte extractions due to the inability to overcome very strong matrix–analyte interactions and/or improve very polar analyte solubility.[28]

1.4 Enhanced Fluidity Liquid Extraction (EFLE)

EFLE is a complementary technique to SFE that can greatly facilitate the extraction of highly polar analytes.[82-84] Similar to SFE, EFLE uses a low viscosity, low surface tension fluid such as liquid carbon dioxide as part of the extraction fluid. However, in contrast to SFE, EFLE mixes in relatively high proportions (e.g. 30–50% v/v) of a polar organic cosolvent.[82-84] For example, methanol is also a common cosolvent in EFLE because of its polarity and its high solubility in carbon dioxide.[85] The main advantage of having such large proportions of both carbon dioxide and methanol in EFLE is that the resulting extraction fluid has relatively low viscosity and increased analyte diffusivity compared to that of pure methanol, yet maintains a solvent strength that is similar to methanol.[84,85] Therefore, these EFLE features can provide very rapid and efficient extractions of a variety of polar analytes, while still reducing organic solvent consumption.[28,77] In this way, EFLE may also be thought of as a specific form of SFE, where a relatively high proportion of organic solvent modifier is employed to create the extraction fluid, which is formally in the liquid state.

1.4.1 EFLE Operation and Apparatus

EFLE instrumentation is similar to SFE instrumentation. Normally, the equipment for EFLE consists of an oven, which is used to heat the extraction chamber, and two

pressure pumps, one to deliver liquid CO_2 and the other to deliver the cosolvent. EFLE operation is also similar to SFE operation and can be operated in two modes, static extraction and dynamic extraction.

Overall, EFLE provides a successful technique for replacing conventional sample preparation procedures with a fast, safe, and significantly less solvent intensive procedure. Additionally, since EFLE normally employs moderate temperatures for extractions (e.g. 50-100 °C),[28,77,86] it can also potentially be used for the sample preparation of thermally labile analytes.

1.5 Pressurized Liquid Extraction (PLE)

Another technique that was developed over the last two decades in order to reduce organic solvent consumption during extractions is PLE. PLE, also known as pressurized fluid extraction, uses organic solvents under pressure at temperatures higher than their boiling point to extract analytes from solid or semi-solid samples. The elevated pressure and temperature used in PLE affect the solvent, the solute, and their interactions. The solvent boiling point is increased under high pressure so the extraction can be conducted at higher temperatures. The high pressure also allows the solvent to penetrate deeper into the solute matrix, thus facilitating the extraction of analytes trapped in matrix pores. At elevated temperatures, analyte solubility increases and the mass transfer is faster. This is due to the fact that high temperatures weaken the solute-matrix interactions and reduce solvent surface tension as well as viscosity, which enhances solvent penetration into the matrix.[87] In general, relatively fast extractions using small volumes of organic solvents are obtained when PLE is employed. For example, extracting organic analytes from solid

samples often requires 12-15 minutes and 25 mL of solvent when PLE is employed.[2,88] In contrast to this, it takes 12-24 h and 150-300 mL of organic solvents for recovering the same analytes when Soxhlet extraction is employed. [45, 46]

1.5.1 PLE Operation and Apparatus

Similar to EFLE and SFE, PLE can be carried out in a static, a dynamic, or often a combination of both modes. Most of the PLE applications reported in the literature are performed in a static extraction mode followed by a dynamic extraction.[30,33,38,42,51,87-94] A schematic diagram of a conventional PLE system is depicted in Figure 1.4. It typically consists of a solvent pump, an extraction vessel, a heating oven, a collection vial, and a nitrogen tank. In such a PLE system the selected solvent (or a mixture of solvents) is pumped to fill the cell containing the sample. Then, the cell is pressurized and heated for the predetermined extraction time (typically 12-15 min).[2,20] After the extraction is completed, a few mL of fresh solvent is dynamically pumped through the extraction cell and the connective tubing. This step displaces the extraction solvent and the majority of the extracted compounds. A nitrogen purge (typically 1–2 min) to guarantee the complete removal of the solvent from the system is then a common final step in the PLE process.[21]

Overall, PLE offers great advantages over conventional sample preparation procedures in terms of reducing solvent consumption and increasing the speed of analysis. However, it still has some significant limitations. For example, PLE normally operates at relatively high temperatures (e.g. 150 °C), which in certain instances can degrade thermally labile analytes such as active pharmaceutical ingredients (APIs) before quantitative extraction can be achieved.[33,89]

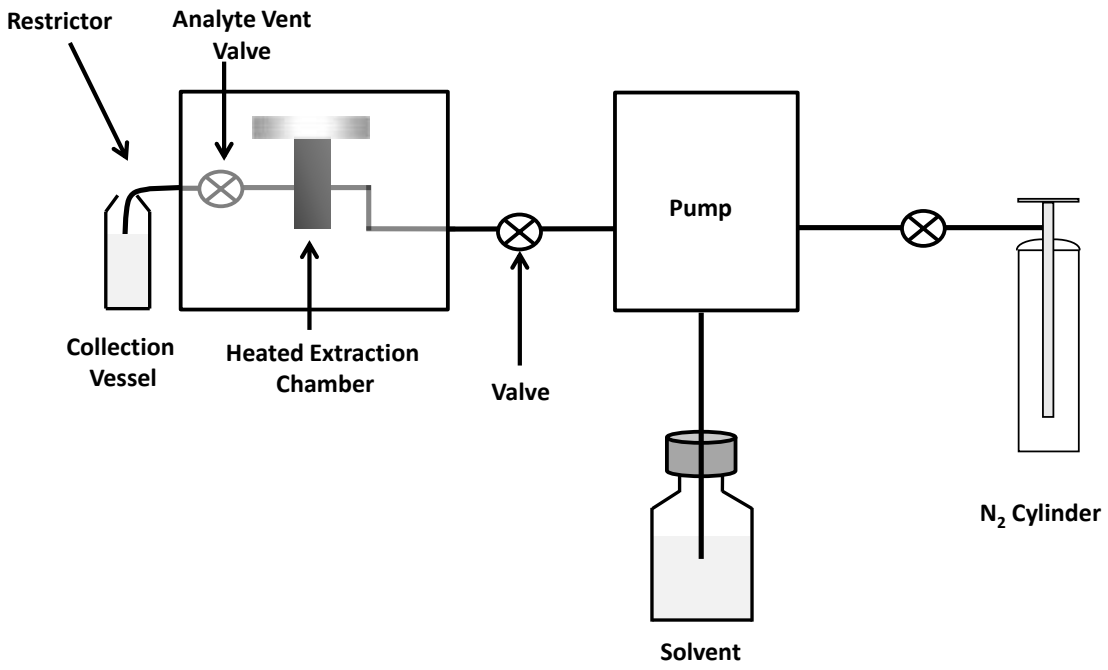

Figure 1.4: Schematic diagram of a typical PLE system

1.5.2 Water in PLE

An interesting solvent that takes unique advantage of temperature and can be used as a PLE solvent is water. Using water in PLE is also known as subcritical water extraction (SWE). SWE employs pure water as the extraction fluid since, among other things, it is inexpensive, safe and environmentally friendly. Since the polarity of subcritical water uniquely decreases dramatically with increasing temperature,[95-97] it can be used to dissolve a wide range of analytes from polar to non-polar depending on the conditions used. The main advantage of SWE is that temperature alone can be used to control the polarity of the solvent, and hence the selectivity of the extraction.[98-101] As well, efficient extractions are equally obtained by this method using only water without the need for harmful organic solvents.[102,103]

1.5.2.1 SWE Description

The term subcritical is used when a fluid is heated above its boiling point and pressurized enough to maintain it in the liquid phase. For example, the region of the water phase diagram (Figure 1.5) defined as subcritical is above the boiling line and between the atmospheric pressure boiling temperature (100 °C) and the critical temperature (374 °C). Although the definitions of sub/supercritical appear concrete, the two are very similar in practice. For instance, since there is often no sudden change in physical properties when moving from sub to supercritical conditions either of these regions may be utilized successfully in an extraction system.[104-111] However, the upper end of the temperature spectrum may prove troublesome since supercritical water (T= 374 °C) can readily degrade organic analytes.[112]

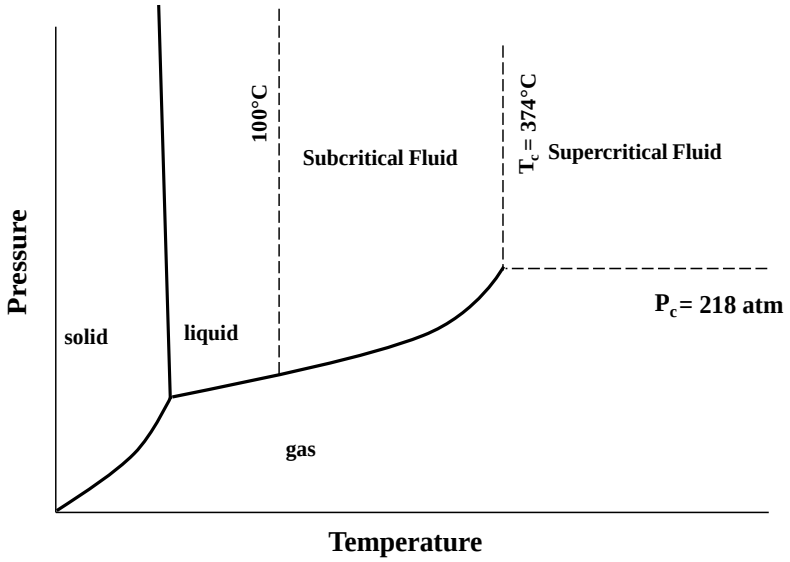

Figure 1.5: Phase diagram of water.

As stated earlier the polarity of water significantly decreases as temperature is increased. This is a very advantageous property of subcritical water. For instance,

subcritical water mimics the polarity of pure room temperature methanol at about 200 °C, and that of pure room temperature acetonitrile at about 160 °C.[95-97] Therefore, a primary advantage of SWE as an alternate extraction method is the potential of eliminating conventionally toxic, expensive, and environmentally hazardous organic extraction solvents with simple, relatively inexpensive, pure water utilized at select temperatures. Other benefits of this approach include the relatively low cost, high purity and environmental compatibility of water relative to typical organic solvents. In addition, similar to PLE, since SWE is often operated at elevated temperatures, viscosity and surface tension of water are reduced which allows for relatively faster extractions.

Overall SWE offers a good substitute to conventional extraction procedures as it eliminates the use of toxic organic solvents. However, it still has major limitations. The greatest disadvantage of SWE is the extreme temperatures needed to extract certain very non-polar analytes, which may lead to thermal degradation issues. For example, subcritical water mimics the polarity of the nonpolar solvent dichloromethane at a temperature of 425°C.[97] This temperature is extremely high and many analytes will degrade before quantitative extractions can be achieved at such high temperatures. As such, SWE is conventionally often ineffective at extracting non-polar analytes. Further, even at lower temperatures certain analytes may also be reactive with the solvent and prone to hydrolysis. A comparison between Soxhlet, PLE, CO_2-based techniques and SWE in terms of various extraction parameters is summarized in Table 1.2.

Table 1.2: A comparison between Soxhlet, PLE, CO_2-based techniques and SWE in terms of various extraction parameters.

Extraction technique	Average organic solvent used per sample	Average extraction time per sample	Advantages	Disadvantages
Soxhlet	200-500 mL	4-48 h	Robust, inexpensive, and the setup is simple	Very lengthy and requires large amount of organic solvents
CO_2-Based	10-20 mL	10 min + 1 h	Relatively rapid, efficient at extracting nonpolar analytes, and does not normally require high temperatures	Not effective for polar analytes and the setup is expensive
Pressurized Liquid Extraction	12-15 mL	12-15 min	Relatively rapid and requires moderate amounts of organic solvents	Not suitable for thermally labile analytes and the setup is expensive
Subcritical Water Extraction	0 mL	10-30 min	Minimum use of organic solvents	Not suitable for nonpolar and thermally labile analytes

1.6 Miniaturized PLE

Another interesting approach that has recently been used in order to make solid sample preparations more efficient is miniaturized PLE. This approach focuses on scaling down the PLE process by using miniaturized cells.[113] This is often done by scaling down

sample size, while using modified conventional instruments in static/dynamic extraction modes. For example, a homemade miniaturized PLE setup was used for extracting polychlorinated biphenyls from foodstuffs using hexane as an extraction solvent.[114] This approach showed that using somewhat smaller samples (i.e. 100 mg) could reduce solvent consumption to 3.5 mL per extraction. While this is beneficial, quantitative extractions required relatively long total extraction times of more than 14 minutes per sample. Alternatively, this approach was used for extracting polycyclic aromatic hydrocarbons (PAHs) from even smaller samples (10 mg of sediment).[115] Even though a 2.2 mL extraction vessel was used and 30 mL of solvent was required for extractions, water was used as a fluid so organic solvent consumption was minimum. However, the time required to quantitatively extract the PAHs was 30 minutes per extraction. In a very interesting approach, Ramos and coworkers used a homemade miniaturized PLE setup that greatly reduced solvent consumption to 100 μL. In this approach a very small vessel (10 × 3 mm I.D.) filled with a moderate amount of sample (50 mg) was used for extractions. Again, however, the time required for quantitative extractions of PAHs from soil samples was relatively long as more than 10 minutes were required for each extraction.[116]

Overall while such efforts have been used to reduce sample size and solvent consumption, extraction times are still relatively long, and are in fact, similar to conventional PLE procedures.[91,117] Further, the instrumentation involved in these setups can be cumbersome, costly, and complex especially at smaller dimensions. Thus, continual development in this area would overcome the current limitations and further facilitate sample preparation procedures. Overcoming these issues could be useful to many areas

that require rapid solids analysis such as food and beverages, environmental matrices, forensic samples, and pharmaceuticals.

1.7 Routine Analysis of Pharmaceuticals

The drug development process is of great concern to the pharmaceutical industry and the global population. Before a potential drug product can make it to the marketplace, it must first undergo an enormous amount of testing to prove the identity, efficacy, and safety of a drug before it is packaged or distributed. For each of these tests, the pharmaceutical formulation must be analyzed for its contents in order to know if any effects are noted on the composition. The degree of testing that a drug product requires depends on the characteristics of the compound, the number of components in the product, and the dosage form. Pharmaceutical tablets are the most common dosage forms of self-medications. Currently, the actual analysis of such tablet contents is relatively rapid. However, preparing the tablet contents for such an analysis is quite lengthy and represents a major delay in analytical laboratory efforts to support the drug development process. As a result, increasing the speed of such sample preparation methods can directly increase the speed of the drug development process.

1.7.1 Conventional Sample Preparation Procedures in Pharmaceuticals

The formulations of pharmaceutical products are very complex and contain different excipients, including fillers, binders, disintegrants, coatings, flavours, colors and lubricants, which can have similar chemistry to APIs. APIs usually exist at low concentrations in pharmaceutical formulations and conventional sample preparation procedures needed to extract and isolate the APIs from complex matrices are very lengthy

and require large amounts of toxic and expensive organic solvents. Conventional sample preparation methods first require weighing and grinding of several tablets, then the homogeneous ground powder is again weighed to acquire the representative contents of a single tablet. The samples are then dissolved in a relatively large amount of organic solvent (e.g. 500 ml) and normally sonicated for a certain period of time, followed by centrifugation, decantation and finally preconcentration.[118-121] At the conclusion of these steps, the sample is ready for analysis by high performance liquid chromatography (HPLC) or other methods.[119,122-125] Most of the steps in the sample preparation procedure need to be done separately for every individual sample, making it a very long process when a series of samples need to be analyzed in a routine laboratory setting.

As such, current pharmaceutical sample preparation protocols involve lengthy, inefficient procedures that are not always effective at extracting analytes from solid oral dosage forms.[126-128] Also, the current methodologies do not address the long term throughput needs currently facing the industry. Further, the large amounts of organic solvents used in such procedures contribute to hazardous waste concerns and considerable difficulties in detecting low-dosage drugs and impurities at the concentration realized from potentially complex matrices.[127,128] Considering this, the development of new methods that can rapidly and efficiently extract APIs from solid dosage forms in a format directly ready for analysis, will have a significant impact on sample throughput in this important routine analytical procedure.

1.7.2 Conventional Chromatographic Analysis Techniques in Pharmaceuticals

Gas chromatographic methods (GC) are rarely used in the field of pharmaceutical analysis. This is because GC is usually operated at high temperatures which are not often

suitable for nonvolatile and/or thermally labile pharmaceuticals.[129] Therefore, a lot of pharmaceutical compounds cannot be analyzed directly by GC without derivatization which is needed to convert them to stable and volatile forms.[130-134] Although derivatization can overcome certain issues of thermal stability and/or volatility, it may not be possible for some analytes. Further, it is often lengthy and adds several extra steps to the sample preparation procedure making analysis of pharmaceuticals even more detailed and slower.

On the other hand, HPLC is the most widely used separation system in the field of pharmaceutical analysis.[135-138] This is because it has the ability to separate, identify, and quantitate wide ranges of APIs of varying polarities and molecular weights. Further, since HPLC is normally operated at ambient temperatures or slightly above, it is ideally suited for pharmaceuticals with limited thermal stability. In HPLC the choice of the detection method is critical to guarantee that all the components are detected. One of the widely used detectors in HPLC is the ultraviolet-visible (UV-vis) detector. While UV-vis often provides good sensitivity and linear dynamic range it generally requires analytes to possess an appropriate chromophore and does not respond equally to all compounds. This can cause a problem when non UV-vis absorbing APIs or unknown API degradants need to be analyzed and quantified.

In general, the use of HPLC in routine pharmaceutical analyses is often successful in analyzing pharmaceuticals. There are, however, significant limitations to using HPLC in this field. For example, the consumption of solvents commonly used as HPLC mobile phase components poses a significant economic impact to those continually employing this separation technique. In addition to the economic concerns, many of the most frequently employed mobile phase solvents are toxic, which can cause health risks to the operator and

a serious negative impact on the environment in their subsequent disposal. Another major limitation to HPLC is its lack of a simple universal detection method. For instance, the well-known flame ionization detector (FID) is widely used in GC for this purpose due to its sensitive and uniform response toward carbon-containing compounds. However, HPLC is not normally compatible with the FID. This is because conventional HPLC mobile phases are composed of organic solvents and as a result, these organic mobile phases will overwhelm and blind the detector from responding to analytes.[139,140]

1.8 Motivations for this Research

This thesis describes advances in solid-liquid extraction methodology on several fronts from novel applications of existing extraction techniques, to the development of novel extraction methods. Initially in Chapter Three, EFLE is explored as a novel application for the sample preparation method of pharmaceutical formulations. Given its mild rapid extraction properties and low solvent requirements, EFLE is a very promising alternative for API extractions, since many APIs can often be too thermally labile for certain other approaches. As EFLE has never been explored in this area before, the extractability and the optimum EFLE parameters for a model pharmaceutical from various matrices is examined in an attempt to explore the possibility of adapting this technique to solid dosage form pharmaceuticals. The second part of this chapter then describes the extraction of several APIs from various pharmaceutical formulations using EFLE combined with other enhancement strategies (e.g. water as a ternary modifier) to further enhance the recovery efficiency and the speed of the extraction process.

Next, a new mode of PLE is introduced. The improved fluid dynamics that is demonstrated by PLE can offer significant improvements over traditional methods for solid-liquid extractions, and can greatly improve the efficiency of sample preparations. [42-44] However, as the speed of analysis continues to grow for many methods [141] and the demand for reduced analytical chemistry waste expands [142], any measures that can further decrease sample preparation times and the organic solvent usage involved would be beneficial. For instance, while average PLE extraction times are significantly smaller than those of Soxhlet methods, they could still potentially pose problems for high sample throughput concerns.[42] As well, the cost and size of the specialized equipment needed to perform PLE can also hamper efforts to maintain multiple extraction units to help facilitate this.[143] Further, PLE procedures frequently consume about 10 to 25 mL of solvent per sample,[42,2,20] which is a fraction of that utilized in Soxhlet procedures, but can still cause excessive waste to readily accumulate in routine analytical settings involving numerous samples.[21] Therefore, efforts that can facilitate even faster and less solvent intensive extractions can help to further address these concerns. In Chapter Four, the operating characteristics of a novel micro PLE technique that uses minute amounts of organic solvents at elevated pressures and temperatures to remove analytes from small quantities of solid samples in only seconds will be detailed and discussed. Chapter Five then presents the results of exploring this novel method in extracting a wide variety of analytes from various solid samples.

Chapter Six then demonstrates the ability of combining this novel sample preparation technique with an ultra-short column GC for the analysis of ASA and its degradation products in a very rapid manner. The advantage of employing an ultra-short

column GC approach for the analysis of ASA is that the elution temperature of this analyte is lowered when this mode of GC is employed. Lowering the elution temperature is very important for ASA (a thermally labile analyte) because it allows for this analyte to be analyzed directly without a derivatization step which is often needed in conventional GC analysis.[118,121,144] Chapter Two describes the experimental setup for all of the experiments discussed in this thesis. Finally, Chapter seven is comprised of a summary and thoughts on future work in the area of sample preparation. Each chapter also presents additional introductory remarks with a particular focus on the topical area being covered.

Chapter Two: **EXPERIMENTAL**

2.1 Characterization of the Sample Preparation of Prozac® Capsules and Other APIs Using EFLE

2.1.1 EFLE Apparatus and Procedure

A schematic diagram of the EFLE instrument used in Chapter Three is presented in Figure 2.1. Extractions were performed using an ISCO model SFX-220 carbon dioxide extraction unit, equipped with two model 260D syringe pumps (ISCO, Lincoln, NE, USA) for controlling the delivery of fluid through the system. This provided an EFLE setup that consisted of an oven, used to heat the extraction chamber, and two pressure pumps, one to deliver CO_2 and the other to deliver the methanol cosolvent. A 2.5 mL stainless steel extraction vessel equipped with 0.5 µm frits on each end was employed in these experiments. Glass beads were also placed in the vessel along with the sample to decrease the void volume of the chamber. The actual vessel volume after subtracting the volume occupied by the glass beads was 1.1 mL. Various lengths of 50 µm i.d. fused silica capillary tubing were used as restrictors in all extractions to maintain backpressure in the system and establish a desired flow rate.

Weighed samples were sealed in the extraction vessel and placed into the preheated chamber. The extraction fluid was then introduced to a constant desired pressure and was allowed to flow through the vessel for a set period of time, during which the contents of this dynamic extraction process were collected at the restrictor outlet in a glass sample tube. There, the carbon dioxide depressurized to a gas leaving the concentrated sample behind. During this dynamic extraction process the EFLE extracts were collected in a glass vial, then quantitatively transferred to a 10 mL volumetric flask and diluted to the mark for

subsequent HPLC analysis. Normally, the carbon dioxide mobile phase was modified dynamically by adding methanol to it using a second pump. However in some experiments, where indicated, the fluid was modified statically by adding a designated volume of methanol directly to the vessel prior to extraction with pure carbon dioxide using a single pump.

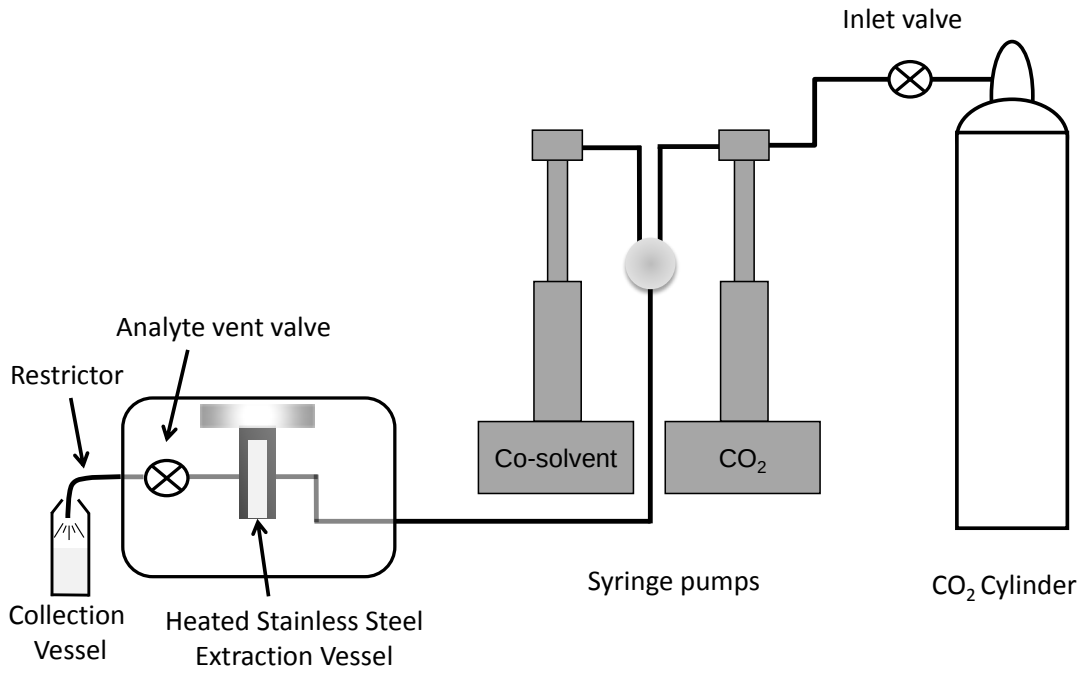

Figure 2.1: A schematic diagram of the EFLE system used in Chapter Three.

2.1.2 Extractability Enhancement Strategies of Certain APIs Using EFLE

Various EFLE enhancement strategies were explored in Chapter Three. These strategies included using water as a ternary modifier, increasing the extraction temperature, replacing the methanol solvent with acetonitrile as a cosolvent, and finally using methanol only as the extraction fluid in a conventional PLE mode. The EFLE system and the extraction procedure employed in this part of the chapter are the exact same as the ones

used previously and fully described in section 2.1.1 above. For PLE experiments, however, only one pump was needed and used to deliver the extraction fluid. For experiments using water as a ternary modifier, water was premixed with methanol in a desired composition before filling the cosolvent pump with the fluid.

2.1.3 Extract Analysis

Analysis of all Fluoxetine Hydrochloride extracts and standards was performed on an HPLC system comprised of an LKB BROMMA model 2150 reciprocating pump (LKB Instruments, Mt. Waverley, AUS), a Valco model C4W injector with a 0.5 µL internal sample loop (Valco Instrument Company Inc., CAN), a Zorbax SB-C_{18} column (15 cm x 4.6 mm; 5 µm particles; Agilent Technologies, Mississauga, CAN), and a fixed wavelength (254 nm) Waters model 440 UV-Vis absorbance detector (Waters, Milford, USA). The mobile phase consisted of 80% acetonitrile and 20% water containing 0.043 mol/L ammonium formate and 0.24 mol/L formic acid (pH 3), and was delivered isocratically through the system at a flow rate of 1.0 mL/min as previously described.[145] Data acquisition was performed using Peak Simple software (SRI Instruments, Torrance, USA)

For all other APIs examined in this chapter, the HPLC analysis was performed using the exact same HPLC system described before except for the mobile phase compositions and, in some instances, the chromatographic columns used. For ascorbic acid standards and extracts, the analysis was done using a Zorbax SB-C_{18} chromatographic column (150 mm × 4.6 mm i.d., 5 µm; Agilent) and a mobile phase of 0.1% aqueous trifluoroacetic acid solution that was delivered isocratically through the system at a flow rate of 1.0 mL/min. For ASA standards and extracts the analysis was performed using a Zorbax SB-Phenyl column (Agilent), and a mobile phase consisting of 40% methanol /

60% aqueous acetic acid (1.5% wt/wt) that was delivered isocratically at a flow rate of about 1.0 mL/min.

Quantification of each extract was achieved through calibration with external standards to determine the analyte mass present. For standard trials, this was the amount of pure API that was weighed and placed in the vessel, and for commercial tablet/capsule trials this was the amount of API present in the pharmaceutical tablet/capsule according to the manufacturer. Commercial Prozac® samples were extracted by opening a capsule and pouring the entire contents (227 mg) into the EFLE extraction vessel. Commercial ascorbic acid and ASA tablets were extracted by crushing and pouring the entire contents of the tablet (a description of each tablet is listed in Table 2.1) into the EFLE extraction vessel. Extraction recovery values were calculated according to the following formula:

% Recovery = (API mass in extract / API mass originally present) x 100…..Equation 2.1

2.1.4 Reagents and Standards

HPLC grade methanol was purchased from EMD Chemicals Inc. (Gibbstown, USA). HPLC grade water, ammonium formate (97%) and formic acid (≥ 96%) were each purchased from Sigma-Aldrich (Oakville, CAN). Instrument-grade carbon dioxide (99.99%) was obtained from Praxair (Calgary, CAN). Acetonitrile and Glacial acetic acid (99.7%) were both purchased from VWR International (Edmonton, Canada). Trifluoroacetic acid (97%) was obtained from Fisher Scientific (Ottawa, CAN). Ascorbic acid (99%), ASA (99%) used for standard solutions were all from Sigma-Aldrich. A Fluoxetine Hydrochloride reference standard and Prozac® capsules (containing 11.2 mg of Fluoxetine Hydrochloride, the equivalent of 10.0 mg of Fluoxetine) were each provided by Eli Lilly and Company (Indianapolis, USA). Mannitol, starch, microcrystalline cellulose,

and lactose stock excipients were also provided by Eli Lily and Company. A set of reference standards and formulations for a variety of different intellectual property (IP) protected APIs were provided by Eli Lilly and Company. Commercial tablets of vitamin C and ASA were purchased from a local drugstore. All other details and variations are described in Chapter Three.

Table 2.1: Descriptions of the pharmaceutical tablets and capsules studied in Chapter Three

Tablet/ Capsule	Listed API Load per Tablet (mg)	Measured Single Tablet/capsule Mass (mg)	Label Listed Excipients
Vitamin C	500	648.0	Microcrystalline cellulose, magnesium stearate
ASA	81	237.0	colloidal silicon dioxide, lactose anhydrous, methacrylic acid copolymer type b, sodium lauryl sulfate, sorbitan tristearate, stearic acid, talc, titanium dioxide, triethyl citrate
Fluoxetine Hydrochloride	11.2	227.0	Starch, dimethyl polysiloxane

2.2 A Novel Ultra-Rapid Micro PLE Technique for Solid Sample Preparation

2.2.1 Micro PLE Apparatus

All extractions were performed using vessels fabricated in-house from two different materials. Each measured 55 mm long x 2.1 mm I.D. x 2.9 mm O.D. and was sealed at one end. The first was formed from a quartz tube which was capped with a tight fitting rubber

septum that covered the top 4 mm of the open end. This extraction vessel could only be used with temperatures up to 120 °C as explained in the text. The second design was subsequently made from stainless steel tubing which was threaded to accommodate a septum-lined stainless steel cap. This extraction vessel could be used with temperatures up to 275 °C.

A schematic of the micro PLE apparatus is shown in Figure 2.2. The resistively heated extraction apparatus was composed of a glass tube (65 mm long × 3.0 mm i.d.) with two open ends that acted as an extraction vessel holder. The upper opening widened to 7.0 mm i.d. at about 8 mm below the edge to fully accommodate the extraction vessel and cap. Nickel-chromium wire (0.25 mm O.D.) was coiled around the entire holder with approximately 1 mm spacing between each wind. The exterior of this coiled housing was then thermally and electrically insulated by coating with a thin layer (2.0 mm) of ceramic adhesive (Cotronics Corp, Brooklyn, USA). Power to resistively heat the coils was supplied through a standard laboratory variable transformer (120 V supply, 10 A max; model 3PN 1010, Staco, Dayton, USA) coupled to a step down transformer (16 V, 1.5 A, Hammond, CAN) connected to the exposed heating coil leads by alligator clips.

2.2.2 Extraction Process

Extractions were performed by placing a weighed solid sample (see below) into the vessel followed by 125 µL of the organic extraction solvent. The vessel was then capped and placed directly inside the pre-heated extraction apparatus. When a set extraction time had elapsed, the vessel was removed with tweezers and immediately cooled in room temperature water. The contents were mixed by inverting the extraction vessel once and then the solvent extract was removed using a 250 µL syringe. This was immediately filtered

through an Acrodisc® syringe filter with a polyethersulfone membrane (13 mm diameter, 0.45 µm porosity; VWR International) and collected for subsequent HPLC analysis. Extraction times and temperatures were optimized for each individual sample investigated here.

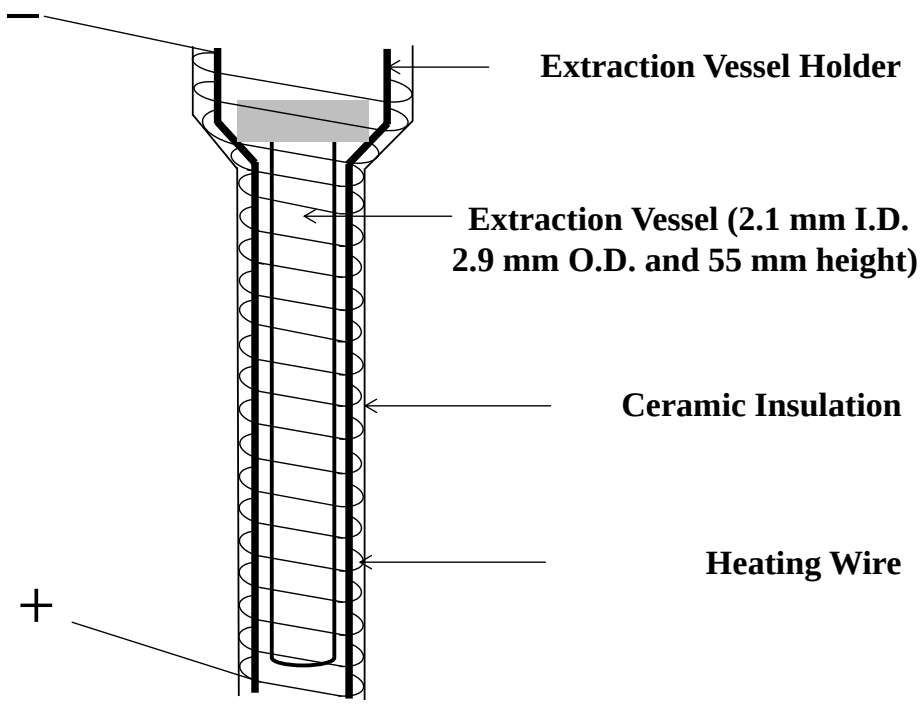

Figure 2.2: Schematic diagram of the µPLE apparatus employed.

2.2.3 Samples

Pharmaceutical tablets (delayed-release acetylsalicylic acid (ASA) and acetaminophen) were crushed and an accurately weighed small piece (5-10 mg) was placed inside the vessel for each extraction. For green tea, dry leaves were first ground and mixed as described previously,[146,147] then a 5-10 mg portion was placed in the vessel for extraction.

2.2.4 Extract Analysis

Analysis of all standards and extracts was performed using an HPLC system comprised of a Waters model 515 HPLC reciprocating pump (Waters Corporation), a Valco model C4 injector with a 0.5 µL internal sample loop (Valco Instrument Company Inc., CAN), and a dual wavelength Waters model 2487 UV-Vis absorbance detector (Waters corporation). Data acquisition was performed using Peak Simple software (SRI Instruments). All analyses were performed using a Zorbax SB-C_{18} column (15 cm x 4.6 mm i.d.; 5 µm particles; Agilent) except for ASA analysis, which employed the same brand and dimensions in a Phenyl phase format. The mobile phase flow was normally set near 1.0 mL/min and the composition varied for each analysis. For ASA a mobile phase consisting of 40% methanol / 60% aqueous acetic acid (1.5% wt/wt) was used. For acetaminophen 85% water / 15% acetonitrile was used. For caffeine standards and green tea leave extracts 70% water / 30% methanol was used. Quantification of each extract was achieved through calibration with external standards to determine the analyte mass present. For all samples, this value was then referenced against the quantity of analyte originally placed in the extraction cell (according to the experiment and/or the manufacturer's specifications) in order to gauge the extraction efficiency/recovery. Extraction recovery values were calculated using Equation 2.1.

For green tea leaves, since exact caffeine levels were not specified on the label, a bulk tea sample (3 g) was exhaustively extracted and analyzed in order to determine the total caffeine quantity present in the leaves for use as a reference.

2.2.5 Conventional PLE Trials

Comparisons with conventional PLE were also explored in this chapter and employed a temperature controlled ISCO model SFX-220 extraction unit, equipped with a model 260D syringe pump (ISCO) for delivery of the methanol solvent. A 2.5 mL stainless steel sample extraction vessel equipped with 0.5 µm frits was used. Glass beads (6 mm diameter) were placed in the vessel with the sample to further decrease the chamber volume down to 1.1 mL. A fused silica capillary (20 cm x 50 µm i.d.) was used as a system restrictor.

Weighed samples (i.e. one full crushed tablet for the ASA and acetaminophen trials, and about 400 mg of ground leaves for the green tea trials) were sealed in the extraction vessel and placed into the preheated chamber. Solvent was then introduced to a constant pressure of 150 atm and was allowed to flow through the vessel for a set period of time (as specified in the text). During this dynamic extraction process the PLE extracts were collected in a glass vial, then quantitatively transferred to a 25 mL volumetric flask and diluted to the mark for HPLC analysis.

2.2.6 Reagents and Standards

HPLC grade acetonitrile was purchased from VWR International. HPLC grade methanol and water were both purchased from Honeywell Burdick & Jackson. Glacial acetic acid (99.7%) was purchased from VWR International. Caffeine (anhydrous), acetaminophen (98%), and ASA (99%), were all purchased from Sigma-Aldrich. Commercial tablets of delayed-release ASA (81 mg per tablet) and acetaminophen (325 mg per tablet) were purchased from a local drugstore. Dry green tea leaves were obtained from a local grocery store. All other details and variations are described in Chapter Four.

2.3 Application of µPLE to Some Challenging Matrices

2.3.1 Dried Blood Spot (DBS) Samples

All DBS analyses that were performed in Chapter Five employed commercial FTA® DMPK-A DBS cards (Whatman, Piscataway, USA). After cutting out 3.65 mm diameter circles using a manual hole puncher, an aliquot of methanol containing 300 ng of Fluoxetine Hydrochloride was deposited onto each. This was left to thoroughly dry for 30 minutes at room temperature before accurately spotting 5 µL of whole blood onto each circle using a micropipette. The spotted circles were then left to completely dry at room temperature overnight before they were stored in a sealed plastic bag. For analysis, a DBS circle was transferred into the extraction vessel and 125 µL of chloroform was added to it as an extraction solvent.

2.3.2 Polycyclic Aromatic Hydrocarbons (PAHs) Samples

The extractability of 16 PAHs (see below) from various solid samples was examined Chapter Five. These solid samples included, sand, soil, chicken breast, toasted bread, and biochar amended soil. For each of these samples a 5-10 mg portion was weighed into the extraction vessel and spiked with an aliquot of 50% acetone / 50% hexane containing 800 ng of each of the 16 PAHs. This was left to thoroughly dry in the uncapped vessel for 45 minutes at room temperature before adding 125 µL of toluene which also contained 2 µg of tetradecane as an internal standard for chromatographic analysis. For chicken breast samples one whole breast was steamed for two hours on an electric stove element at medium heat. Then the thoroughly cooked meat was placed on foil in a commercial smoker and smoked at 175°C for 40 minutes. The outer smoked portions of

the meat were used as samples during experiments. For toasted bread, the surface of one slice of bread was charred evenly with a propane hand torch on both sides. The bread was then placed in a commercial toaster for two minutes. The charred bread was next ground with a mortar and pestle as finely as possible before weighing portions into the extraction vessel. For biochar amended soil, a sample of soil was mixed thoroughly with finely ground coconut charcoal to form a homogeneous mixture of biochar soil composed of about 2 % biochar by weight as previously described.[148] All other steps in the extraction procedure are identical to those employed in Chapter Four and described in section 2.2.2.

2.3.3 Chromatographic Analysis

The analysis of Fluoxetine-Hydrochloride standards and DBS extracts was performed using the exact same HPLC system used in Chapter Four and fully described in section 2.3.4. The HPLC column was a Zorbax SB-C_{18} column (15 cm x 4.6 mm i.d.; 5 µm particles; Agilent) and the mobile phase consisted of 80% acetonitrile / 20% water containing 0.043 M ammonium formate and 0.24 M formic acid (pH 3).

For the analysis of all PAHs samples and standards an HP 5890 Series II (Hewlett-Packard Co. USA) gas chromatograph equipped with a splitless injector and a FID was used. Separations were performed using an SPB-5 (5%-phenyl, 95%-methylpolysiloxane) megabore column (30 m × 0.53 mm I.D. × 0.5 µm film thickness, Supelco Bellefonte, USA). A temperature program was developed for the separation of the sixteen analytes consisting of three stages. The first stage was a 2 minute hold at 100 °C followed by a 10 °C/min ramp to 190 °C and hold for 8 minutes. The second stage was a 20 °C/min ramp to 260°C and hold for 10 minutes, starting right after the end of the first stage. The final stage was a 10 °C/min ramp to 290 °C and hold 15 minutes until all analytes had eluted. The

total separation time for each analysis was therefore approximately 50 minutes. High purity nitrogen (Praxair) was used as a carrier gas and operated at about 4 mL/min. High purity hydrogen (Praxair) and medical-grade air (Praxair) were used to support the detector flame at respective flow rates of 30 and 300 mL/min. An injector/detector temperature of 300 °C was normally maintained during the experiments. Data acquisition was performed using Peak Simple software (SRI Instruments).

2.3.4 Quantification of Extracts

Quantification of DBS extracts was achieved through calibration with external standards to determine the analyte mass present. For all DBS samples, this value was then referenced against the quantity of Fluoxetine-Hydrochloride originally placed in the extraction cell (300 ng) in order to gauge the extraction efficiency/recovery. Extraction recovery values were calculated using equation 2.1.

Quantification of PAH extracts was achieved through calibration with PAH standards. For each extract a standard containing all the PAHs was prepared and analyzed to establish response factors for all analytes relative to the tetradecane internal standard. These factors were then applied to the same ratios from the extracted sample for quantification.

2.3.5 Reagents and Standards

HPLC grade water was purchased from Honeywell Burdick & Jackson. Chloroform (99.94%) was purchased from EMD Chemicals. Fluoxetine-Hydrochloride (≥98%), ammonium formate (97%), and formic acid (≥96%) were all purchased from Sigma-Aldrich. A standard mixture containing 16 PAHs dissolved in 50% methylene chloride / 50% benzene solvent at a concentration of 2 mg/mL for each, was purchased from VWR

International. The 16 PAHs included naphthalene, acenapthene, acenapthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene and benzo[ghi]perylene. Tetradecane (99%) was obtained from Aldrich. Toluene (99.5%), hexanes (98.5%) and acetone (99.5%) were all purchased from Merck (EMD chemicals). Ottawa sand (20-30) mesh was purchased from Fisher Scientific (Fair Lawn, USA). Coconut charcoal (mesh 8-12) was purchased from BDH chemicals (Toronto, CAN). Soil samples were purchased from a local gardening center. Chicken breast and white bread were both purchased from a local grocery store. All other details and variations are described in Chapter Five.

2.4 μPLE Combined with Ultra-Short GC Column Analysis for Monitoring ASA Tablet Degradation

2.4.1 Instrumentation

A schematic diagram showing the ultra-short GC column apparatus employed in Chapter Six is given in Figure 2.3. In this design a 240 mm long × 6 mm O.D. × 1 mm I.D. quartz tube was used to contain a 200 mm long column. The quartz tube had a taper blown into it 40 mm from one end. This taper was used to friction fit the megabore ultra-short GC capillary column to it. The other side of this taper was flared out to aid in guiding a syringe through it to just inside the megabore column for on-column injection. The entire quartz tube assembly was connected to a ¼ inch Swagelok tee union using a graphite/Vespel ferrule. This tee was mounted onto the top of a Shimadzu model 8A-GC-FID (Kyoto, Japan) frame to lend physical support and supply electrical detector

connections. The side port of the tee was connected to high purity hydrogen (Praxair, Calgary, Canada), which was used as the carrier and flame gas at a flow rate of approximately 20 mL/min. The other port of the tee was sealed off with a rubber septum, through which samples were injected.

For controlling the temperature of the ultra-short GC columns a nickel-chromium wire (0.25 mm O.D.) was coiled around the quartz tube (with approximately 2 mm spacing between each wind) and attached to a variable transformer to provide resistive heating. Rubber washers were placed on either side of the Ni-Cr wire to ensure safe insulation between the column holder and the heating wire. Additionally, these washers also provided electrical insulation and a lower background noise in the on-column FID when heat was applied.

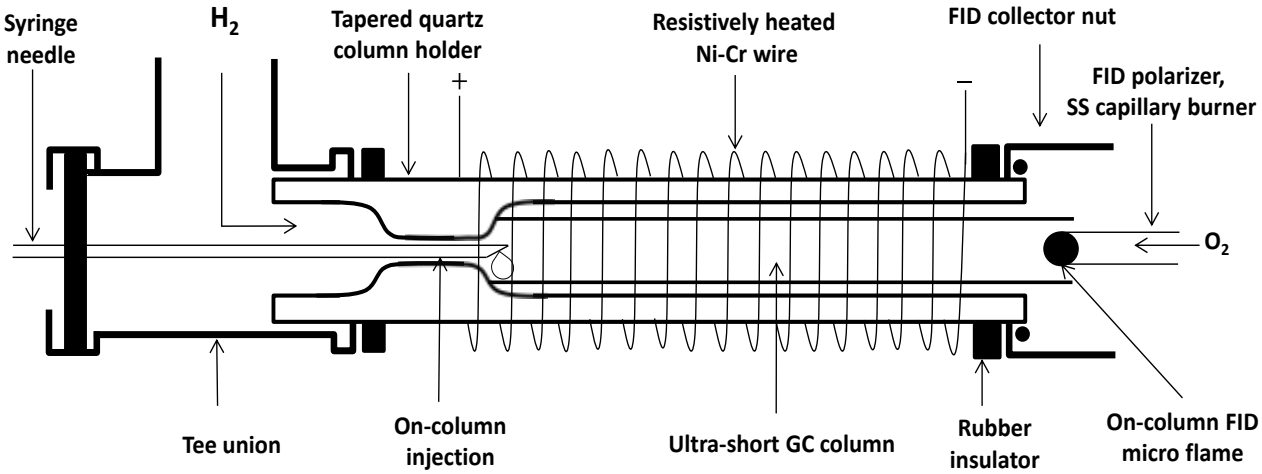

Figure 2.3: Schematic diagram of the ultra-short column GC apparatus with on-column injection and detection.

On-column detection was achieved by using a μFID that was previously described.[149,150] Briefly, the μFID collector was made from a ¼ inch stainless steel (SS) nut that was positioned around the end of the quartz tube and held in place by a rubber o-ring. The μFID polarization was achieved through a SS capillary burner (0.46 mm O.D. × 0.25 mm I.D.) delivering medical-grade oxygen (Praxair) at a flow rate of 7 mL/min to the flame in a direction opposing the hydrogen column flow. A potential difference was supplied to the polarizer and collector by using the existing electrical connections of the original GC-FID mainframe. The counter-current flame of the μFID was normally situated about 5 mm inside the end of the megabore column. This in turn resulted in burning off the stationary phase and polyimide coating at the column outlet. In some instances operating the flame at the column outlet also provided equivalent results. All ultra-short GC column separations were performed using a 20 cm long EC-5 (5% phenyl-95% methylpolysiloxane) megabore column at 0.53 mm I.D. and 2.65 μm film thickness (Alltech, Deerfield, USA).

2.4.2 Ultra-Short Column Operation Procedures

Operation procedures used for this method were as follows. Samples were injected (1 μL volumes) on-column at room temperature. The solvent would instantly elute off the column, leaving behind the analyte of interest. Once the solvent fully eluted and response returned to the baseline (about 60 s), a voltage was applied to the resistive heating wire in order to heat the column to the desired temperature and elute analytes. The applied heat then mobilizes the analyte resulting in its elution. The most common temperature program that was used during separations was room temperature (RT) for two minutes, then heating at a rate of approximately 90°C/min to a final temperature of 180 °C until all analytes are

eluted. The point when this heating rate was initiated will be referred to as applying “heat” to the system in the text.

2.4.3 ASA Degradation

All ASA degradation experiments were performed using a home-made SS cell (1.56 cm I.D. × 1.91 cm O.D. × 2.86 cm height) that was sealed from one end and capped with a Swagelok end cap from the other end. For ASA standards, a 1 mL aliquot of about 10 mg ASA /mL (80% water: 20% methanol) was loaded into the SS cell and the open end was capped with the Swagelok fitting. Next, the cell was placed vertically into a Hewlett Packard 5890A GC oven that was preheated to the desired temperature. After a set amount of time had elapsed, the cell was then removed from the oven and immediately cooled in ice-water. Subsequently, the solution was removed from the cell and 10 times diluted with methanol solvent for further chromatographic analysis.

For ASA tablets, a full crushed tablet was loaded into the SS cell and 100 μL of water was added to the tablet before capping the cell. Then, the cell was heated and cooled down in an identical manner to the ASA standards. The next step, was uncapping the cell and allowing the contents of the cell to dry at room temperature for about two hours before taking (5-10 mg) portions for subsequent μPLE and chromatographic analysis.

2.4.4 HPLC Analysis

In some experiments involving ASA, the ultra-short column GC analysis results were compared with conventional HPLC analysis results of the same analyte. All HPLC analysis of ASA standards and degradants was performed using the exact same HPLC system used before and described in section 2.2.4.

2.4.5 Chemicals and Reagents

Various test analytes were used in characterizing this system. The specifics of these standards such as composition and concentration are shown in Table 2-2 below. These included Dodecane, Hexadecane, and Octadecane (all 99% purity; Aldrich, Oakville, CAN). ASA (99%) and Salicylic Acid (SA) (99%), were additionally obtained from Sigma-Aldrich. Lab-grade Phenol was also used (Anachemia, Vancouver, CAN). For the HPLC analysis of ASA and its degradation products, HPLC grade methanol and water were both used and purchased from Honeywell Burdick & Jackson. Glacial acetic acid (99.7%) was also used in the HPLC analysis and was purchased from VWR International. ASA tablets were purchased from a local drug store. All other details and variations are described in Chapter Six.

Table 2.2: Composition of analyte standards employed in Chapter Six.

Solution	Analyte	Concentration (mg/mL)[a]	Solvent
Alkanes	Dodecane Hexadecane Octadecane	1.05 1.10 1.02	Acetone
ASA and standards of its degradation products	Phenol SA ASA	1.03 1.23 1.12	Methanol

a. Balance uncertainty = ±0.1 mg

Chapter Three: **CHARACTERIZATION OF THE SAMPLE PREPARATION OF PROZAC® CAPSULES AND OTHER APIS USING EFLE**

3.1 Introduction

Fluoxetine Hydrochloride is a second generation antidepressant drug that is chemically known as N-methyl-3-(4-tri-fluoromethylphenoxy)-3-phenylpropylamine.[151,152] Its structure is shown in Figure 3.1. Fluoxetine Hydrochloride acts as a selective serotonin reuptake inhibitor, which helps in treating a variety of depression cases.[153-155] Further, it has good efficacy and very low toxicity compared to other antidepressants, such as tricyclics, and it is therefore one of the most widely used drugs in treating severe depressive disorders.[152,156]

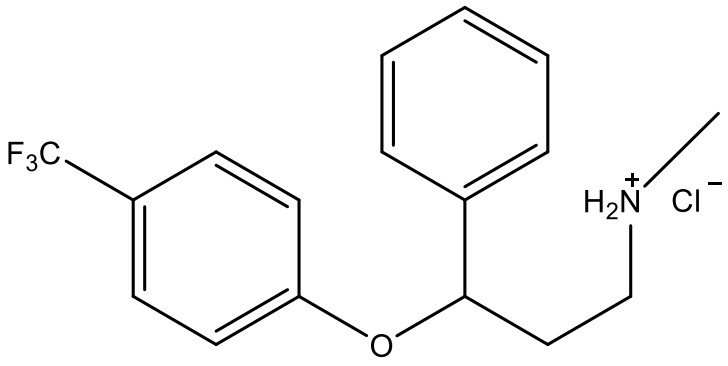

Figure 3.1: The structure of Fluoxetine Hydrochloride.

Currently Fluoxetine Hydrochloride is widely marketed in different countries as capsules under the commercial name of Prozac.® Therefore, to comply with regulatory agency requirements for verifying pharmaceutical composition, dosage, and purity, the analysis of such capsules is of great importance for both quality control and quality assurance purposes.[119,157] Conventional techniques for analyzing Fluoxetine Hydrochloride

formulations normally include HPLC methods,[119,122,123] but have also employed others such as spectrophotometry and GC.[124,125] However, while these instrumental techniques are relatively fast, the conventional sample preparation steps that are also employed for this pharmaceutical often require lengthy procedures (e.g. sonication and/or mechanical stirring) and frequently use significant amounts of organic solvent.[158-161] Indeed, solvent usage and time efficiency is of increasing importance in analytical methodology. Of note, sample preparation, as stated earlier, is the most time consuming and solvent intensive process during chemical analyses. Therefore, faster, more solvent-efficient methods of sample preparation continue to be of great interest.

Carbon dioxide-based fluids are an attractive alternative for sample preparation. For example, for many years SFE techniques have taken advantage of the beneficial low viscosity and high solvation properties of carbon dioxide by using it as a mobile phase fluid in the preparation of a great variety of samples.[4] This is because these properties allow the fluid to better penetrate matrices, dissolve target analytes, and efficiently extract them in a rapid manner. As well, since carbon dioxide has relatively mild critical parameters, a low cost, a wide range of available purities, and is fairly inert and environmentally compatible, it is a useful alternative to conventional organic solvents in analytical procedures. Therefore, methods employing carbon dioxide as an extraction fluid can potentially benefit from greater speed and lower solvent reliance. However, the primary limitation to carbon dioxide is that it is a non-polar fluid and is therefore often ineffective at solvating polar analytes. To alleviate this problem, many SFE methods modify the carbon dioxide with a small amount (e.g. up to 10%) of a polar organic solvent (e.g. methanol) to facilitate the extraction of polar analytes. Instrumentally, such modifiers can be manually added directly

to the extraction vessel or incorporated with the carbon dioxide mobile phase. However, as stated earlier, this approach is not always fully effective at polar analyte extractions due to the inability to overcome very strong matrix–analyte interactions and/or improve analyte solubility.

On the other hand, EFLE fluids, have relatively low viscosities, low surface tensions, high analyte diffusivities, and increased solvent strengths compared to SFE fluids. Therefore, EFLE features can provide very rapid and efficient extractions of a variety of polar analytes, while still reducing solvent consumption.

Despite the potential benefits of EFLE, little if anything has been reported on its usage to extract APIs from pharmaceutical formulations. In particular, Fluoxetine Hydrochloride sample preparation has never been explored using EFLE (or any other carbon dioxide-based extraction technique). Considering the possible advantages, it would be useful to examine the properties of Fluoxetine Hydrochloride extractions using EFLE. For example, such efforts would further knowledge regarding the capabilities of EFLE in pharmaceutical applications, and could also potentially provide an alternate route for improving the specific performance of Fluoxetine Hydrochloride sample preparation methods. This Chapter focuses mainly on exploring the analytical potential of EFLE as a novel means of extracting Fluoxetine Hydrochloride. Extractions are examined both in the presence and in the absence of a variety of model pharmaceutical matrices. Several key EFLE method parameters are investigated for their effect on extraction efficiency, and the capacity of EFLE to prepare commercial Prozac® capsule samples for analysis is presented and discussed. This Chapter also highlights the potential of this technique for extracting a variety of IP protected APIs and Ascorbic Acid and ASA from tablets. It further shows for

the first time the benefits of using water as a ternary modifier in improving the EFLE extraction recovery of hydrophilic APIs.

3.1.1 Effect of Methanol Composition on Extraction Efficiency

Initial experiments were aimed at establishing the efficiency with which Fluoxetine Hydrochloride could be extracted. To examine this, samples of the pure API were extracted using different compositions of methanol in carbon dioxide under constant conditions of 200 atm pressure, 40 °C, 10 minute extraction time, and a flow rate of 1.1 mL min^{-1} (50 cm restrictor). The results of these experiments are shown in Figure 3.2

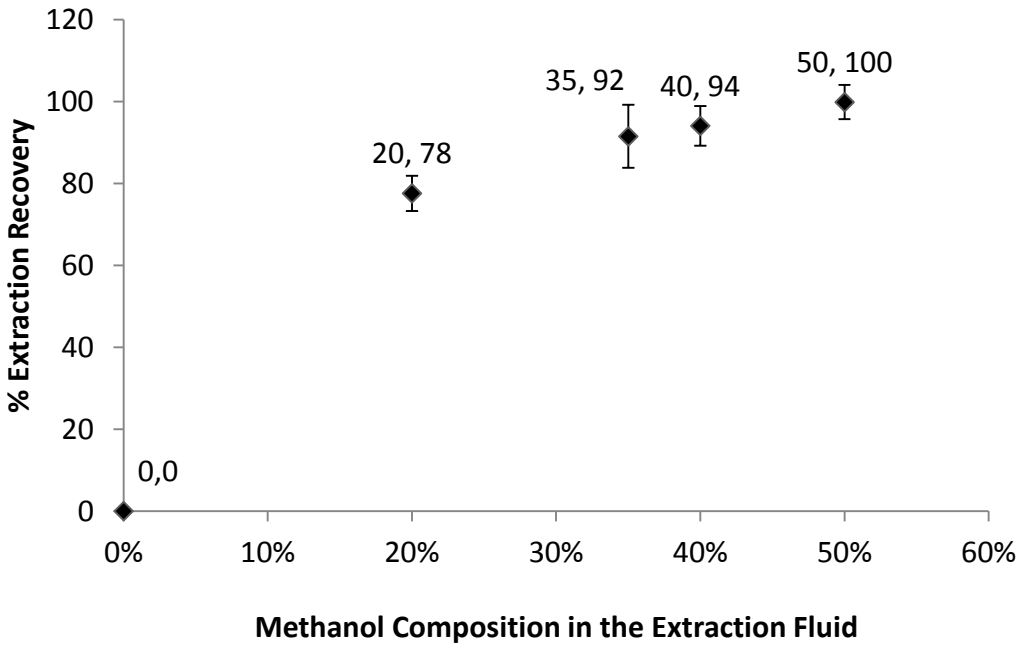

Figure 3.2: The effect of methanol composition on the extraction of pure Fluoxetine Hydrochloride. Conditions are 200 atm pressure, 40 °C, 10 minutes extraction time, and a flow rate of 1.1 mL min^{-1} (50 cm restrictor). Each data point is based on 3 replicate measurements, where the error bar denotes the standard deviation.

As can be seen, pure carbon dioxide was completely ineffective at removing any of the Fluoxetine Hydrochloride from the extraction vessel. However, as methanol was added to the mobile phase, the extraction recovery improved dramatically. For example, even 20% (v/v) of methanol added to the carbon dioxide retrieved almost 80% of the total Fluoxetine Hydrochloride from the extraction vessel. This trend continues up to methanol levels of 50%, where Fluoxetine Hydrochloride was quantitatively recovered. Therefore, Fluoxetine Hydrochloride can be extracted by methanol modified carbon dioxide, and a composition of 50% methanol and 50% carbon dioxide (v/v) was optimum for recovering the API. This demonstrates that the increased solvent ratio in EFLE can be quite effective in this regard, due to the polarity of Fluoxetine Hydrochloride and its solubility in methanol.

3.1.2 Effect of Extraction Time on Extraction Efficiency

Using the optimum fluid composition of a 50/50 mixture of carbon dioxide–methanol, it was then necessary to investigate what effect the extraction time had on the recovery of Fluoxetine Hydrochloride. As such, under the conditions of Figure 3.2, dynamic extractions were conducted for periods ranging from 3 to 15 minutes, and the samples were collected and analyzed. The results are shown in Figure 3.3. It was found that after 3 minutes of extraction only about 20% of the Fluoxetine Hydrochloride had been recovered, and after 6 minutes of extraction about 75% had been recovered. Ultimately, it took a minimum of 10 minutes extraction time to extract 100% of the total Fluoxetine Hydrochloride from the extraction vessel, indicating that the pure API can be mobilized quite readily by EFLE. Also, as expected, it can be seen in the figure that recovery values

beyond 10 minutes do not further increase, but rather stabilize within experimental error, indicating that no further API was recovered at longer extraction times.

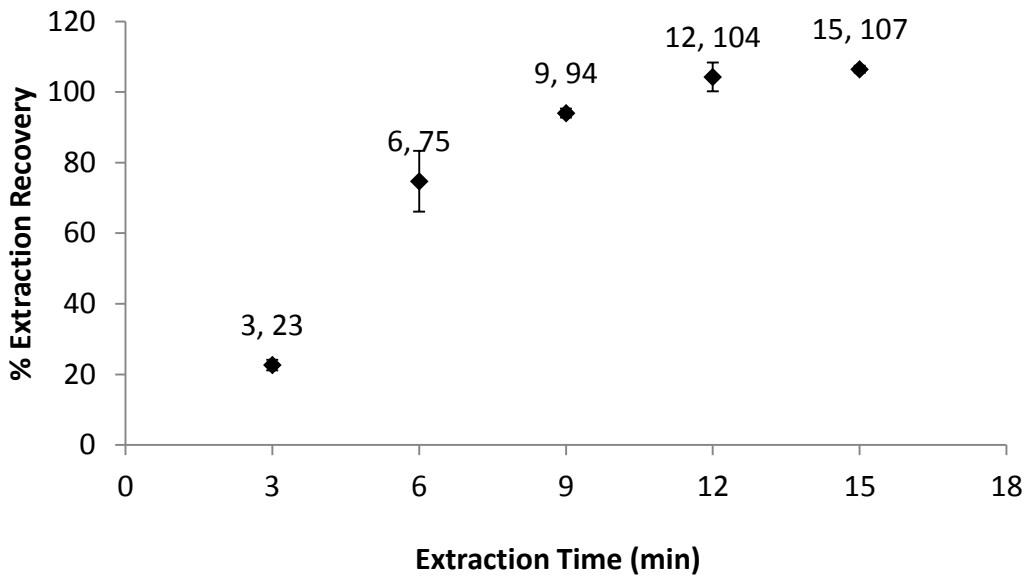

Figure 3.3: The dynamic EFLE extraction profile of pure Fluoxetine Hydrochloride as a function of time, using a 50% carbon dioxide–50% methanol mobile phase. Conditions are as in Figure 3.2. Each data point is based on 3 replicate measurements, where the error bar denotes the standard deviation.

3.2 Extraction from Excipients and Prozac® Capsules

3.2.1 Effect of Sample Matrix on Extraction Efficiency

Given the EFLE results for the pure API, the presence of a sample matrix was next probed for its effect on extraction efficiency. To examine this, Fluoxetine Hydrochloride was individually pre-mixed with various excipients that are commonly

used in pharmaceutical formulations and each sample was then extracted by EFLE. The results are shown in Figure 3.4.

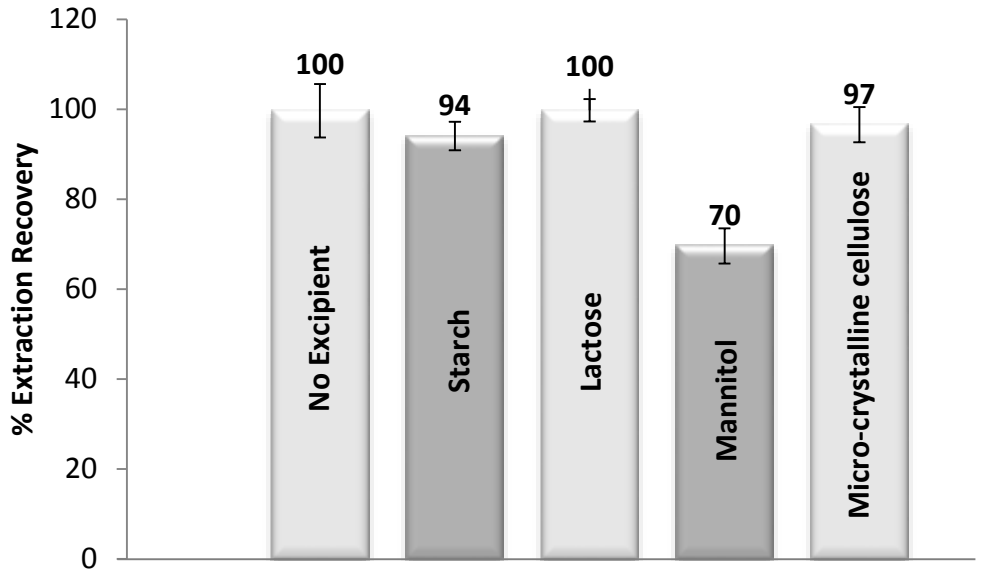

Figure 3.4: Extraction recovery of Fluoxetine Hydrochloride from different excipient matrices using a 50% carbon dioxide–50% methanol as an extraction fluid. Extraction conditions are as in Figure 3.2. Fluoxetine Hydrochloride mass: 20 mg, excipient mass: 180 mg. Each data point is based on 3 replicate measurements, where the error bar denotes the standard deviation.

As can be seen in Figure 3.4, most of the excipients did not greatly alter the extraction efficiency of Fluoxetine Hydrochloride compared to equivalent trials without excipient present. These include starch, lactose, and micro-crystalline cellulose, which each produced extraction recoveries of about 95% or greater. However, the one notable exception in this regard was mannitol which produced a significantly reduced extraction

recovery of about 69% for Fluoxetine Hydrochloride. While the exact reason for this is unknown, it is believed to be possibly due to the unique physical state that mannitol acquired during extraction. For example, unlike the other excipients, after extraction it was found that mannitol left a paste/gel-like residue in the sample chamber. Therefore, it could be that this hindered recovery of the analyte and created a barrier to quantitative extraction. So, with the exception of mannitol, it appears that the excipient should not greatly interfere with the extraction of Fluoxetine Hydrochloride by EFLE.

3.2.2 Extraction from Prozac® Capsules

Next, Prozac® capsules were extracted under the exact same conditions as those of Figures 3.3 and 3.4. It should be noted that the commercial capsules provided were produced using a starch excipient formulation according to the manufacturer. As such, considering the data in Figure 3.4, minimal interference was anticipated in the recovery of Fluoxetine Hydrochloride from the capsules, and this indeed was observed to be the case. Of note, after only 10 minutes of extraction by EFLE, the recovery of Fluoxetine Hydrochloride from a single capsule formulation was determined to be 104 ± 3%. Therefore, EFLE can quantitatively extract Fluoxetine Hydrochloride from commercial Prozac® capsules in a relatively short period of time. It is worth noting here that such values beyond 100% are common in the extraction literature[4,77,82,83,94,162] and are frequently interpreted as complete analyte recovery. In this case, the most likely sources of these extraction values lie in the standard error associated with separately weighing different amounts of analyte for calibration and extraction, preparing stock solutions, and the actual product specifications. For instance, the US Pharmacopeia Monograph for Fluoxetine capsules states that they contain “an amount of Fluoxetine Hydrochloride equivalent to not

less than 90.0% and not more than 110.0% of the labeled amount of Fluoxetine Hydrochloride".

3.2.3 Improving the Extraction Efficiency from Prozac® Capsules

Subsequent efforts focused on further reducing this extraction time. The main parameter explored in this regard was the fluid flow rate through the vessel. This was directly controlled by increasing the system pressure and/or altering the restrictor length. It is worth noting here that the effect of pressure on extractions employing relatively large modifier concentrations is negligible,[82] and so this served as a useful means of altering the flow rate. It was found that as the flow rate was increased from 1.1 mL min^{-1} (at 200 atm, 50 cm restrictor) to 2.0 mL min^{-1} (at 250 atm, 25 cm restrictor) the time required to quantitatively extract all of the Fluoxetine Hydrochloride from the Prozac® capsules decreased from 10 minutes to 5 minutes. Efforts to further exploit this trend in terms of time using a slightly larger flow rate of just 2.3 mL min^{-1} for 3 minutes (at 300 atm, 50 cm restrictor) produced lower extraction recoveries of about 40%, and so further increases in flow rate alone were not investigated.

Another parameter explored was extraction temperature. In general it was found that extractions done at 80 °C would often improve recoveries of Fluoxetine Hydrochloride by a factor of about 1.4 versus those performed at 40 °C. For example, the extraction recovery of Fluoxetine Hydrochloride from Prozac® capsules was found to be 49% at 40 °C and 69% at 80 °C when all other extraction conditions (200 atm pressure and 50% carbon dioxide–50% methanol mobile phase) were maintained the same. However, this same increase in the extraction temperature could also raise the observed flow rate by a factor of about 1.3 if different lengths of restrictors were not used to compensate for this.

Therefore, considering this effect of increasing the temperature on raising both the flow rate and the extraction recovery, several combinations of restrictor, pressure and temperature were examined. Ultimately, as can be seen in Figure 3.5, the best conditions were obtained when using an extraction temperature of 80 °C, a pressure of 375 atm, and a 15 cm restrictor, which yielded a flow rate of about 3.5 mL min^{-1}. These parameters produced an extraction recovery of Fluoxetine Hydrochloride from the Prozac® capsules of 99 ± 2% in just 3 minutes using only about 5 mL of total methanol. Therefore, EFLE conditions can be adjusted to efficiently and quantitatively extract Fluoxetine Hydrochloride from Prozac® capsules quite rapidly.

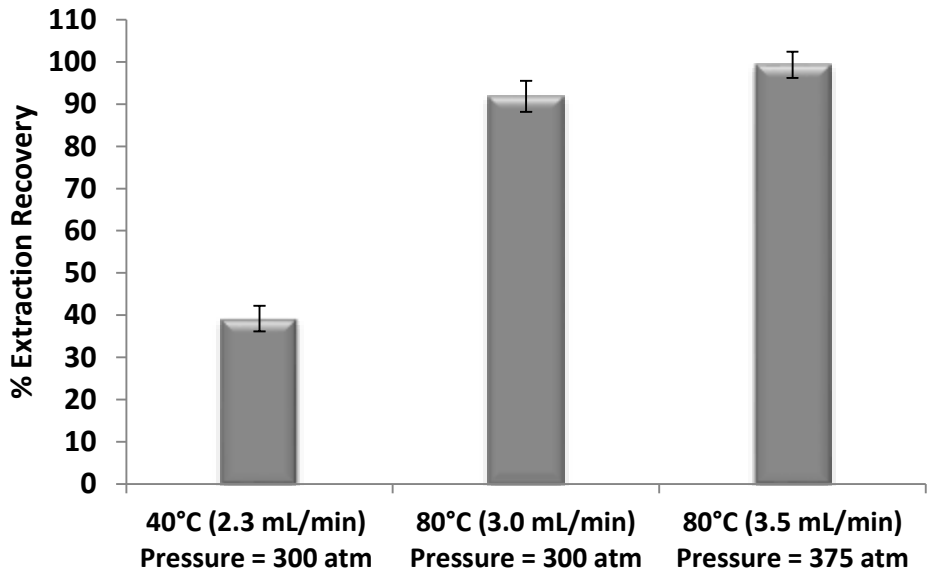

Figure 3.5: Typical effect of different conditions on the extraction recovery of Fluoxetine Hydrochloride from Prozac® capsules. Each data point is based on 3 replicate measurements, where the error bar denotes the standard deviation.

3.3 Pure Methanol Extractions

In view of the rapid and quantitative Fluoxetine Hydrochloride extractions obtained using EFLE, it was interesting and necessary to also directly compare the results with extractions that employed only pure room temperature methanol in a conventional liquid–solid sample preparation method. To examine this, methanol was added directly to the contents of one capsule that was emptied into a volumetric flask, and the solution was thoroughly mixed by inverting the flask a minimum of ten times. The solution was then filtered and the filtrate was analyzed for its Fluoxetine Hydrochloride content. As can be seen in Figure 3.6, for methanol volumes of 10, 25, and 50 mL, it was found in each case that only about 80% of the Fluoxetine Hydrochloride present in the capsule could be recovered by this approach.

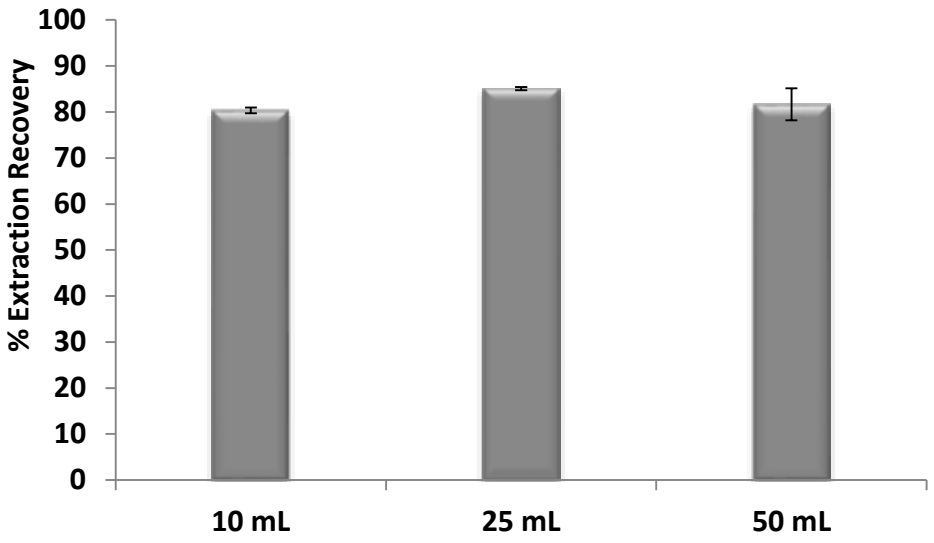

Figure 3.6: Room temperature pure methanol extractions of Fluoxetine Hydrochloride from Prozac® capsules using 10, 25 and 50 mL of methanol added directly to the capsule contents and shaken for 10 minutes and then filtered and analyzed.

The same recovery was also observed when using a 10 mL volume of methanol and constantly shaking the solution for 10 minutes prior to analysis. Ultimately, in our experience, a minimum of 15 minutes of constant shaking was required to quantitatively extract Fluoxetine Hydrochloride in this manner using pure methanol. Therefore, extraction of this API using EFLE can significantly improve upon the amount of time and solvent used in the process.

Another interesting observation was made regarding the chromatograms obtained for the above methanol extracts. Specifically, there appeared to be other material being extracted by the pure methanol trials that was not present in the EFLE extracts. Figure 3.7 shows the HPLC-UV chromatograms from both EFLE and pure methanol extracts for comparison. As seen in Figure 3.7A, the EFLE extract is fairly simple and displays only the injection solvent at about 1.5 minutes and the Fluoxetine Hydrochloride peak at about 3.25 minutes. Since the concentration of API in this chromatogram is about five-fold larger than that of the pure methanol trial, the inset in Figure 3.7A also shows the same chromatographic region with the response expanded to match the range used in Figure 3.7B. As seen, it remains that no other peaks are apparent. By comparison, the pure methanol extract (Figure 3.7B) shows these same features and additionally other peaks primarily in the region between 1.5 and 2.5 minutes. Examination of blanks and analysis of this same sample by LC-MS reveal that these extra peaks are attributed to starch excipient that is also present in the sample.

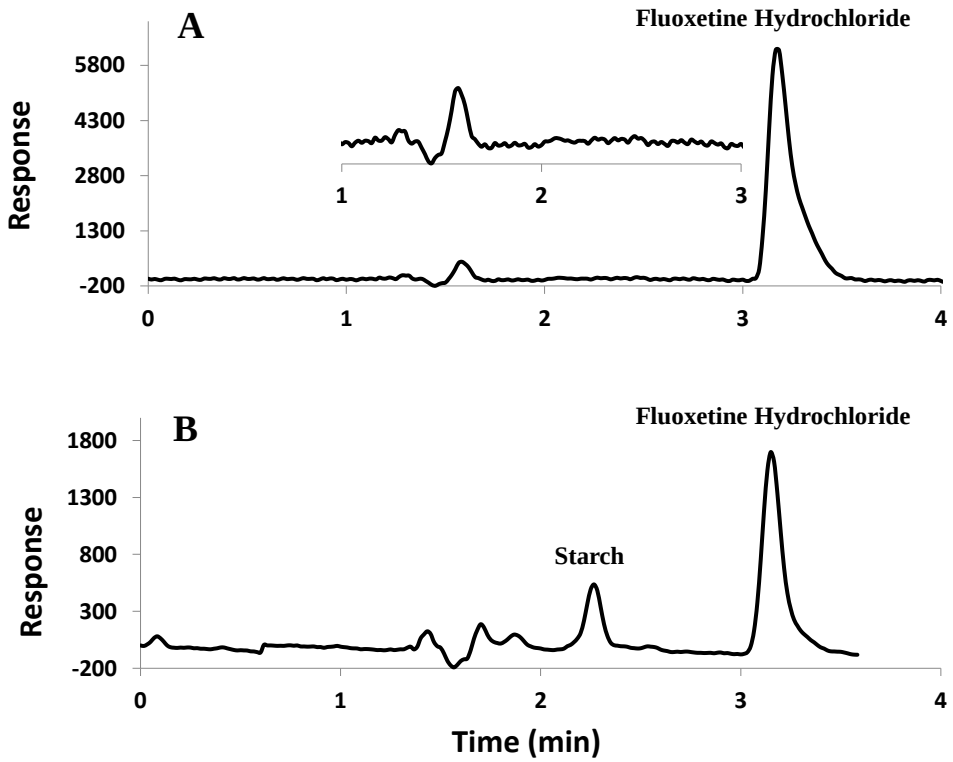

Figure 3.7: Chromatograms of Prozac® capsule extracts obtained by (A) EFLE under conditions of Figure 3.2, and (B) 50 mL of pure methanol. The amount of API in each extract is (A) 1.12 mg mL^{-1} and (B) 0.224 mg mL $^{-1}$. The inset region in (A) shows an expanded response range that matches that of the chromatogram in (B) for comparison.

It is worth noting here that these starch peaks become more apparent when larger amounts of methanol are used for capsule extractions. For example, as can be seen in Figure 3.8 these peaks in the 50 mL extract volume are significantly larger than those for the 25 mL extract. This indicates that the amount of starch being extracted with 50 mL methanol

is larger than that with 25 mL methanol. Therefore, not only can EFLE potentially reduce solvent usage in extractions, but this feature can also offer more selective extractions in this instance as well compared to conventional extractions using pure solvents.

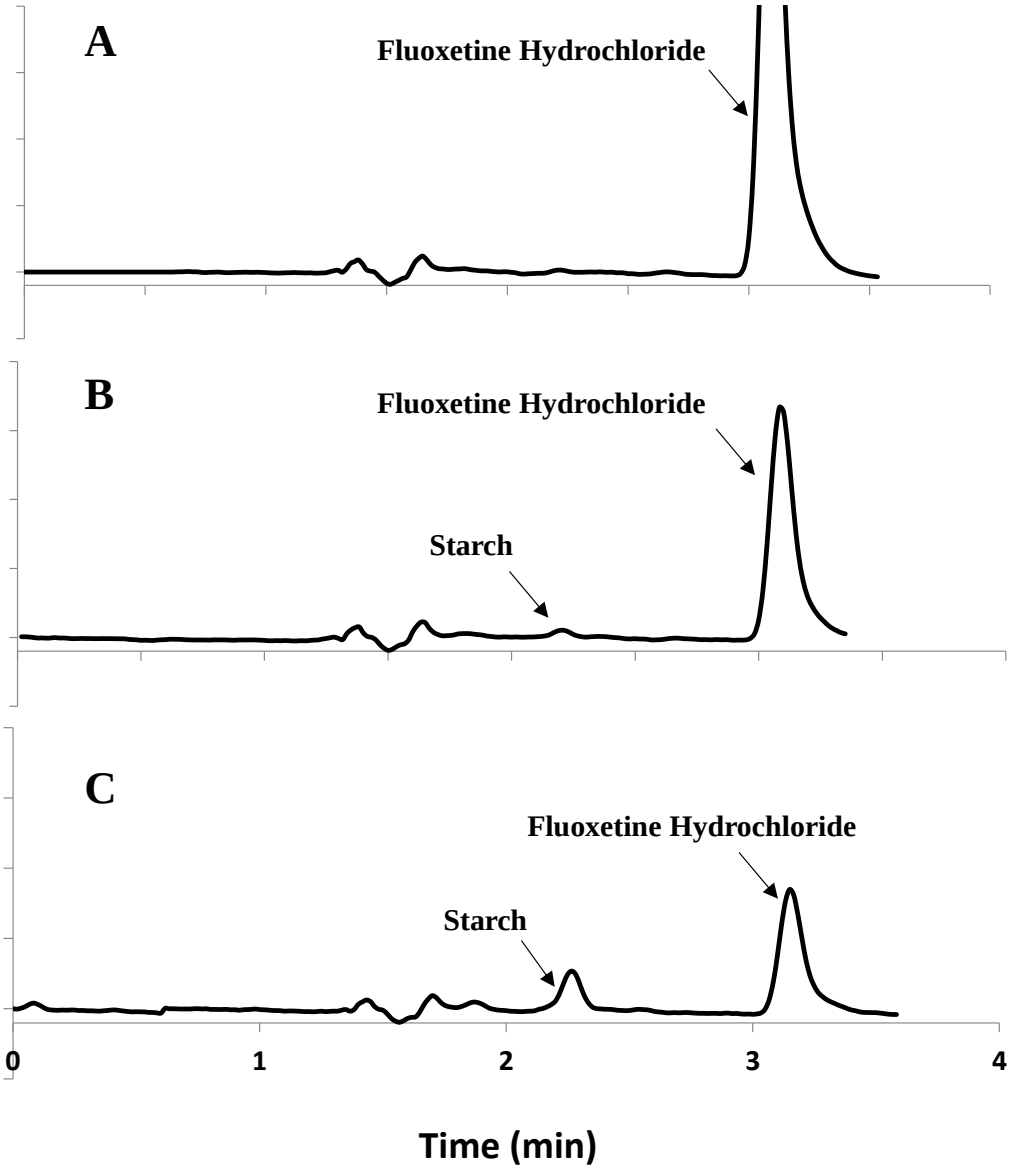

Figure 3.8: Chromatograms of extracts of a single Prozac® capsule obtained by room temperature methanol extraction in solvent amounts of A) 10, B) 25, and C) 50 mL.

3.4 Static Modifier Addition

In a final set of experiments for Fluoxetine Hydrochloride, methanol was added directly to the sample chamber before sealing it and pressurizing the vessel. This represents a static modifier addition mode, where only a primary carbon dioxide pump is used to perform the extraction. This is of interest since such an approach, if viable, can provide both a simpler and less expensive means of carrying out carbon dioxide based extractions. In this method, methanol was directly added to the extraction vessel after loading it with the contents of one Prozac® capsule. Next, pure carbon dioxide was pumped though the extraction vessel under extraction conditions of 375 atm carbon dioxide and 80 °C (yielding a flow rate of about 3 mL min^{-1}). Under these conditions, as can be seen in Figure 3.9, it was found that only 15 drops (~400 μL) of methanol added to the vessel resulted in Fluoxetine Hydrochloride recoveries of 104±5%, 98±8%, and 89±3% after 10, 5, and 3 minutes respectively (n=3) of extraction, indicating that quantitative recoveries can be obtained in as little as 5 minutes by this approach. However, when either less extraction time or less methanol was explored, the resulting recoveries were not quantitative (i.e. over 90% extraction recovery). Therefore, a static modifier addition mode can allow even greater solvent reduction without compromising extraction recovery. The various optimum EFLE conditions developed in this chapter for the extraction of Fluoxetine Hydrochloride from Prozac® capsules are presented in Table 3.1 for comparison.

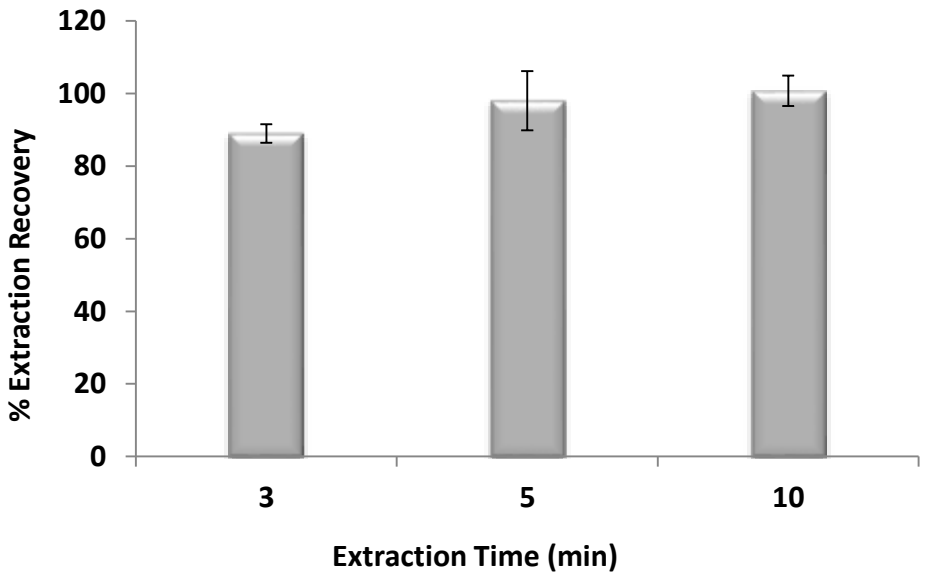

Figure 3.9: Extraction recovery of Fluoxetine Hydrochloride from Prozac® capsules at various extraction times using a static modifier addition mode. 400 µL methanol added to each. The error bar denotes the standard deviation for 3 replicate trials.

Table 3.1: Various optimum conditions for EFLE extraction of Prozac® capsules

Extraction Fluid[a]	Extraction Pressure	Extraction Temperature (°C)	Flow Rate (mL min^{-1})	Extraction Time (min)	Extraction Recovery (%)
A	200	40	1.1	10	104 ± 3
A	375	80	3.5	3	99 ± 2
B	375	80	3.0	5	98 ± 8

[a] A =50% CO_2–50% methanol delivered dynamically through the extraction vessel (about 5 mL total methanol). B = 400 µL of total methanol added directly to the extraction vessel (i.e. static mode) then extracted with 100% CO_2.

3.5 Probing the EFLE Extraction Recoveries and Dynamic Solubilities of Other Pure APIs

Based on the positive results of Fluoxetine Hydrochloride extractions obtained by using EFLE, it was interesting to investigate the capability of this technique to solubilize and extract other APIs. To examine this, the dynamic solubilities and the analyte recoveries after 10 minutes of extraction for 8 IP protected pure APIs were tested here (Table 3.2).

Table 3.2: Dynamic solubilities and % extraction recoveries of 8 IP protected APIs in 50% CO_2/50% methanol extraction fluid. Conditions are 200 atm pressure, 40 °C, 10 minutes extraction time, and a flow rate of 1.1 mL min^{-1} (50 cm restrictor)

Name of API	% Recovery after 10 min[a]	Dynamic solubility after 10 min[a,b,c]
API A	12±2%	0.10±0.00
API B	39±1%	0.29±0.01
API C	48±5%	0.30±0.02
API D	52±1%	0.39±0.00
API E	56±9%	0.50±0.07
API F	57±7%	0.32±0.03
API G	36±6%	0.27±0.04
API H	7±2%	0.04±0.01

a: n=2
b: mg/mL
c: Calculated as the amount of analyte that was removed from an excess of solid sample per volume of the extraction fluid that was continuously passing through the sample for the full extraction time

These APIs were under investigation by a pharmaceutical industrial partner and varied in properties although their structures were not available to us. All EFLE extractions were performed under the Fluoxetine Hydrochloride optimum extraction conditions for comparisons. These conditions are, 40 °C, 200 atm, 50 cm restrictor, and 50% CO_2/50% methanol extraction fluid. Results of this experiment, as can be seen in Table 3.2, showed that fully quantitative extractions of these APIs after 10 minutes were not possible under these conditions. This is because the dynamic solubilities of these APIs in the extraction fluid were not high enough. For example, most of the APIs have moderate solubilities and a few of them have very low solubilities in the extraction fluid mixture (e.g. API H). The relationship between the API extractability and its dynamic solubility in the extraction fluid is demonstrated in Figure 3.10. These findings suggest that the extraction fluid mixture is not optimum for all of these APIs and therefore, strategies that can improve the fluid dynamics of the EFLE procedure should be considered here. As such, further improvements for many of these APIs were pursued below.

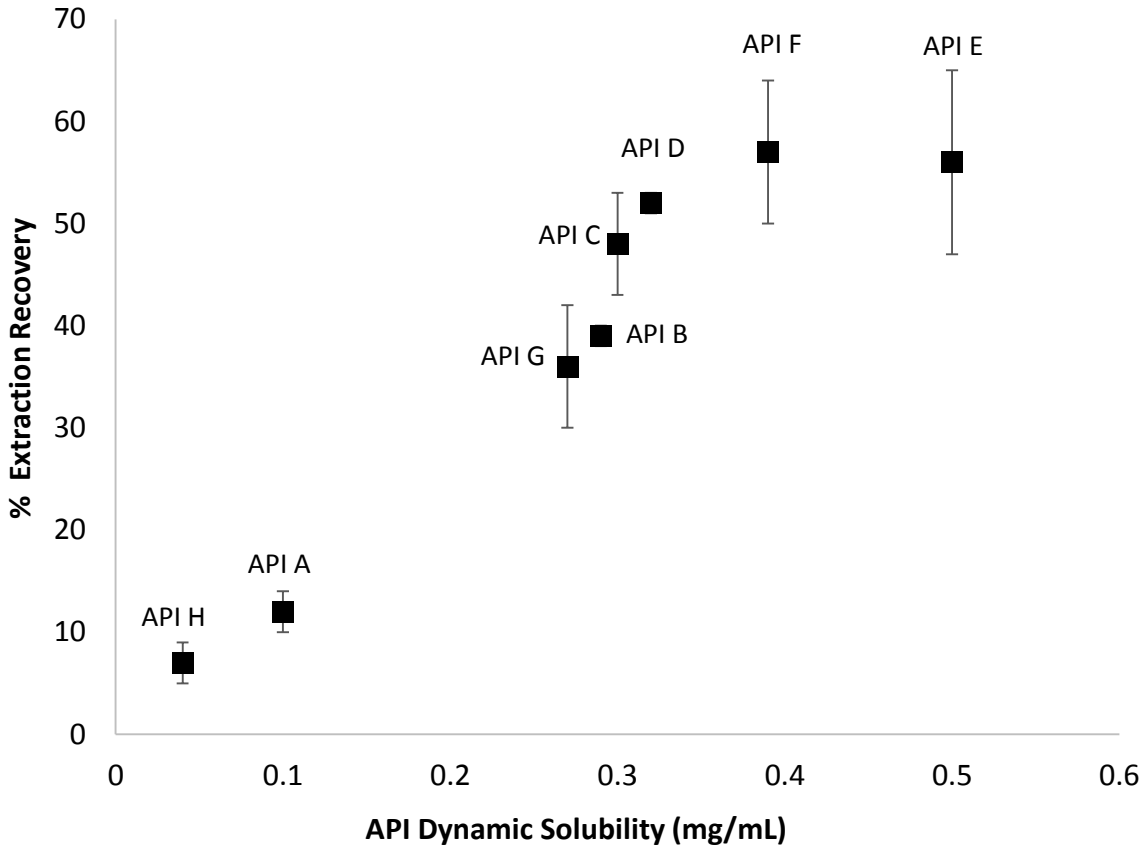

Figure 3.10: The relationship between the API dynamic solubility and its percentage extraction recovery after 10 minutes using 50% CO_2/50% methanol extraction fluid. Extraction Conditions are 200 atm pressure, 40 °C, 10 minutes extraction time, and a flow rate of 1.1 mL min^{-1} (50 cm restrictor). Each data point is based on 2 replicate measurements, where the error bar denotes the standard deviation.

3.6 Extractability Enhancement Strategies of Certain Pure APIs

3.6.1 Using Pure Methanol as an Extraction Fluid to Enhance the Extractability of Certain APIs

The extractability of APIs A, B, C, and D using 50% methanol/50% CO_2 extraction fluid was not very successful due to the relatively low solubility of these APIs in the extraction

fluid (. However, the solubility of these APIs is higher in pure methanol solvent. Therefore, using pure methanol as an extraction fluid (i.e. PLE mode) was next probed for these APIs. Results of this experiment, as can be seen in Table 3.3, showed that using pure methanol as an extraction fluid was very important and beneficial for these analytes. This is because the extraction recoveries of these APIs significantly improved by only changing the extraction fluid composition from 50% methanol/50% CO_2 to 100% methanol and maintaining the other extraction conditions the same (200 atm, 50 cm restrictor, and 10 minutes extraction time). It is worth noting here that obtaining quantitative extractions for some of these APIs (e.g. API A) required additionally raising the extraction temperature from 40 °C to 80 °C. Thus, while not an EFLE approach, these data show that PLE can be also very useful for API extractions, which is beneficial information since little is known of PLE capabilities in this area.

Table 3.3: Improving the extractability of 4 APIs by changing the extraction fluid from 50% methanol/50% CO_2 to 100% methanol. Conditions are 200 atm, 50 cm restrictor, and 10 minutes extraction.

Name of API	EFLE % Recovery after 10 min at 40 °C[a]	PLE % Recovery after 10 min at 40 °C[a]	PLE % Recovery after 10 min at 80 °C[a]
API A	12±2%	87±3%	105±6%
API B	39±1%	96±2%	99±5%
API C	48±5%	99±1%	103±2%
API D	52±1%	95±1%	98±8%

a: n=2

3.7 Using Water as a Ternary Modifier in EFLE

One of the most interesting approaches in enhancing the extractability of certain polar APIs is using water as a ternary modifier. Using water as a ternary modifier in EFLE is very beneficial because it allows for incorporating relatively large amounts of water in the extraction fluid. Incorporating these large amounts of water is not normally possible in conventional SFE due to the very low solubility of water in CO_2 (0.1% wt/wt).[4] However, when methanol is used as a substantial cosolvent in modified SFE or EFLE, up to 10% wt/wt of water can be incorporated with the methanol cosolvent as a part of the extraction fluid. This is particularly important in EFLE because large proportions of cosolvent (methanol) are normally employed which in turn allows for the incorporation of relatively large amounts of water that might be important to enhance the extractability of hydrophilic analytes. Using water as a ternary modifier has previously been used in conventional modified SFE,[163] however, it has never been explored in the field of EFLE. Therefore, it was interesting to investigate this approach in extracting target APIs that are not highly extractable with 50% methanol/50% CO_2 and are highly soluble in water.

3.7.1 The Effect of Using Water as a Ternary Modifier on the Extractability of API E

In order to improve the extractability of API E, water was used as a ternary modifier in the extraction fluid. API E has a relatively low solubility in methanol but a very high solubility in water. Therefore, water was used here as a ternary modifier by premixing it in the desired proportion with the methanol cosolvent. The effect of different water proportions in methanol on the extraction recovery were examined in this experiment. As can be seen in Figure 3.11, incorporating water with the extraction fluid, significantly improved the extractability of API E. For example, when 1% water/99% methanol v/v

mixture was used as a cosolvent in the extraction fluid (50% cosolvent/50% CO_2) instead of 50% methanol/50% CO_2, it improved the extraction recovery of this API from 56 % to 85% in only 10 minutes at 40 °C, 200 atm, and 50 cm restrictor. It was also seen in this experiment that higher water proportions in the cosolvent resulted in further improvements in the extraction recovery of this API. This is mainly due to the high solubility of this API in water. Thus, this can be a very useful approach.

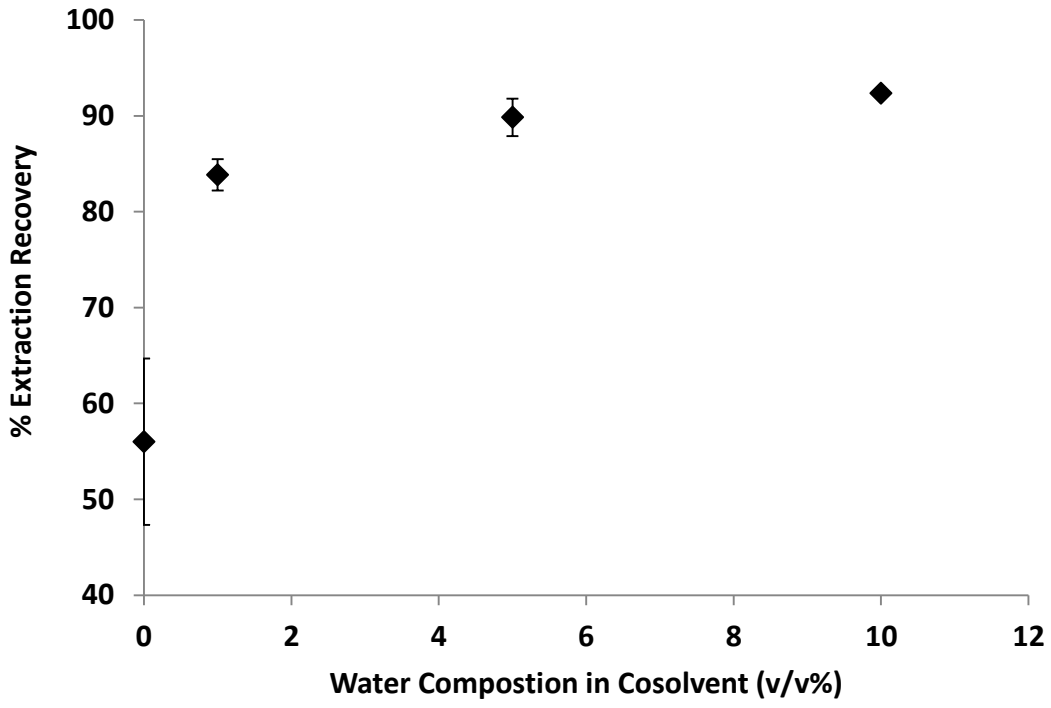

Figure 3.11: The effect of water composition in methanol cosolvent on enhancing the extractability of pure API E. Conditions are the same as those in Table 3.2. The error bar denotes the standard deviation for 2 replicate trials.

3.7.2 The Effect of Using Water as a Ternary Modifier and the Temperature on the Extractability of API F

In order to improve the extractability of API F, water was also used here as a ternary modifier since this API has a slightly higher solubility in water than methanol. Various water proportions in the cosolvent were also examined here for their effects on the extraction recovery. Results of this experiment showed that using water as a ternary modifier did actually improve the extraction recovery of this API but to a lesser extent than that was seen for API E. For example, when 5% water/95%methanol v/v mixture was used as a cosolvent the extraction recovery of API F improved from 57% to 74%. However, further increases of water in the extraction fluid were not beneficial. Therefore, and in order to improve the extraction recovery of this API, a higher extraction temperature was next pursued. Results of this experiment (Figure 3.12) showed that increasing the extraction temperature from 40 °C to 80 °C while maintaining all other extraction conditions the same (50% cosolvent/50% CO_2), 10 minutes extraction, and a 50 cm restrictor) significantly improved the extraction recovery of API F from 74% to 97%.

The findings of this experiment show that raising the temperature and using water as a ternary modifier can be very powerful tools in improving the EFLE extraction recoveries of certain APIs according to their properties.

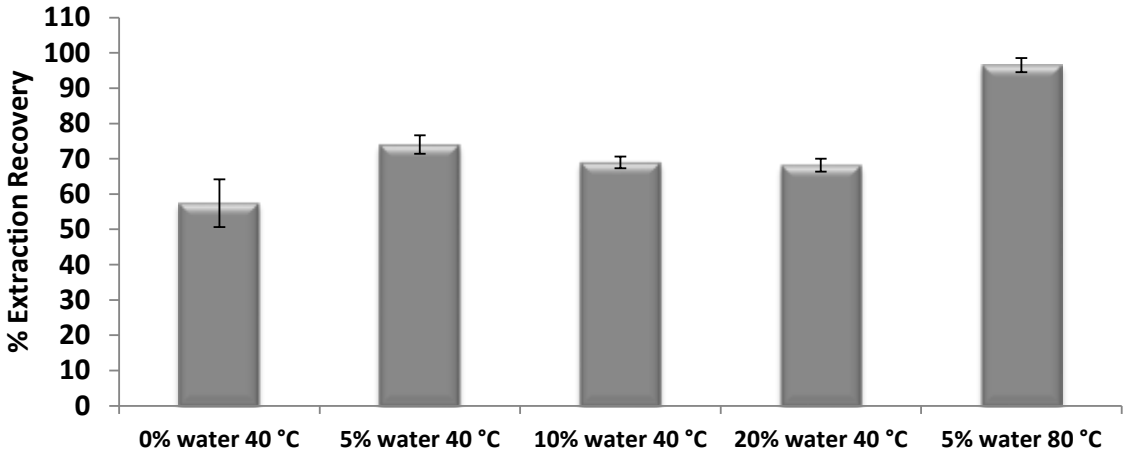

Figure 3.12: The effect of using water as a ternary modifier and increasing the extraction temperature on the extraction recovery of pure API F. The error bar denotes the standard deviation for 2 replicate trials.

3.8 Application of EFLE to Commercially Available Tablets: Ascorbic Acid

Given the results obtained from using EFLE in extracting various APIs from samples, it was interesting to apply these principles to the extractability of common pharmaceuticals. The first of these was Ascorbic Acid from tablets. This is because pharmaceutical tablets are normally composed of complex matrices and conventional methods for extracting target analytes from such matrices are frequently lengthy. In order to examine this, a preliminary experiment aimed to extract Ascorbic Acid from tablets using 50% methanol/50% CO_2 as the extraction fluid was performed. Results of this experiment, as can be seen in Figure 3.13, show that extracting Ascorbic Acid from tablets under these conditions was not very successful. For example, only about 10% of Ascorbic Acid was recovered from tablets after 10 minutes at 40 °C and 200 atm pressure (50 cm

restrictor). This extraction recovery only slightly improved to 27% when the temperature was raised to 80 °C while maintaining the other extraction conditions the same.

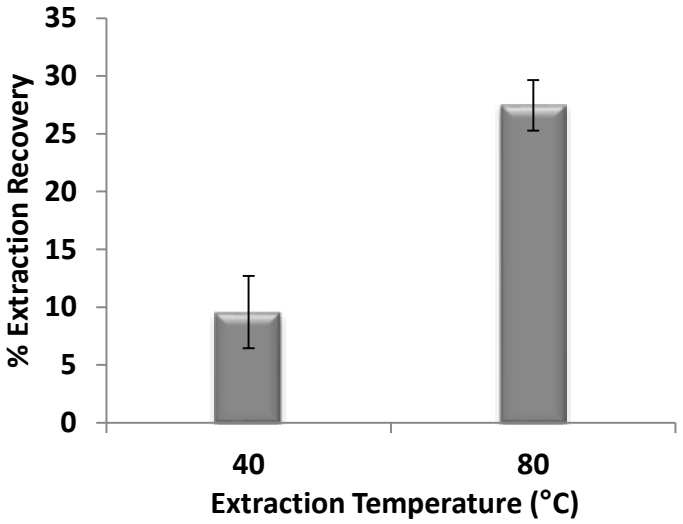

Figure 3.13: Extraction recoveries of Ascorbic Acid from tablets at different temperatures. Conditions are 50% methanol/ 50% CO_2, 200 atm pressure, 10 minutes extraction time, and 50 cm restrictor. Each data point is based on 3 replicate measurements, where the error bar denotes the standard deviation.

Next, in order to improve the extractability of Ascorbic Acid from tablets, water was used as a ternary modifier. This approach was pursued here because Ascorbic Acid is 10 times more soluble in water than methanol and therefore, it is anticipated that water will enhance the extractability of this API using EFLE. Results of this experiment, as can be seen in Figure 3.14, showed that the extraction recovery of Ascorbic Acid significantly improved when 10% water/90% methanol mixture was used as a cosolvent instead of pure methanol. For example, 63% of Ascorbic Acid was recovered from tablets after 10 minutes

and quantitative extractions of this API were obtained after only 15 minutes when the 10%water/90%methanol cosolvent was employed.

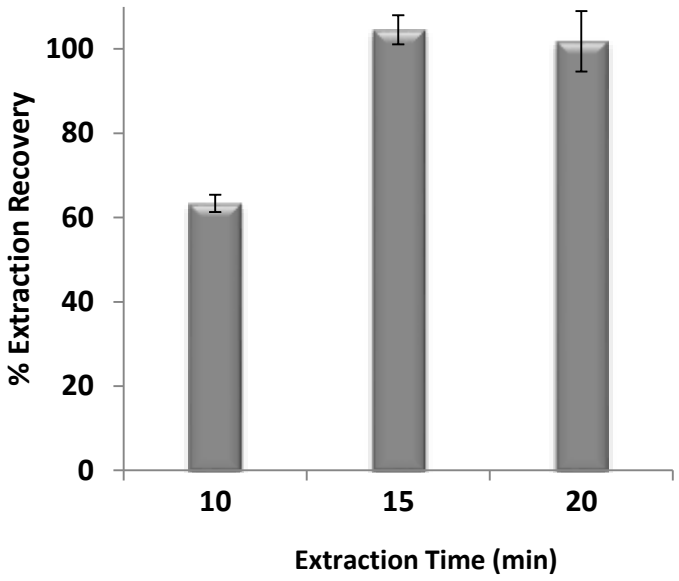

Figure 3.14: Extraction recoveries of Ascorbic acid from tablets using water as a ternary modifier in the extraction fluid. Extraction fluid is 50% (9 methanol/1 water v/v) / 50% CO_2 at 40 °C and 200 atm pressure. A 50 cm long restrictor was used. The error bar denotes the standard deviation for 3 replicate trials.

3.9 Application of EFLE to Commercially Available Tablets: ASA Delayed-Release Tablets

In a final set of experiments, the extractability of ASA from Delayed-Release tablets using EFLE was examined. This pharmaceutical formulation is particularly interesting to explore because it is a Delayed-Release formulation and it contains a large number of excipients that make the extractability of ASA challenging. Extracting ASA from

Delayed-Release tablets using the CO_2/ methanol EFLE extraction fluid was very successful in 10 minutes. For example, as can be seen in Figure 3.15, quantitative extractions of ASA from tablets were obtained in just 10 minutes at 40 °C.

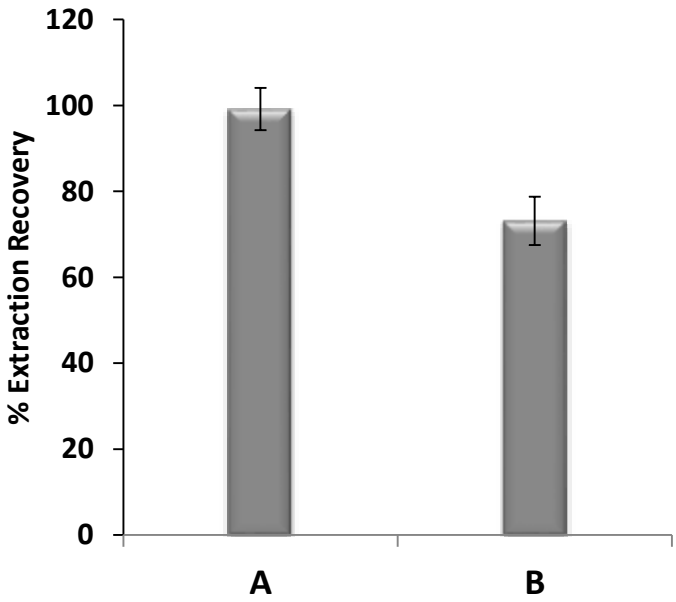

Figure 3.15: EFLE extraction recoveries of ASA from tablets using 50% methanol/50% CO_2 extraction fluid at various extraction conditions. A) 10 minutes, 40 °C, 200 atm pressure, 1.2 mL/min flow rate, and a 50 cm restrictor. B) 3 minutes, 80 °C, 375 atm pressure, 3.7 mL/min flow rate, and a 15 cm restrictor. The error bar denotes the standard deviation for 3 replicate trials.

It was also found in this experiment that extraction time could be much shorter if the extraction temperature and flow rate (via higher pressure) were both increased. For instance, after only 3 minutes at 80 °C, nearly 73% of the API was recovered. Therefore, quantitative recovery is likely to be attained after 3 minutes but well below 10 minutes.

3.10 Conclusions

From these experiments, it is clear that EFLE can significantly (and greatly in a number of cases) improve upon sample preparation processes in the pharmaceutical industry. The primary modes of facilitation are in organic solvent /hazardous waste reduction, and increased speed.

Fluoxetine Hydrochloride can be efficiently and quantitatively recovered from commercial Prozac® capsules using EFLE employing carbon dioxide/methanol fluids. Compared to conventional methods, the extractions use only minimal organic solvent and extraction time and can deliver more selective extractions of the API for analysis. For instance, it was found that greater amounts of solvent present in conventional liquid-solid extractions of the API caused more starch excipient to be co-extracted in the process. Conversly, EFLE was not found to extract any starch. While EFLE was found to be compatible with most excipients, it was not able to quantitatively extract Fluoxetine Hydrochloride from mannitol due to paste formation in the extraction vessel. Therefore, mannitol could be problematic with EFLE in other pharmaceutical formulations. The results also suggest that EFLE in either a dynamic or static modifier addition mode could be a useful alternative sample preparation method for Fluoxetine Hydrochloride and potentially for many other APIs.

Using water as a ternary modifier in EFLE is also found to be a very powerful approach in enhancing the extractability of water soluble analytes. For example, results showed that modest water additions to the EFLE cosolvent significantly improved the extraction recovery of several hydrophilic APIs compared to using pure methanol as a cosolvent.

Chapter Four: **A NOVEL ULTRA-RAPID MICRO PRESSURIZED LIQUID EXTRACTION TECHNIQUE FOR SOLID SAMPLE PREPARATION**

4.1 Introduction

As stated earlier, PLE is one of the most effective improvements introduced in the area of sample preparation. This is because this technique uses moderate amounts of organic solvents to extract analytes from solid matrices in a relatively rapid manner. Nonetheless, while the time and the solvent consumption in PLE are significantly smaller than those of conventional methods (e.g. Soxhlet), they can still potentially impede efforts to further improve sample throughput and solvent waste reduction in routine analytical settings. Even more, the cost and size of the specialized equipment needed to perform PLE can also hamper the acquisition of multiple extraction units to facilitate this.

In this regard, efforts to miniaturize PLE have been further beneficial in terms of significant organic solvent reduction (e.g only a few mL required).[113,115] However, other issues with this promising approach still need to be addressed and improved. For instance, several aspects of the instrumentation employed for miniaturization remain similar to conventional PLE. In particular, since the methods typically use static and dynamic extraction modes, conventional components such as pumps, valves, and fluidic connections are required. This increases the cost and complexity of the technique and can inhibit its wider implementation. More importantly, many of the extraction times realized by these approaches are still on the order of 15 minutes or more, which are the same as conventional PLE.[115,116] As a result, such efforts do not provide any sample throughput benefits for potential users. Thus, new miniaturized methods that are simple, fast, and solvent efficient are of great benefit to explore.

In this chapter, a novel micro pressurized liquid extraction (µPLE) procedure that uses microliter amounts of solvent to prepare milligram quantities of solid samples in only seconds using a simple static operating mode is introduced. The method generates little to no solvent waste after extraction and the apparatus used is small, inexpensive, and easily accessible. The operating properties of this µPLE approach are characterized here and compared with conventional PLE using some common pharmaceutical tablets and green tea leaves as model sample matrices.

4.2 General Operating Characteristics

In order to better understand the µPLE operating parameters, it was important to initially examine the heating characteristics of the two extraction vessels used. For instance, since temperature is a fundamental force for extraction efficiency in PLE, it is useful to explore the relative ability of the sample vessels to achieve the set extraction temperature and any differences between the two designs in this regard.

To examine this, a thermocouple was put inside of an open, uncapped extraction vessel that was then placed into the preheated extraction unit. Next, the temperature was monitored over time for various preset extraction temperatures. Figure 4.1 displays the typical results of these experiments with the vessel temperatures observed over time in quartz and stainless steel at both 120 and 225 ^{o}C. As can be seen, for these relatively low and high extraction temperatures, there is little difference between the stainless steel and quartz vessels in terms of the temperatures recorded and the two profiles follow each other very closely. It can also be noted that the vessels both reach the set extraction temperature in a very short amount of time. For instance, at 10 seconds both vessels have reached a

temperature within about 95% or more of the set value. This is reasonable and also agrees with the fact that extractions done under identical conditions with both vessel designs consistently provided the same results.

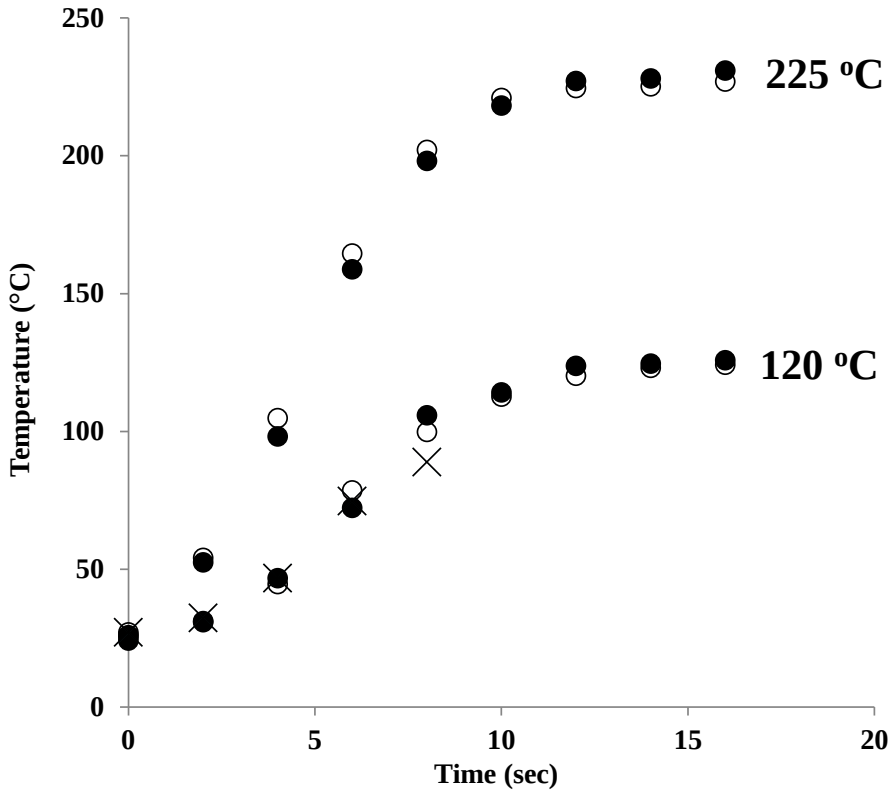

Figure 4.1: Vessel temperature as a function of time after being placed inside of the heated extraction unit for both the quartz (•) and stainless steel (o) designs at 120 and 225 °C. Vessels were empty and uncapped. Another partial trial where the quartz vessel was capped with a thermocouple sealed inside of 125 µL of methanol (X) is also included for a 120 °C set temperature.

In order to gauge how these temperature profiles compare when extraction solvent is present, trials were also performed where the thermocouple was led through a small hole

in the septum cap of the quartz vessel and then sealed with a high temperature silicon adhesive. The thermocouple was immersed in the typical 125 µL volume of extraction solvent and then capped as usual before heating. It was found that this arrangement could only withstand leaking and provide proper measurements for the first eight seconds before the improvised silicon seal failed. Still, the partial data available from the trials performed at 120 ^{o}C is also included in Figure 4.1 and overlaid on the other results for comparison. Here again it can be seen that although the solvent is present, the heating profile still follows very closely to the previous trials. Of note, even at the highest temperature recorded (after eight seconds, just before the seal failed) the solvent reached a temperature of about 74% of the set value. By comparison, the empty vessels at this time point attained values that were about 83% of the set value. Therefore, the vessel contents appear to heat quite rapidly in the µPLE system, which is beneficial since such characteristics can directly impact the extraction efficiencies realized.

Another property to probe was the pressure accumulated inside of the vessel during extraction, since this is primarily utilized in PLE to maintain the heated solvent in the liquid state. Indeed, this was apparent in early µPLE experiments where it was visually clear that the solvent remained liquid inside of the quartz vessels during heating. In particular, though, it was necessary to investigate if any leakage occurred as a result of such pressure buildup. To gauge this, both quartz and stainless steel vessels containing solvent were accurately weighed before and after heating to assess any loss. It was found that the leakage tolerance of the two vessels greatly differed. Specifically, using methanol solvent, the quartz vessels could be heated up to 120 ^{o}C without any issues, but beyond this they readily began to leak. Results of this experiment are shown in Figure 4.2. As can be seen, the

rubber septum can remain sealed for long heating times only at 120 ^{o}C and fails at higher temperatures. For instance, it can remain sealed for only 8 seconds at 180 ^{o}C before leaking. As such, 120 ^{o}C was treated as the maximum operating temperature of the quartz vessels. For this reason the second stainless steel design was pursued so that higher temperatures could be explored. By comparison, the stainless steel vessels remained sealed at temperatures tested up to 275 °C.

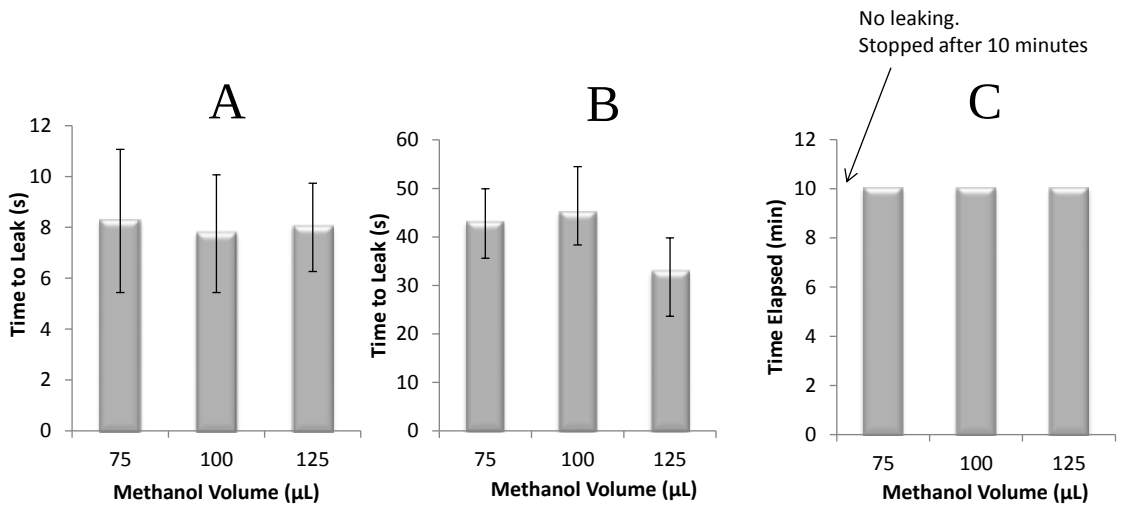

Figure 4.2: Time before leaking occurs when methanol is sealed inside the quartz extraction vessel at various temperatures of A) 180 °C, B) 150 °C, and C) 120 °C.

Leaking arose in the quartz vessels due to the rubber septum failing to withstand the increased vapor pressure of the solvent. To better confirm this pressure, the bottom of a capped quartz extraction vessel was cut open and a nitrogen source was connected to it. The gas pressure was then systematically increased and it was found that the septum cap failed to seal for pressures greater than about 6 atm. Therefore this should reflect the pressure conditions inside the quartz vessel near the maximum operating temperature of

120 ^{o}C. In this regard, it should be noted that the vapor pressure of methanol at 120 ^{o}C also agrees well with this value.[164]

4.3 µPLE Extraction Characteristics

4.3.1 Pharmaceutical Tablets

To probe the extraction characteristics of the µPLE system, preliminary trials were aimed at extracting some target analytes from different model solid sample matrices. The first explored were some common pharmaceutical tablets, which presented a reasonably homogenous and compressed solid sample for extraction. Two different tablets were examined in this study, each with a fairly complex range of excipients present in the matrix. The first were Delayed-Release ASA tablets, which contained colloidal silicon dioxide, anhydrous lactose, methacrylic acid copolymer type b, sodium lauryl sulfate, sorbitan tristearate, stearic acid, talc, titanium dioxide, and triethyl citrate. The second were acetaminophen tablets containing cellulose, corn starch, magnesium stearate, and sodium starch glycolate.

Early experiments indicated that the extraction of such APIs from pharmaceutical tablets was quite fast and efficient, and optimum conditions could be readily identified through altering the primary parameters of temperature and time. For instance, Figure 4.3 displays the typical results of such an optimization for ASA extractions by µPLE in the quartz vessels.

As seen over the ranges shown, higher extraction efficiency is obtained at greater temperatures and times, consistent with what would be anticipated from PLE. As a result, quantitative extractions of ASA are attained in about 60 seconds at 120 ^{o}C with 99 ± 4% (n=4) of the API being recovered. For acetaminophen, using a similar protocol at higher

temperatures in the stainless steel vessels, it was found that 97 ± 4% (n=4) of the API could be extracted from tablets after only 10 seconds at 150 ^{o}C.

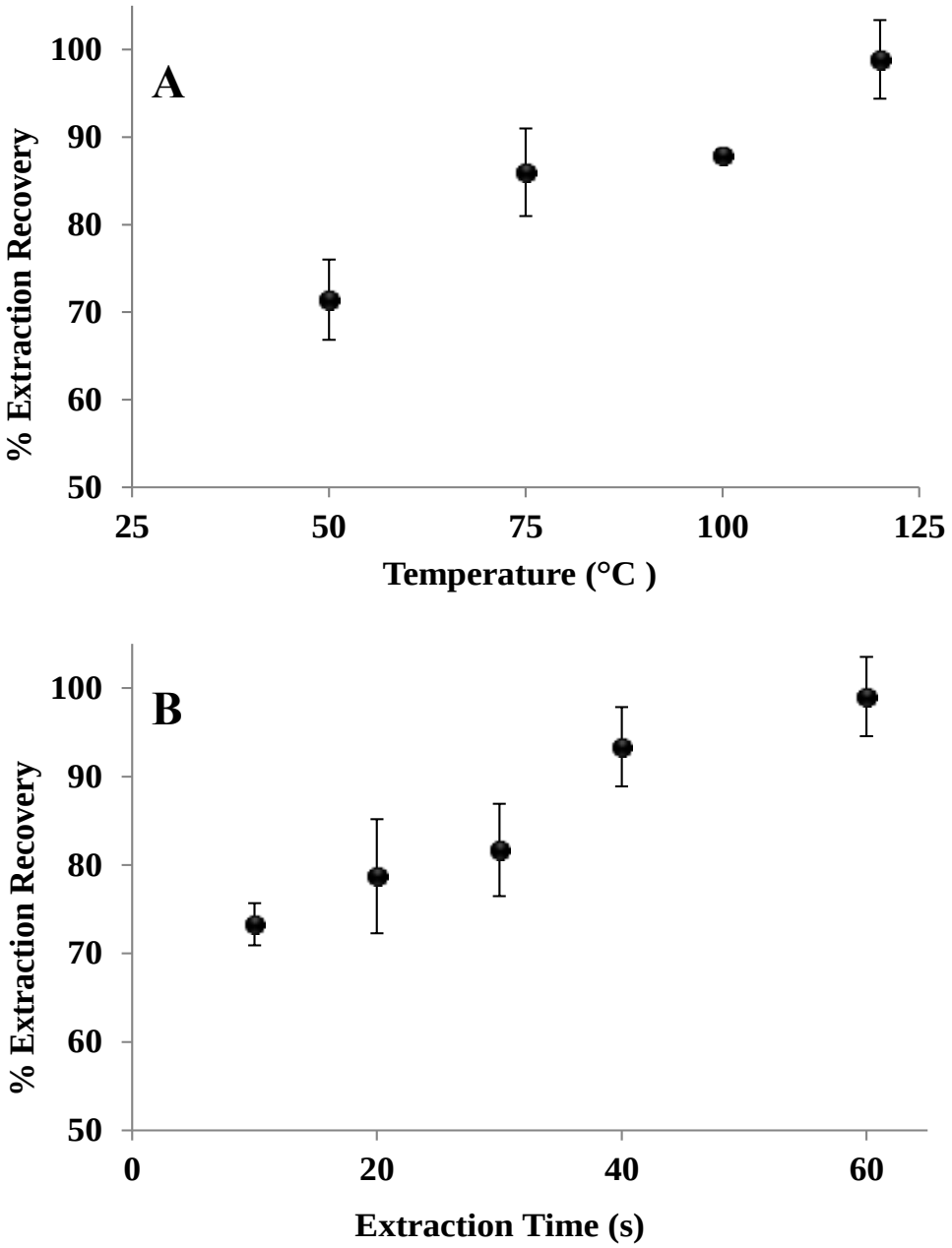

Figure 4.3: Extraction profiles of ASA tablets as a function of A) temperature for 60 seconds each, and B) time at a temperature of 120 °C. Replicate extractions (n=4) were performed using the quartz extraction vessel and methanol as a solvent.

Therefore, such samples can be prepared for analysis in a remarkably short period of time using µPLE. Further, since higher temperatures seemed to enhance many µPLE extractions, the stainless steel vessels were used exclusively for the rest of the study since they had a much wider operating range than the quartz vessels did.

4.3.2 Analyte Degradation

Given the promising results for acetaminophen, efforts were made to further reduce the ASA extraction times by increasing the now stainless steel vessel temperature beyond 120 °C. For this, ASA extractions of 30 seconds each were explored at temperatures ranging from 150 to 250 °C. It was found, as can be seen in Figure 4.4 that as the temperature increased the ASA extraction efficiency decreased dramatically from about 90 to 7%, which indicated thermal degradation of the analyte.

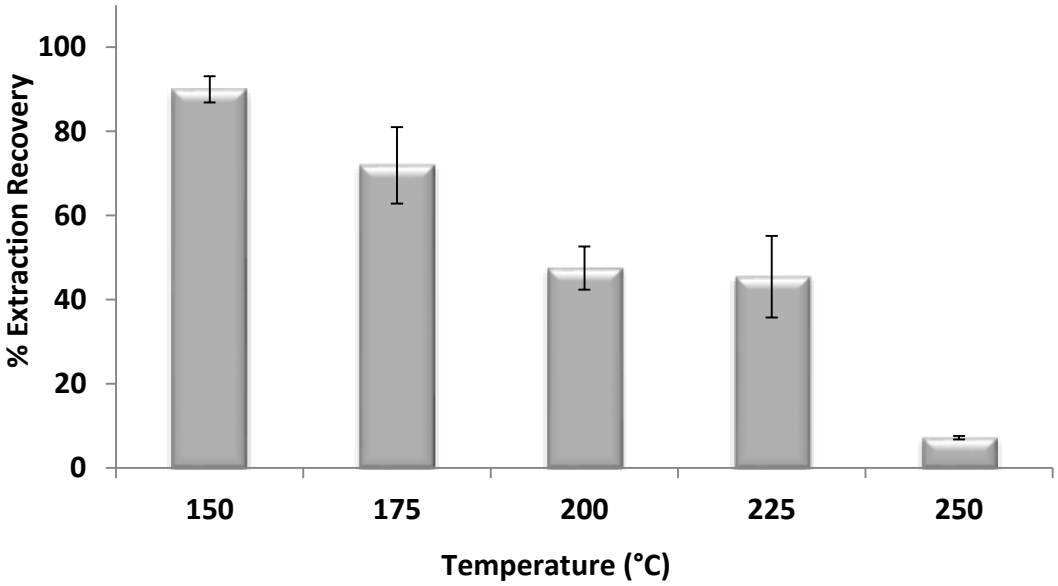

Figure 4.4: µPLE extraction recoveries of ASA from tablets after 30 seconds at relatively high temperatures (150-250 °C). Replicate extractions (n=4) were performed using the stainless steel extraction vessel and methanol as a solvent.

This behavior has been noted previously for ASA[102,165] and was also confirmed here by the appearance of new degradant peaks in the HPLC extract chromatograms, as shown in Figure 4.5.

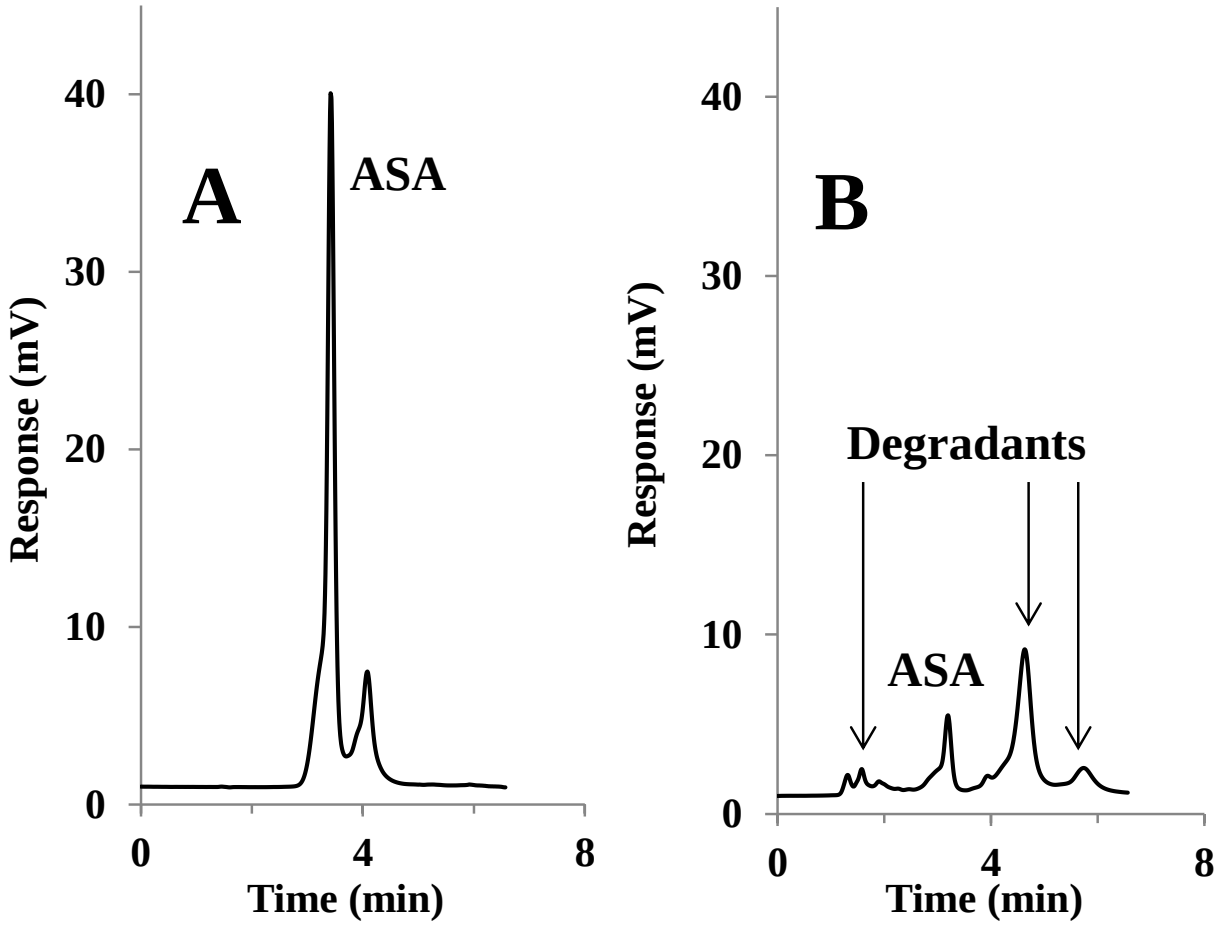

Figure 4.5: HPLC chromatograms of A) an unheated ASA standard and B) a µPLE extract of an ASA tablet after 30 seconds at 250 °C in the stainless steel extraction vessel. Methanol is the solvent used in both cases.

Since degradation of ASA was evident in these results, shorter extraction times were subsequently pursued at a moderately elevated temperature of 150 °C in efforts to avoid this. As shown in Figure 4.6 this approach was found to be successful with 99 ± 5% of the ASA being extracted after just 10 seconds under these conditions. Incidentally, slightly longer extractions of 20 seconds yielded lower recoveries on average, potentially once again indicating the onset of degradation at these times.

Therefore, rapid and quantitative extractions of such thermally labile analytes with the µPLE system can be possible when temperatures are optimized to fully recover the analyte before degradation initiates. Incidentally, in contrast to the ASA findings, it should be noted that acetaminophen was found to be very stable during 10 second µPLE extractions tested up to 200 ºC with no degradation observed. An example of the method validation parameters determined for the analysis of ASA and acetaminophen in this study is presented in Table 4.1. Overall then, these results suggest that µPLE may be potentially useful to explore in these and perhaps other API analyses.

4.4 Green Tea Leaves

Since early work showed that temperatures above 120 ºC often enhanced µPLE extractions, the stainless steel vessels were used exclusively for the rest of the study due to their much wider operating range. Green tea leaf extractions were next examined since they provided a relatively heterogeneous, uncompressed model sample matrix for further exploring the extraction characteristics of the µPLE system. Among the numerous potential extractives of interest in green tea, caffeine is one component that has been widely isolated and analyzed using various techniques.[146,147] As such, the extraction of caffeine by µPLE was examined.

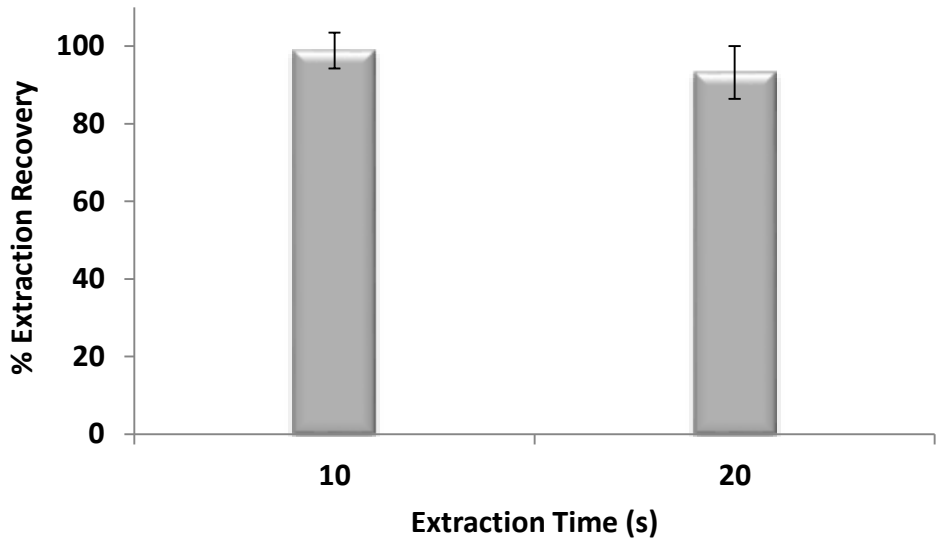

Figure 4.6: µPLE recoveries of ASA from tablets at 150 °C for 10 and 20 seconds extraction times. Replicate extractions (n=4) were performed using the stainless steel extraction vessel and methanol as a solvent.

Table 4.1: Validation results for the determination of acetaminophen and ASA in tablets.

Parameter	ASA	Acetaminophen
Linear dynamic range[a]	2.1 - 1×10^5	0.87 - 4×10^3
Square of correlation coefficient (R^2)	0.9996	0.9956
Limit of detection[a,b]	0.65	0.27
Limit of quantification[a,c]	2.1	0.87

a. µg /mL; methanol solvent
b. calculated as 3 x standard deviation of the blank/the slope of the calibration curve
c. calculated as 10 x standard deviation of the blank/the slope of the calibration curve

Using several combinations of extraction time and temperature, it was again found, as can be seen in Figure 4.7, that efficiency increased with both of these parameters. However, since caffeine is present in small quantities (e.g. 2-4% wt/wt) and is strongly bound to the matrix, temperatures well beyond the conventional PLE temperature of 150 ^{o}C were found to be much more beneficial to analyte recovery. For example, even 30 second µPLE extractions at 150 ^{o}C could not quantitatively recover caffeine from samples. Conversely, at a µPLE temperature of 275 °C, it was found that 100 ± 20% of the caffeine present was extracted from green tea after only 20 seconds.

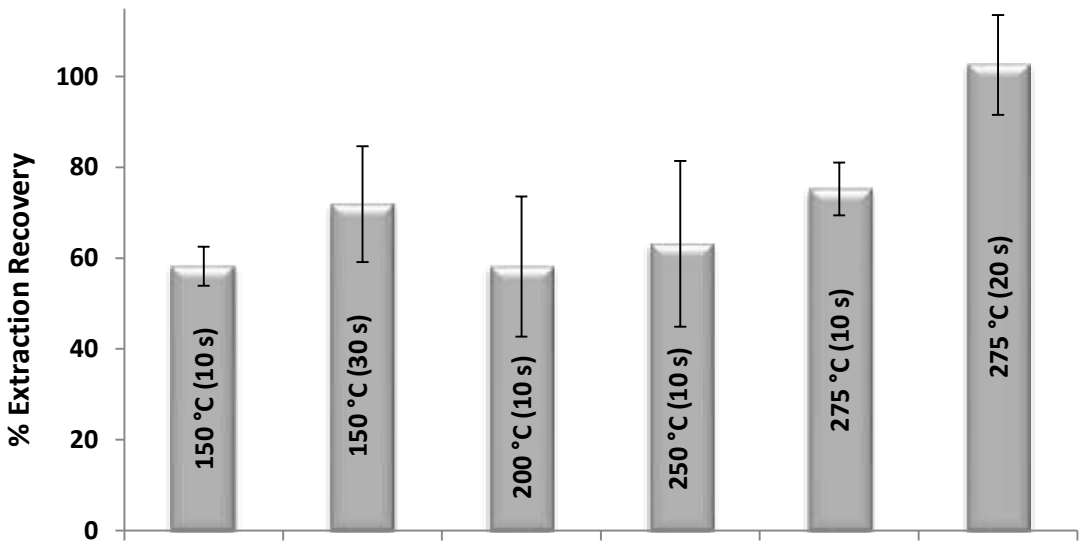

Figure 4.7: µPLE recoveries of caffeine from green tea leaves at various combinations of extraction time and temperature. Replicate extractions (n=4) were performed using the stainless steel extraction vessel and methanol as a solvent.

This finding is quite significant since such green tea extractions by conventional PLE and ultrasonic methods often require 30 to 50 minutes of extraction time per

sample.[146,147] Therefore, although such µPLE temperatures are relatively high compared to conventional PLE, they can potentially provide very rapid extractions for difficult samples that take longer at lower settings. This can also be partly attributed to the rapid heating profile of the extraction vessel as shown in Figure 4.1.

4.5 Comparisons with Conventional PLE

In order to properly evaluate these µPLE results, it was interesting and necessary to directly compare them with conventional PLE extractions performed on the same samples. For this, 5 minute conventional PLE extractions were performed on each sample using the same solvent at the maximum system operating temperature of 150 ºC. The extracts were then similarly analyzed for their content. The results are presented in Table 4.2 as the relative extraction recovery values obtained for the samples using both µPLE and conventional PLE.

Table 4.2: Comparison of µPLE[a] and conventional PLE[b] extraction % recoveries.

Analyte	Matrix	µPLE	PLE
Acetaminophen	Regular Tablets	97±4	90±5
ASA	Delayed-Release Tablets	99±5	108±1
Caffeine	Green Tea Leaves	100±20	90±17

a. 10 seconds at 150 ºC, except green tea for 20 seconds at 275 ºC; n=4
b. 5 minutes at 150 ºC, except ASA for 5 minutes at 120 ºC; n=4

Initially, it was found that conventional PLE extractions at 150 °C for 5 minutes resulted in degradation of ASA and provided recovery values near 83%. Shorter extraction times at this temperature also caused degradation, while 5 to 10 minute extractions at 100 °C were not quantitative. Ultimately, a 5 minute extraction at 120 ºC did not produce any degradation and 108 ± 1% of the ASA was recovered. The results of all ASA PLE experiments are shown in Figure 4.8.

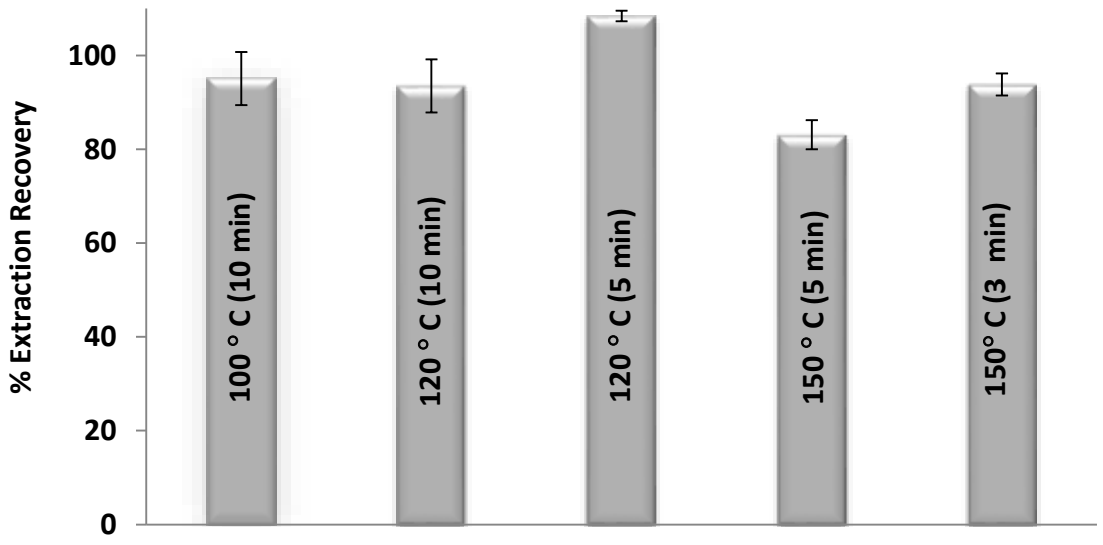

Figure 4.8: Conventional PLE recoveries of ASA from tablets at various combinations of extraction time and temperature using methanol as a solvent. Replicate extractions (n=3) were performed using the conventional PLE system described in Chapter Two.

By comparison, acetaminophen showed no signs of degradation and 90 ± 5% of the analyte was recovered after 5 minutes at 150 ºC. Under these same conditions, conventional PLE recovered 90 ± 17% of the caffeine from green tea leaves. Thus, it can be seen from

the data that the µPLE results compare well to those of the conventional PLE trials of the same samples. For instance, both can quantitatively recover ASA from Delayed-Release tablets under the conditions used. For the acetaminophen and green tea samples, however, 5 minutes at 150 ^{o}C was insufficient for conventional PLE to fully extract all of the analyte. As such, longer and/or higher temperature extractions are likely required. By comparison, µPLE could achieve quantitative results for these samples in 10 to 20 seconds.

It should be noted that the error associated with the green tea determination is relatively large. This is attributed to the greater heterogeneity of the green tea matrix compared to the pharmaceutical tablets, which is further supported by the fact that the conventional PLE results for this sample also had a comparably large error. Incidentally, both methods also extracted very similar components between them as evidenced by their chromatographic profiles. These profiles are shown in Figure 4.9. Therefore, the µPLE and conventional PLE results for green tea are quite similar.

However, this raises an important issue that should be noted about miniaturization in general with regards to sampling error. For instance, the efficiency gains observed for µPLE can be directly attributed to the small sample size that is rapidly extracted. At the same time though, it must be recognized that fundamental sampling error increases as sample size is reduced, and this effect is most pronounced for heterogeneous matrices. Therefore, µPLE may be inherently not well suited for certain applications where a large sample size is required in order to keep the sampling error low.

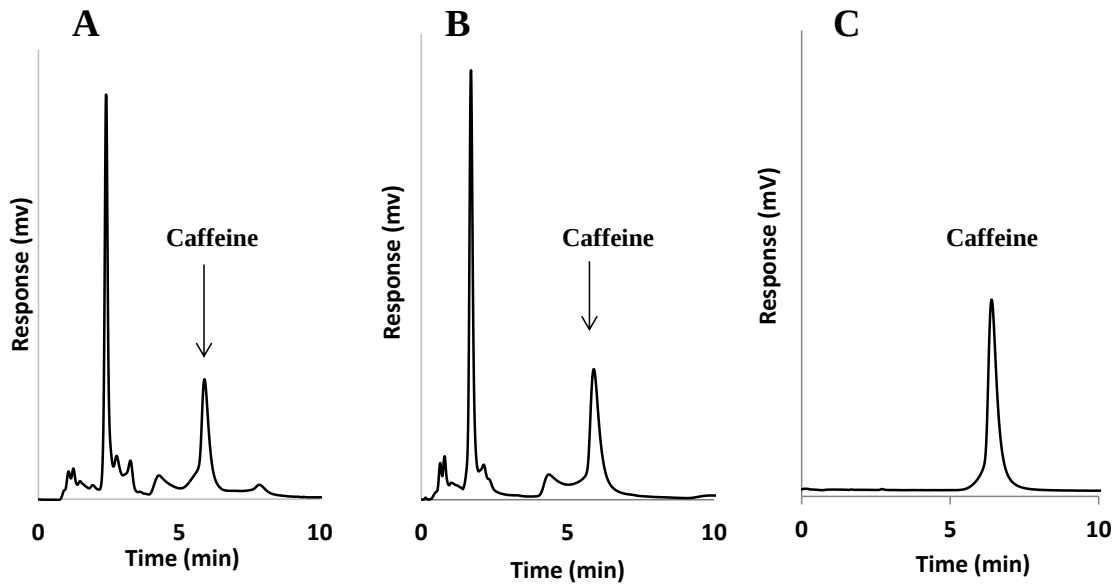

Figure 4.9: HPLC chromatographic profiles of HPLC green tea leaf extracts obtained by (A) µPLE after 30 seconds using the stainless steel vessel and (B) conventional PLE after 5 minutes. Extraction conditions were 150 °C using methanol as the extraction solvent. C is an HPLC chromatogram of a standard caffeine solution.

However, for many scenarios where analyte levels and matrix heterogeneity can support such a smaller sample size, µPLE could offer significant advantages in terms of time and solvent usage relative to conventional methods.

Conversely, Table 4.3 compares the extraction parameters used in each method for these experiments. As can be seen, the µPLE trials were relatively much faster with quantitative results being obtained after 10 to 20 seconds of extraction. By comparison, 5 minute conventional PLE extractions did not produce quantitative results for all of the

samples. In terms of solvent usage, the µPLE method uses only a minor fraction of that required by conventional PLE. Further, the waste solvent remaining after analysis is much larger in conventional PLE than in µPLE, since the small sample size normally employed in the latter requires inherently lower extraction solvent volumes that are fully consumed in the subsequent chromatography injections.

Table 4.3: Comparison of µPLE and conventional PLE extraction parameters

Method	Extraction Time	Total Time	Solvent	Sample	Waste
µPLE	10-20 s	70-80 s	125 µL	5-10 mg	0 mL
Conventional PLE	5 min	10 min	5-10 mL	400 mg	25 mL

4.6 Conclusions

A novel µPLE method was investigated. The method uses very small quantities of sample and solvent in a static extraction mode to remove analytes in as little as 10 to 20 seconds. This approach is also environmentally friendly and uses tremendously lower amounts of organic solvents. The required instrumentation and procedure are relatively simple and readily accessible to most laboratories. The method was explored with different samples and the results compared well to conventional PLE extractions of the same samples. The findings of this research show that µPLE has clear advantages over conventional PLE and other miniaturized PLE methods in terms of reducing the time and the organic solvent requirements for the studied samples. It also compares well with

conventional PLE in terms of the analysis error associated with this technique. Overall, the results indicate that this µPLE approach may be a useful and simple means of rapidly preparing samples for analysis. For instance, it could potentially lend itself well to automation through adapting multi-vial extraction plates to this approach. Alternately, it could also be useful where rapid real-time results are required in areas such as on-line processing. Finally, given its size, it may be further beneficial as a portable method to prepare individual samples *in-situ* in field analyses.

Chapter Five: **APPLICATION OF µPLE TO SOME CHALLENGING MATRICES**

5.1 Introduction

Since µPLE employs very small extraction cells, the method could be potentially well suited to accommodate inherently small and/or precious samples. For instance, some samples may be difficult to obtain, or do not naturally occur in a larger size. Dried blood spot (DBS) analysis is a very interesting area that falls into this category, since only a few drops of blood are normally spotted onto a cellulose-based card and allowed to dry. This relatively minute spot is then excised and analyzed for trace components. In this way, DBS analysis produces both a very small and precious sample for extraction. Another category of sample preparation that µPLE could also be well suited for is extracting polycyclic aromatic hydrocarbons (PAHs) from various solid samples. This is because PAHs can be present in small quantities (ng levels / g sample) and are often very strongly bound to matrices and therefore, the relatively high µPLE operating temperatures could prove beneficial for recovering these analytes. Therefore, it would be interesting to explore the use of µPLE in DBS and PAH analyses in this chapter.

5.2 Application of µPLE to DBS Analysis

Due to its practical and economical advantages over conventional wet blood plasma analysis, DBS analysis has grown rapidly in recent years.[166] For example, DBS analysis is increasingly being used to determine drug levels present in native blood samples[167]. However, the extraction of drug substances from DBS samples is very time consuming and commonly spans one hour or more in length.[167,168] Therefore, it was interesting to examine if µPLE could be useful in preparing such samples. To investigate this, the extraction of

the second generation anti-depressant Fluoxetine Hydrochloride from DBS samples was attempted using this method.

Preliminary experiments were conducted to determine the optimum extraction conditions for Fluoxetine Hydrochloride using DBS cards with only a standard solution spotted on them (i.e. without whole blood present). One of the best solvents for Fluoxetine Hydrochloride is methanol, while water and chloroform are also exceptionally good in this regard. As such, methanol was initially employed as the extraction solvent. However, under all elevated temperatures examined, it was found to remove both the target analyte and a significant portion of the cellulose card matrix. This subsequently created considerable interference in the chromatographic analysis of Fluoxetine Hydrochloride. Further, additions of 25 to 75% water into the methanol also produced similar or worse results. Therefore, methanol and water were not useful for DBS analysis by this method, which is consistent with the cellulose decomposition reported to occur at high temperatures in such solvents.[169] Next, chloroform was used and it was found to fully extract the analyte without removing any significant amount of the cellulose matrix or interfering. Figure 5.1 illustrates this difference for extractions performed in methanol, 50:50 methanol/water, and chloroform. Therefore, given its advantages, chloroform was used as the extraction solvent for this application.

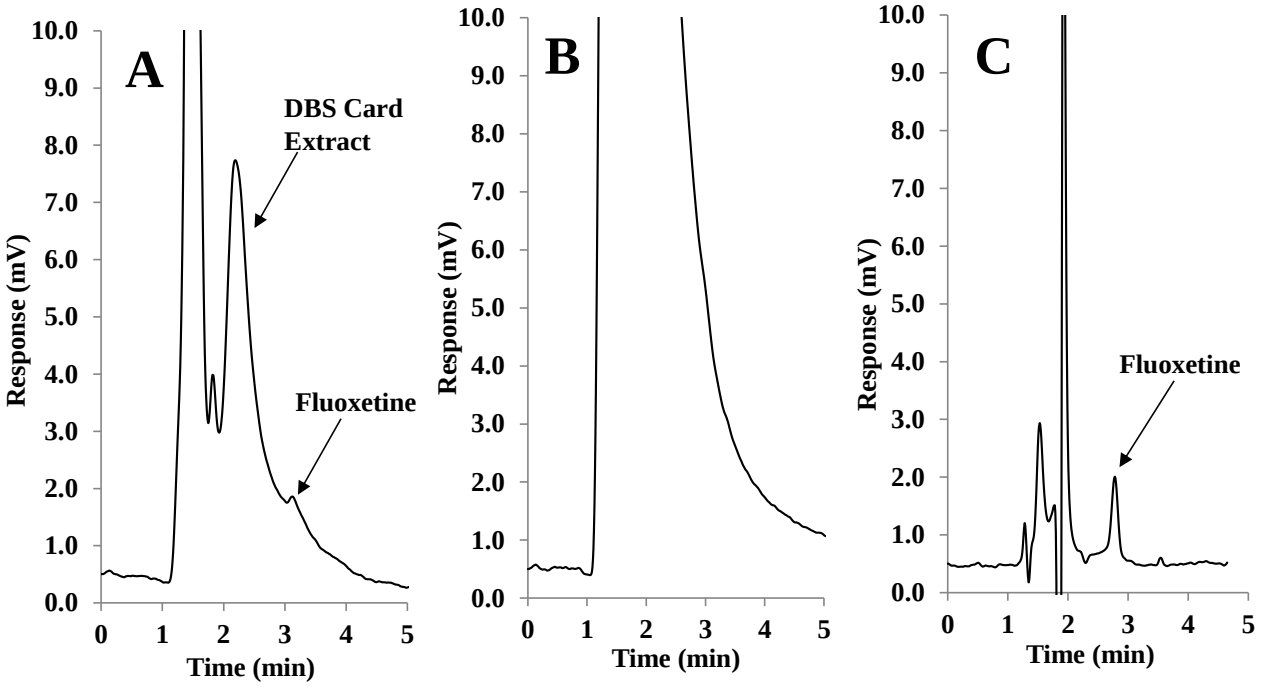

Figure 5.1: HPLC profiles of extracts from DBS cards containing Fluoxetine Hydrochloride (2.4 ng/µL) without blood present. The µPLE solvent and conditions used are A) methanol at 200 ºC for 30 seconds, B) 50:50 methanol/water at 200 ºC for 30 seconds, and C) chloroform at 250 ºC for 20 seconds.

In order to find optimum DBS extraction conditions for Fluoxetine Hydrochloride, various times and temperatures were investigated with and without blood present. The typical results of these trials are shown in Figure 5.2. As can be seen, even without blood present, 30 second extractions at 150 ºC could only recover about 65% of the analyte from the DBS card and similar extractions at 200 ºC were required to fully extract all of the

Fluoxetine Hydrochloride. However, when blood was also present, it introduced significant interference and only about 74% of the analyte could be recovered under the same conditions. In order to circumvent this issue, even higher extraction temperatures were investigated. Ultimately it was determined that only 20 seconds of extraction at 250 °C was adequate to recover 101 ± 7% (n=6) of the Fluoxetine Hydrochloride from the DBS samples. Therefore, as with the certain samples in the previous chapter, only a brief exposure to this unusually high temperature can greatly improve analyte recovery and provide very efficient extractions in µPLE.

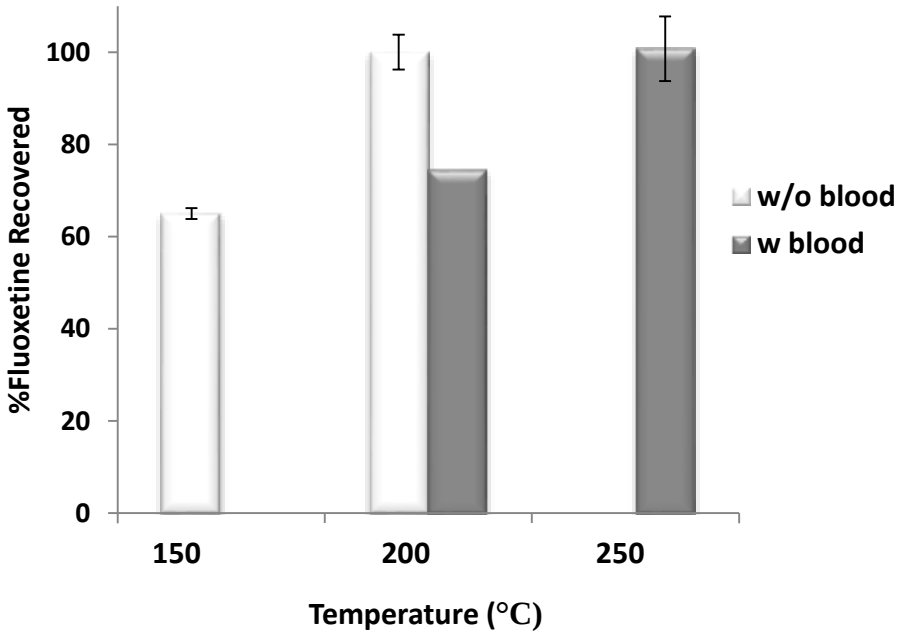

Figure 5.2: Example of optimization results of µPLE extractions of Fluoxetine Hydrochloride from DBS cards as a function of temperature. Samples are both without (white) and with (grey) whole blood present. All extractions are 30 seconds long except 250 °C, which is 20 seconds long. Replicate extractions were performed using the stainless steel extraction vessel and chloroform as a solvent.

This is a very significant finding since conventional DBS preparations can consume up to 1 hour per sample.[167] Additionally, the error associated with this result agrees well with conventional techniques and is within the margin suggested by the U.S. Food and Drug Administration in their guidelines for industry concerning such analyses.[167] Therefore, considering the notable savings in the time required, µPLE may potentially offer a very simple and effective means of rapidly preparing such DBS samples for analysis, which could be further beneficial for obtaining real-time results of blood samples taken during time point analyses.

5.3 Application of µPLE to PAH Extractions

PAHs are ubiquitous carcinogenic pollutants that essentially enter the environment through incomplete combustion of organic material. Sources of PAHs may be natural or anthropogenic. Fossil fuel combustion is a major source of environmental contamination, while on smaller scales activities like food preparation such as smoking and grilling can also contribute to human exposure. Accordingly, the bioaccumulation of PAHs in food from environmental contamination is also a major concern.[170]

Since they are highly hydrophobic and sorb strongly to carbon containing materials,[171] this toxic nature of PAHs has prompted monitoring and regulation of their distribution.[172] Many studies concerning the extraction of PAHs from diverse matrices have been conducted using methods such as Soxhlet, ultrasound-assisted solvent extraction, PLE, and others. Since many of these methods require relatively long extraction times and large amounts of solvent, it would be interesting to know if the developed µPLE technique could facilitate the preparation of such solid samples for analysis.

The next part of this chapter demonstrates the use of μPLE for the extraction of 16 US EPA priority pollutant PAHs from various matrices. These matrices included foodstuffs, sand, soil, and char-containing solids. The chemical structures of the 16 PAHs are shown in Figure 5.3.

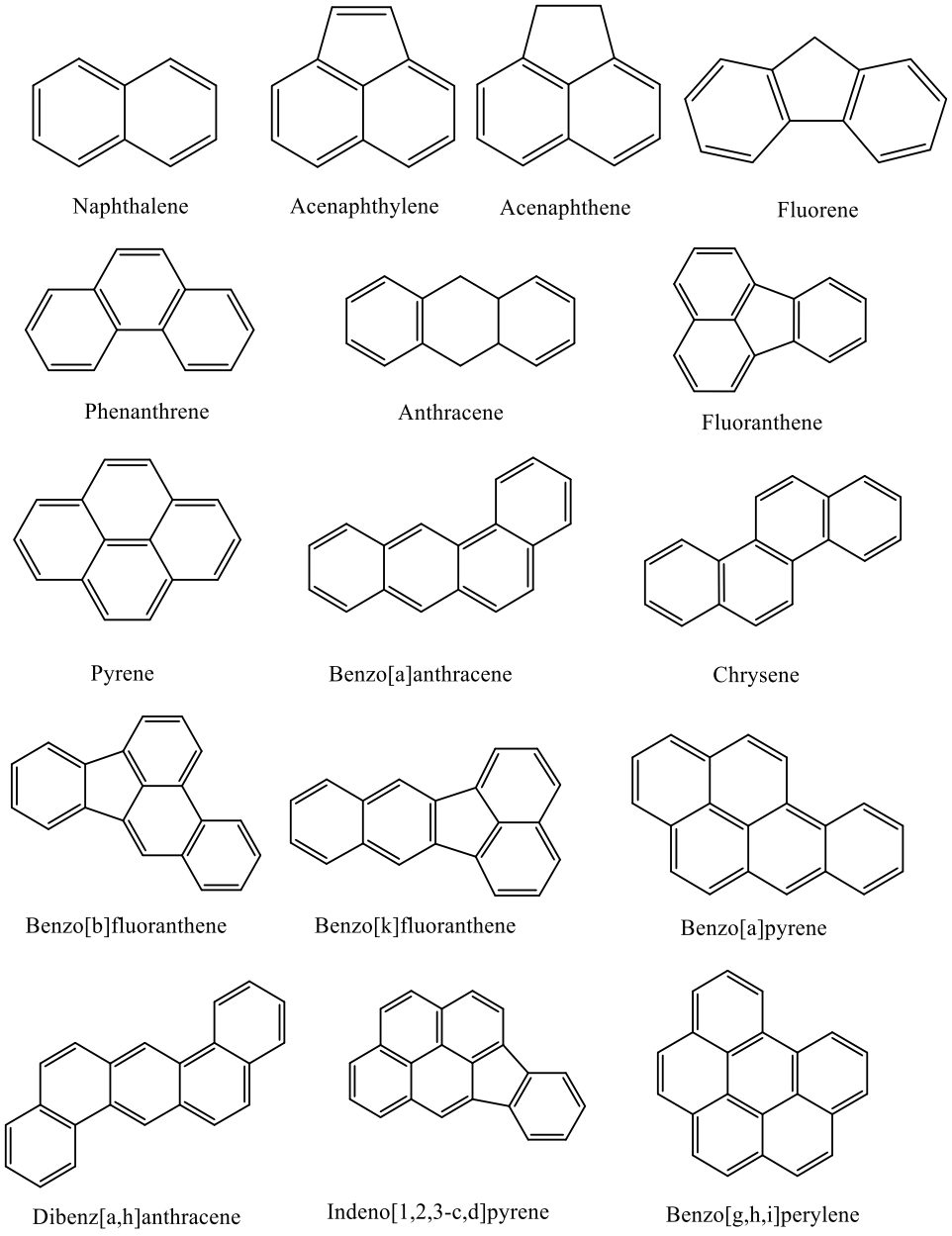

Figure 5.3: The chemical structures of the 16 EPA PAHs used.

5.3.1 Ottawa Sand

In order to test the capability of the μPLE technique to extract PAHs from various solid samples, the extractability of the 16 EPA PAHs from an Ottawa sand matrix was first probed. This matrix was chosen here because it provided a reasonably homogeneous sample to which PAHs can weakly bind. Therefore, examining the extractability of these analytes from such a matrix would indicate whether more challenging matrices should next be pursued or not. Further, extracting PAHs from Ottawa sand using conventional PLE has previously been reported[173] and therefore, it would be interesting if improvements in time and solvent requirements could be attained using our μPLE technique.

Results of these experiments (as presented in Table 5.1) indicated that the extraction of these PAHs from Ottawa sand was quite fast and efficient. For example, quantitative extractions of the 16 PAHs were attained in only about 15 seconds at 200 ^{o}C using 125 μL of 1:1 hexane acetone extraction solvent with 110% average extraction recovery for all the PAHs tested (n=3). These findings are quite significant since such PAH extractions by conventional PLE methods often require about 7 minutes of extraction time and 30 mL of organic solvent per sample.[173]

It is also worth mentioning that the error associated with these μPLE extractions (average RSD of 8%) is similar or even slightly better than the error associated with the previously described conventional PLE procedure (average RSD of 11%).[173] This is probably due to the homogeneous nature of this matrix which can make the error associated with such extractions not as pronounced as other heterogeneous samples.

Table 5.1: Extraction recoveries of 16 PAHs from Ottawa sand by µPLE. Conditions are 200°C for 15 seconds using 1:1 hexane / acetone as an extraction solvent and the stainless steel extraction vessel (n=3).

Analyte	% Extraction Recovery[a]	% RSD
Naphthalene	112	9
Acenapthene	114	6
Acenapthylene	115	5
Fluorene	116	4
Phenanthrene	114	5
Anthracene	113	2
Fluoranthene	110	2
Pyrene	103	4
Benzo[a]anthracene	113	7
Chrysene	112	10
Benzo[b]/[k] fluoranthene	114	12
Benzo[a]pyrene	104	11
Indeno[1,2,3-c,d]pyrene	97	11
Dibenz[a,h]anthracene	104	15
Benzo[g,h,i]perylene	100	14
Average	110	8

a: Extraction blanks were also prepared and their analysis did not show any measurable contamination.

5.3.2 Soil

As stated earlier, PAHs are highly dangerous to human health and to the environment. Therefore, the analysis of PAH levels in soil samples is of concern. Based on the promising µPLE results obtained from the Ottawa sand samples, it was interesting to

examine the extractability of the 16 PAHs from soil samples. Soil samples are particularly challenging because PAHs are lipophilic compounds that strongly bind to humic substances in such matrices. As such, extracting PAHs from soil samples require powerful extraction techniques that are normally lengthy and solvent intensive (e.g. Soxhlet method). [174] Therefore, it would be of a great importance to examine if such extractions are possible using our very rapid μPLE technique.

In order to test the extractability of these PAHs from soil samples, the optimum Ottawa sand extraction parameters (200 °C using 1:1 hexane / acetone as an extraction solvent) were first examined for 1 minute extraction time. Results of this experiment showed that these conditions could not quantitatively recover these PAHs from soil samples. For instance, the mean extraction recovery of the high molecular weight PAHs (Benzo[a]anthracene-Benzo[g,h,i]perylene) under these conditions was found to be only 65%. Therefore, increasing the extraction temperature to 250 °C was next probed. The results of this experiment did not show significant improvements in the PAH extraction recoveries and as a result longer extraction times of 2, 3, and 4 minutes were next probed. Once again, longer extraction times did not significantly improve the extraction recoveries of these APIs and therefore, changing the extraction solvent was next attempted. Changing the extraction solvent from 1:1 hexane / acetone to toluene had the most pronounced effect and resulted in quantitative extractions of all of the PAHs from the spiked soil samples. For instance, as can be seen in Table 5.2, quantitative extractions of the 16 PAHs were attained in only about 30 seconds at 200 ^{o}C using 125 μL of toluene as an extraction solvent with 106% average extraction recovery of all the PAHs tested (n=3).

Table 5.2: Extraction recoveries of 16 PAHs from soil by µPLE. Conditions are 200°C, 30 seconds using toluene as an extraction solvent and the stainless steel extraction vessel (n=3).

Analyte	% Extraction Recovery[a]	% RSD
Naphthalene	92	8
Acenapthene	90	3
Acenapthylene	93	4
Fluorene	92	4
Phenanthrene	92	6
Anthracene	95	6
Fluoranthene	93	6
Pyrene	109	20
Benzo[a]anthracene	113	7
Chrysene	113	11
Benzo[b]/[k] fluoranthene	119	9
Benzo[a]pyrene	116	9
Indeno[1,2,3-c,d]pyrene	130*	-
Dibenz[a,h]anthracene	122*	-
Benzo[g,h,i]perylene	127	12
Average	106	8

* These recoveries were calculated based on a single trial due to chromatographic interference in the other trials.
a: Extraction blanks were also prepared and their analysis did not show any measurable contamination.

These findings are also very significant since such PAH extractions by other miniaturized PLE and SWE methods require about 10-20 minutes of static and dynamic extraction time per sample.[173] Therefore, using µPLE for extracting PAHs from soil

samples provides a very rapid alternative sample preparation technique that can quantitatively recover target analytes from such samples in only seconds.

5.3.3 Biochar-Amended Soil

Biochar is the charred product of biomass pyrolysis, which is widely applied to soils to modulate porosity, water retention, and other factors to improve soil fertility.[175] Recently, it was reported that soil sorption affinity to PAHs significantly increases when small proportions of biochar are present within soil samples.[176] Since μPLE has shown very promising extraction features, it was therefore interesting to examine the extraction characteristics of the 16 PAHs from biochar-amended soil as another challenging matrix.

In order to test this, the optimum soil extraction parameters (200 °C using toluene as an extraction solvent for 30 seconds) were first examined. Results of this experiment, as can be seen in Table 5.3, showed that quantitative extractions of 7 low molecular weight PAHs (Naphthalene-Pyrene) and less than quantitative recoveries for the higher molecular weight PAHs were attained. Therefore, in order to improve the extraction recoveries of these high molecular weight PAHs (Benzo[a]anthracene-Benzo[g,h,i]perylene), various approaches were next pursued. The first approach attempted was increasing the extraction time to 1 minute. However, results of this experiment did not show major improvements in these PAH recoveries. Next, since running multiple extraction cycles on the same sample is normally useful in conventional PLE,[2,20] this approach was next pursued. This was done by running three 30 second extraction cycles on each sample using 125 μL of toluene in each run. Again, however, results of this experiment did not show significant improvements in extraction recoveries of these PAHs. As a result, extracting PAHs from

biochar-amended soil proved to be more challenging than extracting them from soil samples alone (section 5.3.2 above). This can mainly attributed to the very strong sorption of PAHs to biochar compared with soil.[176]

Table 5.3: Extraction recoveries of 16 PAHs from biochar-amended soil by µPLE. Conditions are 200°C, 30 seconds using toluene as an extraction solvent and the stainless steel extraction vessel (n=3).

Analyte	% Extraction Recovery[a]	% RSD
Naphthalene	102	5
Acenapthene	104	3
Acenapthylene	106	6
Fluorene	101	7
Phenanthrene	101	4
Anthracene	97	4
Fluoranthene	92	3
Pyrene	98	8
Benzo[a]anthracene	77	3
Chrysene	79	2
Benzo[b]/[k] fluoranthene	76	6
Benzo[a]pyrene	73	10
Indeno[1,2,3-c,d]pyrene	51	8
Dibenz[a,h]anthracene	53	10
Benzo[g,h,i]perylene	53	5
Average	84	6

a: Extraction blanks were also prepared and their analysis did not show any measurable contamination.

Although quantitative extractions of all of the 16 PAHs from biochar-amended soil samples were not attained, these findings are still significant. This is because conventional methods for extracting these PAHs from such samples are also not quantitative but are very time consuming. For instance, a previously reported Soxhlet method showed that only 6 PAHs from the these PAHs can be quantitatively recovered after 36 hours of extraction time using 160 mL of organic solvent.[176]

5.3.4 Biochar

As stated earlier, biochar is increasingly promoted as a beneficial soil conditioner. However, it may contain residues of PAHs as a result of its production by pyrolysis. Currently, conventional Soxhlet and PLE methods to extract PAHs from biochar need prolonged extraction times and intensive amounts of organic solvents (e.g. 36 hours and 200 mL per sample).[175] Therefore, it would be important to examine if μPLE can recover PAHs from biochar samples.

In order to test this, the extraction recovery of the 16 PAHs from spiked biochar samples was tested using μPLE. Results of this experiment (Table 5.4) showed low extraction recoveries for these PAHs, especially the high molecular weight analytes. For example, the average extraction recovery for the 16 PAHs was found to be only 54 % after 1 minute extraction at 200 °C. These results are still significant though since conventional PLE methods for extracting PAHs from such samples are normally entirely ineffective.[175] For instance, a previously reported PLE method showed that none of the PAHs that are heavier than chrysene can be recovered from biochar samples. Further, the extraction recoveries obtained by the μPLE method are similar to those obtained by the conventional

Soxhlet method for similar samples although the extraction times and the solvent requirements for the Soxhlet method are significantly larger (300 mL and 36 h).

Table 5.4: Extraction recoveries of 16 PAHs from biochar by µPLE. Conditions are 200°C, 60 seconds using toluene as an extraction solvent and the stainless steel extraction vessel (n=3).

Analyte	% Extraction Recoverya	% RSD
Naphthalene	61	7
Acenapthene	60	13
Acenapthylene	66	11
Fluorene	66	18
Phenanthrene	65	18
Anthracene	65	14
Fluoranthene	59	17
Pyrene	58	20
Benzo[a]anthracene	55	17
Chrysene	58	14
Benzo[b]/[k] fluoranthene	47	17
Benzo[a]pyrene	45	15
Indeno[1,2,3-c,d]pyrene	33	25
Dibenz[a,h]anthracene	35	17
Benzo[g,h,i]perylene	38	28
Average	54	17

a: Extraction blanks were also prepared and their analysis did not show any measurable contamination.

5.3.5 Food

Determination of PAHs in food is particularly important since these compounds are highly toxic/carcinogenic and they can enter the food via contaminated soil, polluted air, polluted water, and thermal treatment of the food. As such, accurate determinations of these PAHs in food are routinely required by regulatory agencies. Such determinations normally involve time and solvent consuming sample preparation procedures such as Soxhlet and sonication. Therefore, it will be not only interesting but also important to examine if PAHs can be recovered from such samples using our rapid µPLE technique. In this regard two food samples were investigated. The first one was chicken breast as a low-fat meat model, and the second one was charred bread.

Based on the soil extraction results it was clear that toluene is a better extraction solvent than 1:1 hexane / acetone. Therefore, in order to attain the optimum extraction results, toluene was chosen as the extraction solvent for the chicken breast samples. The samples used in this experiment are the outer smoked portions of a meat sample that was spiked with the 16 EPA PAHs as previously described in Chapter Two. Results of this experiment (as seen in Table 5.5) showed that the extraction of these PAHs from chicken breast samples was very rapid and efficient. For instance, the 16 PAHs were quantitatively recovered with a mean extraction recovery of 101% for all the PAHs tested (n=3) in just 30 seconds at 200 ^{o}C using 125 µL of toluene as an extraction solvent.

The findings of this experiment are substantial since such PAH extractions from meat samples are normally very lengthy and require vast amounts of organic solvents. For example, conventional Soxhlet and ultrasonic methods often require 0.5 to 3 hours of extraction time and 20 to 200 mL of organic solvent per sample.[177] Therefore, µPLE shows

again that it can potentially provide significantly faster and much less solvent intensive extractions than conventional extraction techniques for difficult samples.

Table 5.5: Extraction recoveries of 16 PAHs from Chicken Breast by µPLE. Conditions are 200°C, 30 seconds using toluene as an extraction solvent and the stainless steel extraction vessel (n=3).

Analyte	% Extraction Recovery[a]	% RSD
Naphthalene	114	9
Acenapthene	103	6
Acenapthylene	97	5
Fluorene	96	4
Phenanthrene	93	5
Anthracene	104	2
Fluoranthene	91	2
Pyrene	88	4
Benzo[a]anthracene	93	7
Chrysene	94	10
Benzo[b]/[k] fluoranthene	100	12
Benzo[a]pyrene	110	11
Indeno[1,2,3-c,d]pyrene	108	11
Dibenz[a,h]anthracene	110	15
Benzo[g,h,i]perylene	107	14
Average	101	8

a: Extraction blanks were also prepared and their analysis did not show any measurable contamination.

In order to further investigate the capability of μPLE to extract PAHs from food samples, charred toasted bread was next probed. The samples used in this experiment are obtained from the charred surface of a slice of toasted bread and spiked with the 16 EPA PAHs as previously described in Chapter Two. Results of this experiment (as seen in Table 5.6) indicated that the extraction of these PAHs from charred toasted bread was again quite fast and efficient. For example, an average extraction recovery for the 16 PAHs tested of about 84% was obtained in only 30 seconds at 200 ºC using 125 μL of toluene as an extraction solvent (n=3). Although these recoveries are not quantitative, they are still higher than the recoveries obtained by conventional ultrasound-assisted and Soxhlet methods. For instance, a previously reported ultrasound-assisted method for extracting PAHs from charred bread requires 30 minutes and 30 mL of hexane per sample to recover only 64% average extraction recovery of the high molecular weight PAHs (Benzo[a]anthracene-Benzo[g,h,i]perylene).[178] Therefore, μPLE does not only offer tremendous saving in time and solvent requirements over conventional methods, but also higher extraction recoveries as well.

Table 5.6: Extraction recoveries of 16 PAHs from charred toasted bread by µPLE. Conditions are 200°C, 30 seconds using toluene as an extraction solvent using the stainless steel as an extraction vessel (n=3).

Analyte	% Extraction Recovery[a]	% RSD
Naphthalene	93	6
Acenapthene	87	4
Acenapthylene	92	1
Fluorene	88	3
Phenanthrene	87	2
Anthracene	87	3
Fluoranthene	82	2
Pyrene	83	5
Benzo[a]anthracene	81	7
Chrysene	90	5
Benzo[b]/[k] fluoranthene	78	4
Benzo[a]pyrene	78	3
Indeno[1,2,3-c,d]pyrene	80	3
Dibenz[a,h]anthracene	79	7
Benzo[g,h,i]perylene	82	3
Average	84	5

a: Extraction blanks were also prepared and their analysis did not show any measurable contamination.

5.4 Conclusions

The use of µPLE in two important applications was demonstrated. The method was found to quantitatively extract Fluoxetine Hydrochloride from DBS cards in

only a fraction of the time required conventionally for such samples. It also showed tremendous saving in time and solvent requirements for extracting PAHs from various solid samples relative to current conventional sample preparation techniques. For example, while μPLE requires only seconds to extract PAHs from solid samples, the other conventional techniques require hours and sometimes days to recover these analytes from such matrices. The proposed methodology allowed quantitative recoveries of the 16 EPA PAHs from several solid matrices and accurate determination of the target analytes. Further, the μPLE system described in this chapter provides a low cost technique with minimal instrumental and operational costs. It is easily adaptable to the demands of different analyses and has potential for on-line coupling to other techniques. Additionally, the method has proven to be fairly robust and we have used it for over a year without any major problems.

Chapter Six: **μPLE COMBINED WITH ULTRA-SHORT GC COLUMN ANALYSIS FOR MONITORING ASA TABLET DEGRADATION**

6.1 Introduction

Capillary GC, which was first proposed about 60 years ago by Golay, is currently the main practical method in analytical GC.[178] Accordingly, the main focus of activity in this area often involves the use of long capillary columns (e.g. 10 to 150 m). This is because such long columns demonstrate very high efficiency and resolution for complex samples containing a large number of compounds.[179-181] However, this high efficiency is not always necessary for samples of lower complexity or situations where the resolution of all analytes is not critical. For example, fast GC uses short columns to elute compounds quickly in cases where high efficiency is unnecessary and speed can offset the lower resolving power.

Interestingly, it has also been observed that the elution temperature of analytes can be significantly lowered when reducing the column length.[182,183] Lowering analyte elution temperature in GC is advantageous since it can greatly increase the range of analytes that can be determined. For instance, many non-volatile and/or thermally labile pharmaceuticals cannot be analyzed by conventional GC methods, or require derivatization by lengthy procedures in order to do so.[121,130,134] In this way, a short column GC approach can potentially facilitate this. Recently, Hayward[184] explored the use of a novel ultra-short GC column method to analyze various molecules. The method used cold on-column injection into ultra-short (10 cm) capillary GC columns followed by rapid temperature programming through direct resistive heating. Then, on-column detection was also achieved using a micro flame ionization detector (μFID) that was previously

developed.[149,150,185] Benefits of using this method included the ability to perform unconventional GC separations in a rapid manner. For example, using this technique he was able to very rapidly (less than 4 minutes) analyze the polypeptide Gramicidin which has a molecular weight of 1900 g/mole and is normally impossible to be analyzed by conventional GC. Given these attributes, it was interesting to explore here if such an approach could be combined with the very rapid μPLE technique of earlier chapters and used to monitor pharmaceutical degradation as an extended application of this method. Such analyses are critically important in the pharmaceutical industry since many drugs degrade over time under different combinations of temperature and humidity.[186,187] As a result, since such degradants can pose toxicity issues, manufacturers are required to regularly monitor for product degradation as part of the quality control and drug development process. [186,187] In both cases, since degradants can have very different properties from the parent drug, universal detection is often desired since it can determine the presence and quantity of the compounds involved regardless of their structure. [186,187] In this way, UV-vis methods can be problematic due to their reliance on chromophores, while a universal, carbon-sensing FID would be optimal if GC were possible to perform with the molecules involved. [186,187] While ultra-short columns might be able to facilitate this, to the best of our knowledge they have never previously been attempted to use for monitoring drug degradation. Further, μPLE has not been explored for its ability to rapidly extract such APIs and degradants from pharmaceutical tablets. Given the speed advantages of both, it would be therefore interesting to see if μPLE and ultra-short GC column analyses could combine to yield a powerful tool for such monitoring in the pharmaceutical industry.

In this chapter, the potential benefits of using an ultra-short GC-FID column apparatus to analyze and track degradation products of a model pharmaceutical are presented. Thermally degraded standard solutions of ASA are used for this purpose. Further, to better illustrate the performance attributes of this ultra-short GC column approach in pharmaceutical applications, the method is directly compared with conventional HPLC analysis of the same. Finally, using ASA tablets as a model pharmaceutical formulation, the potential benefits of combining this technique with μPLE in the context of using it as a rapid monitoring tool to rapidly extract and analyze ASA tablet degradation products are also discussed.

6.2 Operating Characteristics of the Ultra-Short GC Column Apparatus

Initial efforts focused on examining the operating characteristics of the assembled ultra-short GC-FID column apparatus in order to better evaluate its performance. For this, a series of alkane standards (i.e. C_{12} and larger) were investigated since they are commonly analyzed in conventional GC and should be readily detected by the on-column μFID. In general, it was found that the injection solvent instantly eluted from the system at room temperature, while low volatility analytes were often fully retained until temperature programming began.

Figure 6.1 illustrates the typical properties of the apparatus with an alkane separation on the 20 cm ultra-short capillary GC column. As can be seen, the on-column μFID yields stable response and produces a steady baseline during operation. Additionally, the acetone solvent appears quickly upon injection, while the alkanes start to elute very soon after temperature programming begins 70 seconds later. Under these conditions, the

analyte retention times are found to be quite reproducible with an average RSD value of 2.4 %. In terms of efficiency, the 20 cm ultra-short GC column provides around 2000 plates and a resolution of 1.6 between C_{16} and C_{18}. Thus, while this is about two orders of magnitude lower than conventional capillary GC columns, it can still be potentially useful for relatively simple mixtures. Most notably, however, Figure 6.1 demonstrates that the speed of separation on the ultra-short GC column is remarkably fast. For instance, the separation of these larger alkanes is complete in 3 minutes, whereas C_{18} alone can often require up to an hour to elute from conventional 60 m long GC capillary columns due to its relatively high boiling point of 317 ºC.

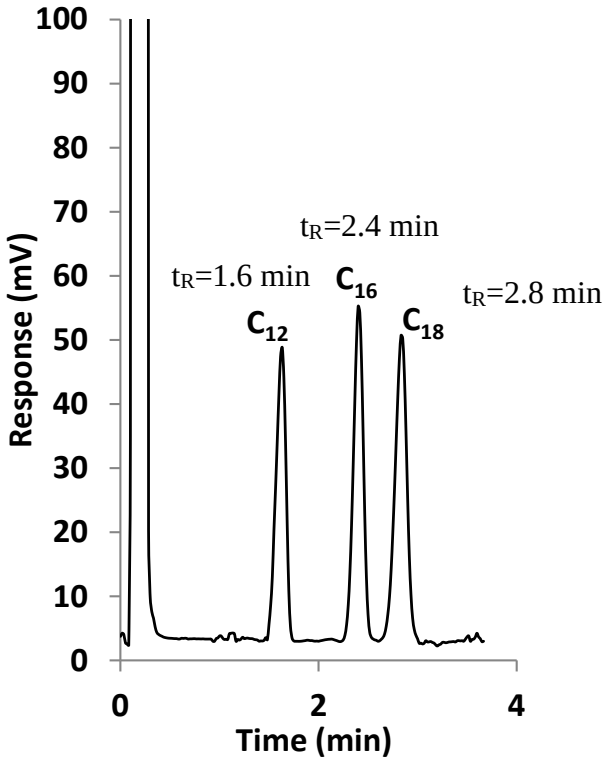

Figure 6.1: An ultra-short GC column separation of C_{12}, C_{16}, and C_{18} alkanes in acetone (about 1 µg each). The temperature program is 25 °C for 70 seconds, then 90 °C/min to 180 °C. A 20 cm EC-5 column (2.65 µm thick film) is used.

6.3 Analysis of ASA Using the Ultra-Short GC-FID Column Setup

With the system confirmed to be operating well, investigations next focused on the feasibility of analyzing standards solutions of ASA using the ultra-short column GC setup. ASA is a common anti-inflammatory drug that is highly thermally labile and rapidly decomposes near its boiling point of 140 ºC.[188,189] As a result, like many other pharmaceutical agents, it cannot be directly analyzed by conventional GC since it is destroyed when subjected to the high temperature conditions of the injector and oven. [129] Accordingly, ASA must be derivatized by lengthy procedures in order to analyze it by conventional GC, [133,134,186,190] and is normally analyzed by HPLC instead. [109] Therefore, it was challenging to examine here with the ultra-short GC column setup.

Figure 6.2 shows the typical result of this experiment, where it can be seen that a prominent ASA peak is readily observed in the system about 1 minute after the temperature program is initiated. Further interesting is that ASA emerges at a column temperature of about 100 ºC. Therefore, the ultra-short GC column method used here can facilitate the rapid and direct analysis of ASA. As such, it could potentially also be useful for other highly thermally labile pharmaceuticals as well.

6.4 Application of Ultra-Short Column GC to Pharmaceutical Degradant Analysis

Given the rapid analysis result for ASA using the ultra-short column setup here, it was next interesting to examine the system's ability to monitor this analyte and its degradation products simultaneously. ASA is known to thermally degrade to salicylic acid (SA), which can then further degrade into phenol at higher temperatures.[102,109] Thus, the separation of a standard mixture of ASA, SA, and phenol was first probed in the system.

The typical result is displayed in Figure 6.3, which shows that the compounds are readily separated in about 3 minutes using the ultra-short GC column setup. It is further interesting to note that phenol elutes shortly after the solvent at room temperature before heating the column, even though this analyte has a boiling point in excess of 100 ^{o}C. This demonstrates how the ultra-short GC column setup can facilitate the elution of high boiling point compounds at significantly lower temperatures than conventional GC methods. Thus, the method can readily separate ASA and its degradants.

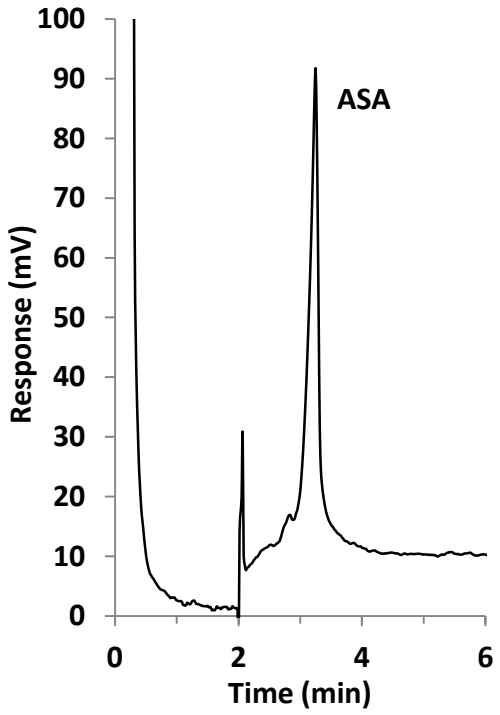

Figure 6.2:Ultra-short GC chromatogram of 2 µg injection of ASA in methanol using a 20 cm EC-5 column (2.65 µm thick film). The temperature program is 25 °C for 2 minutes then 90 °C/min to 180 °C. A minor detector spike/offset is noted when column heating begins.

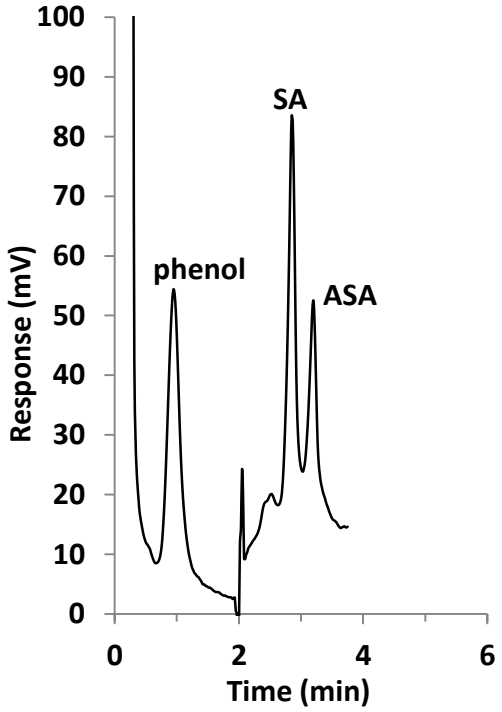

Figure 6.3: Ultra-short GC column separation of 1 µg each of ASA and its primary degradants, phenol and SA in 80:20 methanol/water under the same conditions as those in Figure 6.2. A minor detector spike/offset is noted when column heating begins.

6.4.1 Monitoring ASA Decomposition Products During Controlled Degradation

Next, in order to investigate the system's ability to monitor degradant formation, it was applied to the analysis of ASA standards under conditions of controlled degradation. For this, ASA standard solutions were thermally degraded for 10 minutes each at various temperatures ranging from 25 to 250 °C and the resulting mixtures were analyzed using both the ultra-short GC column setup and conventional HPLC analysis for comparison. The results are displayed in Figures 6.4. As can be seen, both methods produce very similar profiles and are comparably able to track the degradation of this analyte. For instance, both

ultra-short column GC and HPLC indicate that about half of the ASA present is degraded into salicylic acid at temperatures near 150 ºC.

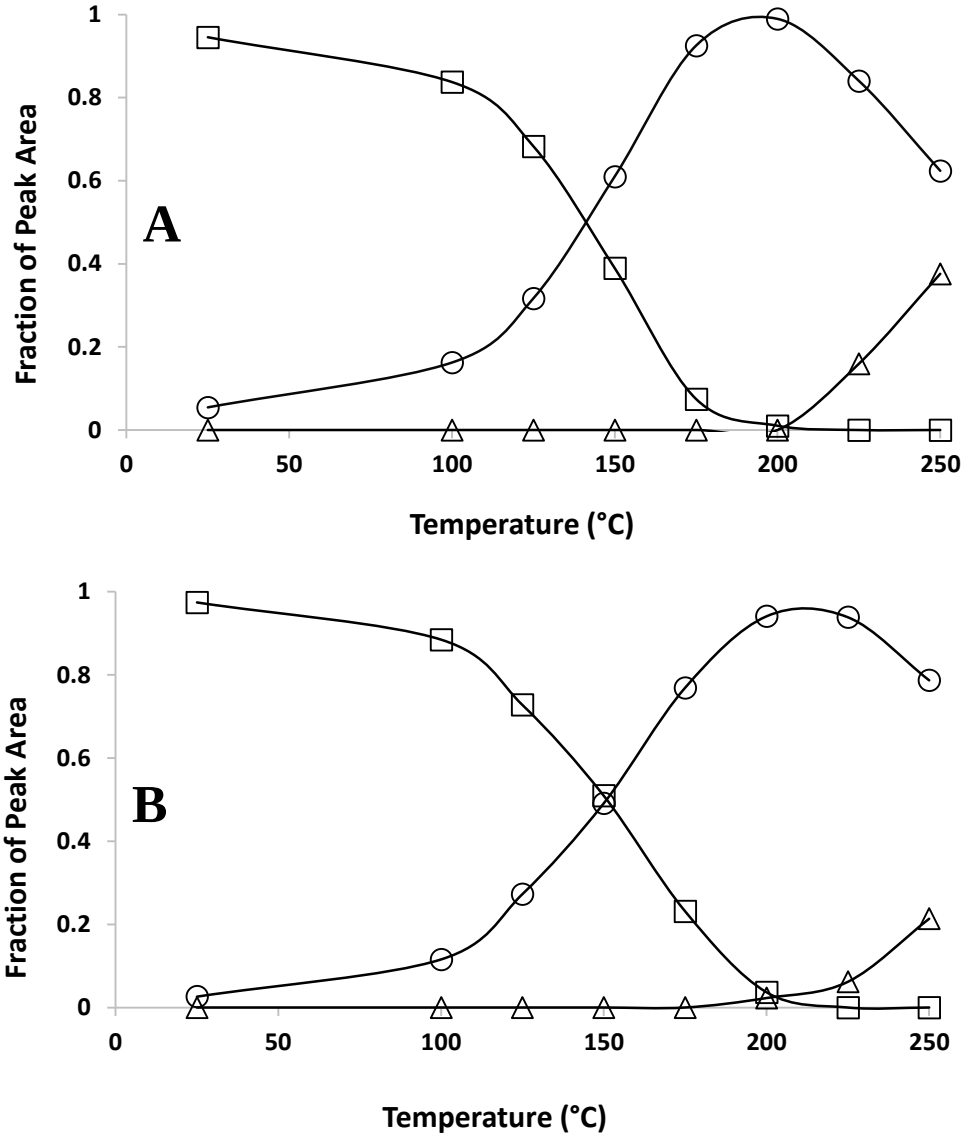

Figure 6.4: Degradation/formation profiles for ASA (□) and its degradants, salicylic acid (○) and phenol (Δ), as monitored by A) ultra-short GC column analysis and B) conventional HPLC analysis. The decomposition of the ASA standard was conducted in a water/methanol solvent at various temperatures for 10 minutes each.

Further, both methods also clearly indicate the total decomposition of ASA at 200 ^{o}C, as well as the onset of salicylic acid conversion into phenol at higher temperatures. Therefore, the ultra-short column GC method compares well to HPLC in monitoring the degradation of ASA and may be useful in such applications for other pharmaceuticals. If so, this could indeed be beneficial, especially in addressing the concern of detecting unknown pharmaceutical degradants during drug development [186,187] since the µFID employed here provides a uniform universal response to carbon-containing compounds that is identical to a conventional FID. Furthermore, the organic solvent requirements associated with conventional HPLC methods are relatively high and therefore, using the ultra-short GC column for such analyses could be additionally beneficial in reducing waste in routine separations for the pharmaceutical industry. For example, Figure 6.5 shows the elution of ASA and its degradation products from each system as a function of degradation temperature. While a single HPLC trial at a flow rate of 1.0 mL/min requires about 10 mL of organic solvent (near 100 mL for the full set) and 10 minutes to elute these compounds, an ultra-short GC column trial requires 0 mL of organic solvent and only 3.5 minutes to elute the same compounds.

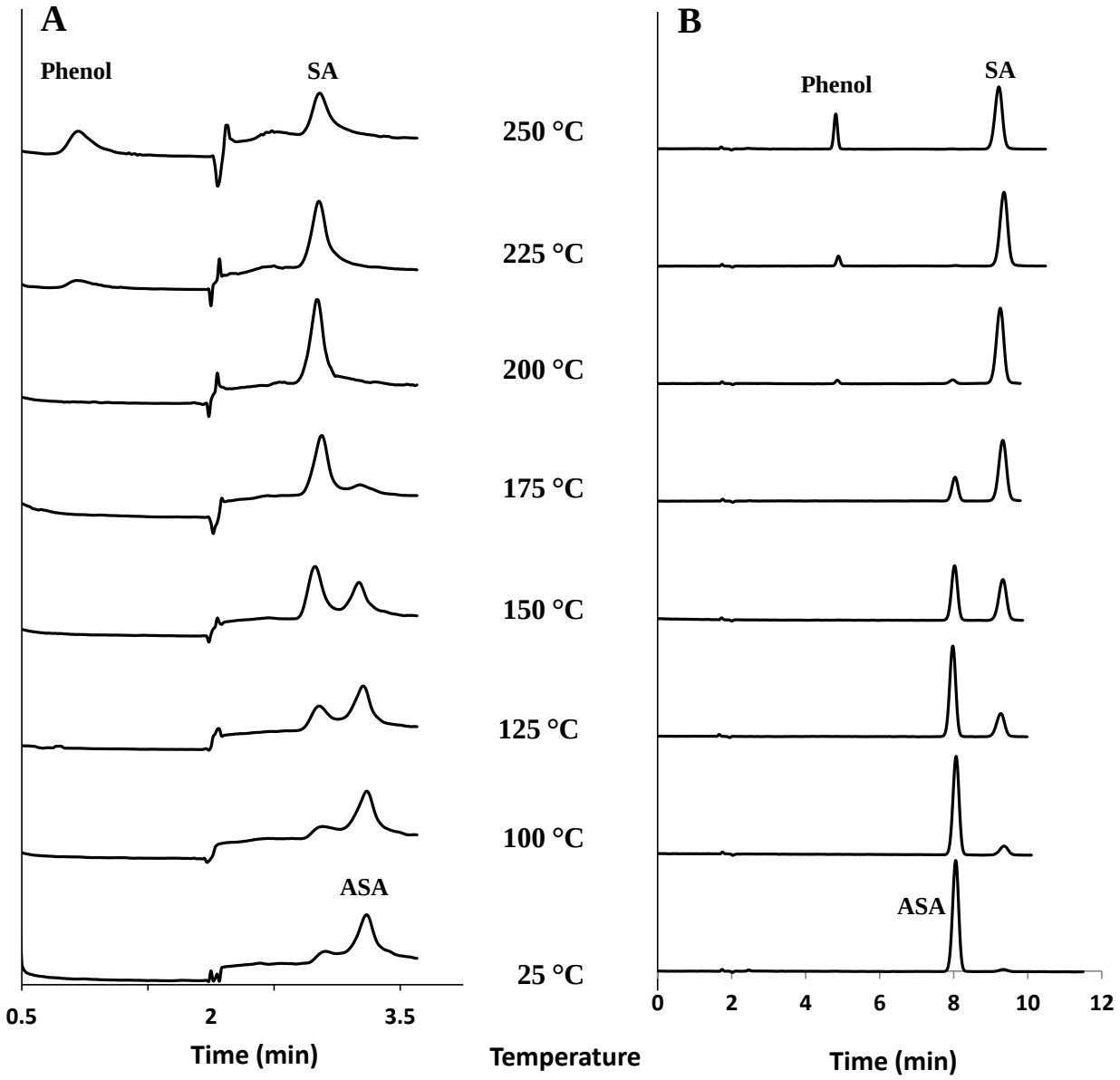

Figure 6.5: Chromatograms of thermally degraded ASA standard solutions as monitored by A) ultra-short GC column analysis and B) conventional HPLC analysis. The decomposition of the ASA standard was conducted in a 80:20 water/methanol solvent at various temperatures for 10 minutes each.

6.5 Monitoring ASA Degradation in Tablets Using µPLE Combined with the Ultra-Short GC Column Setup

As stated earlier, monitoring API degradation products in drugs is very important for the pharmaceutical industry. This is because such degradation products that usually form during storage, could be toxic and therefore need to be monitored for quality control/ quality assurance purposes. Current techniques for monitoring such degradation products in drugs involve prolonged and solvent intensive extraction procedures prior to the chromatographic analysis of these extracts which is routinely done by HPLC. As such, any development in this area that makes such analyses faster, less solvent demanding, and more compatible with the desired FID detection method are of great benefit to explore. Considering the possible advantages of combining the µPLE with the ultra-short GC column setup for monitoring API degradation in tablets, it was next examined if these two techniques can be used together to rapidly extract and analyze ASA and its degradation products in tablets.

In order to do so, an ASA tablet was heated for 10 minutes at a various temperatures in a humidified cell as previously described in Chapter Two. ASA and its degradation products were then extracted by methanol at 150 °C for 10 seconds from a portion of the resulting tablet using the µPLE technique. The resulting extracts were next immediately analyzed using the ultra-short GC column setup. The typical results of this experiment for ASA tablet degradation at 150 °C for 10 minutes can be seen in Figure 6.6. As seen, the findings indicate that µPLE is indeed capable of extracting ASA and its degradation products in a very rapid manner (i.e. 10 sec). For instance, the results indicate that the original unheated tablet contains 0.43 mg ASA per mg tablet, while after heating it contains

only 0.29 mg ASA and 0.11 mg SA per mg tablet, suggesting that about 33% of ASA has degraded to SA under these conditions of humidity and temperature. Incidentally, shorter times of 3 and 5 minutes tablet heating did not show any sign of degradation. Conversely, a more extreme heating condition of 200 °C for 30 minutes produced a fully black tablet residue that completely eroded and could not be analyzed.

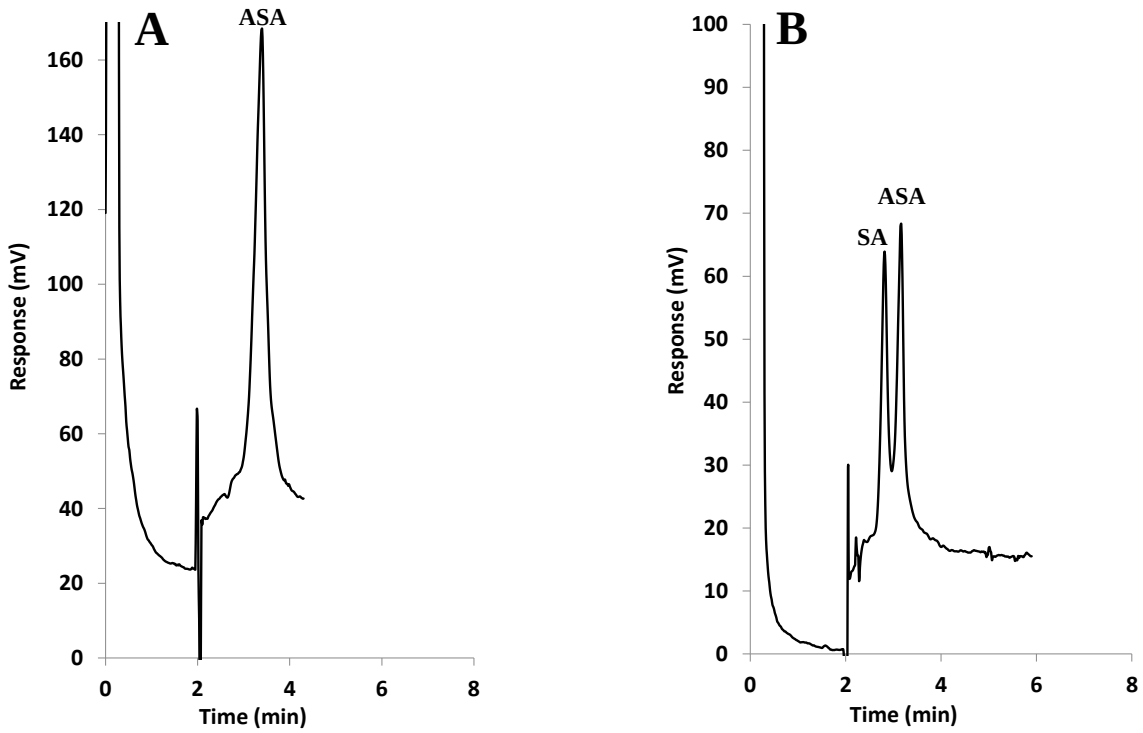

Figure 6.6: Ultra-short GC column profiles of µPLE extracts for A) an unheated ASA tablet and B) an ASA tablet heated at 150 °C for 10 minutes. The GC temperature program is 25 °C for 2 minutes then 90 °C/min to 180 °C. A 20 cm EC-5 column (2.65 µm thick film) is used. A minor detector spike/offset is noted in each when column heating begins. µPLE extraction conditions are 150 °C for 10 seconds using methanol as an extraction solvent.

These findings therefore demonstrate the possibility of combining the μPLE technique with an ultra-short GC column setup to very rapidly analyze ASA and its degradation products in tablets. This could be potentially beneficial to the pharmaceutical industry since employing these two techniques together can provide a very rapid preparation method for pharmaceutical formulations and a simple universal detection method for unknown API degradants. Further, this combination minimizes the use of toxic organic solvents that are commonly employed for the sample preparation and HPLC analysis of such pharmaceutical formulations. Therefore, using μPLE along with ultra-short GC column analysis can potentially offer pharmaceutical manufacturers considerable savings in time and waste, which in turn can enhance the drug development process.

6.6 Conclusions

The previously developed on-column injection/detection ultra-short GC column setup was used in this chapter for a pharmaceutical degradant application. The method provides reasonably efficient and very rapid separations for relatively simple mixtures. The method allows for the analysis of the highly thermally unstable analyte ASA and therefore potentially others as well. Further, as an application, the method was also successfully applied to monitoring pharmaceutical degradant formation as a function of temperature. The results indicate that the ultra-short GC column apparatus described here yields findings that compare well to those obtained by conventional HPLC and therefore it could be useful in such analyses. Accordingly, this would be further beneficial considering the environmentally hazardous and costly solvent consumption typically associated with HPLC, as well as the unique ability of such GC methods to use the desirable universal FID detection method for sensing unknown analytes.[139,140] Finally, the ultimate combination of

the novel μPLE technique with the ultra-short GC column setup to extract and analyze ASA degradants in tablets also proved to be a potentially useful means for online monitoring of such species. Accordingly, this combination may be useful to monitor API degradation in various other pharmaceutical formulations as well.

Chapter Seven: **SUMMARY AND FUTURE WORK**

7.1 Summary

This thesis describes successful advances in solid-liquid extraction methodology on several fronts from novel applications of existing extraction techniques, to the development of novel extraction methods. Such advances provide an alternative to conventional solid sample preparation methods, many of which are very slow and solvent intensive. As such, the developed methods are rapid, significantly less solvent demanding, and environmentally compatible alternatives.

One such development involved exploring EFLE for its ability to extract Fluoxetine Hydrochloride and various other APIs from pharmaceutical solid samples. The use of liquid CO_2/methanol extraction fluids results in a significant reduction of the viscosity and the surface tension of the extraction solvent compared to pure methanol while maintaining a solvent strength that is similar to methanol. As such, these features provide rapid and efficient extractions of a variety of polar analytes, while still reducing solvent consumption. For example, a significant reduction in extraction time and solvent requirements for extracting polar APIs from solid pharmaceutical formulations was observed. In addition, successful extractions of hydrophilic APIs were also attained using this method when water was used, for the first time, as a ternary modifier in the extraction fluid. Therefore, using water as a ternary modifier could potentially extend the range of EFLE to hydrophilic analytes while maintaining the technique's desirable speed and low solvent usage.

Next, a novel µPLE method was introduced. The method uses very small quantities of sample and solvent in a static extraction mode to remove analytes in as little as 10 to 30 seconds. The required instrumentation and procedure are relatively simple and readily

accessible to most laboratories. The method was explored with different samples and the results compared well to conventional PLE extractions of the same. As applications, the method was explored in DBS and PAH samples and was found to be very efficient at recovering target analytes from various matrices in a very rapid manner. For example, Fluoxetine Hydrochloride was quantitatively extracted from DBS cards in only 20 seconds which is a minor fraction of the time normally required for such samples using conventional extraction methods.

Finally, further studies were also performed to evaluate the ability of combining the rapid μPLE technique with an ultra-short GC-FID column setup as a novel tool to monitor API degradation in ASA tablets as a model pharmaceutical formulation. Results of these studies showed that ASA and its degradants can successfully be analyzed using an ultra-short GC-FID column setup and the results compared well with HPLC for monitoring degradation of the analyte as a function of temperature. Additionally when coupling this with μPLE, it was found that a thermally degraded ASA tablet could rapidly be extracted and analyzed for its degradant contents.

7.2 Future Work

7.2.1 EFLE for Extracting Hydrophilic APIs from Solid Pharmaceutical Formulations

Chapter Three demonstrated the benefits of using methanol as a CO_2 cosolvent in EFLE for extracting polar APIs from various pharmaceutical formulations. One such benefit was the ability to incorporate modest amounts of water with methanol in order to extract hydrophilic APIs even though pure water is insoluble with pure CO_2. While incorporating such amounts of water with methanol cosolvent showed to be very successful

at enhancing the extractability of certain hydrophilic APIs, using larger water proportions could be further beneficial for more hydrophilic APIs and/or for more challenging matrices. However, the maximum amount of water that can be incorporated with methanol is limited to 10% v/v, and therefore, using other EFLE cosolvents that allow for incorporating larger water proportions would be interesting to explore. In this regard, one cosolvent that could be employed and allows for incorporating larger water proportions (up to 50% v/v) is isopropyl alcohol. Therefore, it would be beneficial to explore the extractability of a wide range of hydrophilic APIs from various pharmaceutical formulations using water/isopropyl alcohol mixtures as EFLE cosolvents. This, if done, could potentially extend the range of EFLE to highly hydrophilic analytes in more challenging matrices and/or make the extraction of such analytes even faster.

7.2.2 μPLE Combined with Large Volume Injections for Trace Analysis Applications

The advantages of using μPLE for extracting a wide variety of analytes from various solid matrices in a very rapid manner was demonstrated in this thesis. However, extracting trace analytes from solid samples was only preliminary probed here. Therefore, it would be interesting to examine the extractability of such analytes using μPLE. One issue that is normally encountered in trace analysis is the detection of very small quantities of analyte. One approach analysts occasionally employ in order to address this issue is to introduce more sample to the chromatographic system using large volume injections (LVI) ranging normally between 50 to 250 μL. By introducing more sample into the chromatographic system, the mass of analyte reaching the detector will be proportionally increased resulting in higher analyte response. Such an approach could be perfectly suited for μPLE since the volume of μPLE extracts is only 125 μL, and therefore, the whole

extracted sample can be used for subsequent LVI chromatographic analysis. Thus, using LVI along with μPLE could be a very useful approach when trace sample components at low concentration levels need to be determined. Further, coupling these two techniques together could potentially be useful for online analyses of μPLE extracts.

It should also be mentioned here that despite the potential benefits of μPLE there are still some areas that could be further improved in future developments. For instance, the current procedure for cleaning extraction vessels is relatively troublesome. This is because the extraction vessels are very small in dimensions (2.1 mm I.D. and 2.9 mm O.D.) and sealed from one end which makes it challenging to rinse the vessels from inside. Therefore, it will be useful to try different approaches that could overcome this issue. One way that could potentially facilitate this is using another design of the extraction vessel that is threaded and capped from both ends. Such a design will make cleaning the vessels much easier as the cleaning solvent can easily and rapidly flow through the uncapped vessels. In addition, this issue may also be addressed if disposable extraction vessels (made of cheaper materials such as glass or vespel) are used in future experiments.

Another area that could also be further improved in future work is introducing multiplex optimization experiments in order to efficiently and rapidly optimize the extraction conditions of solid samples. In this regard, the suggestion of standard extraction conditions for solid samples can be useful. For example, an analyst can first try extracting analytes from solid samples for 30 seconds at a temperature 150 °C using an extraction solvent that the target analyte is highly soluble in. Next, if these conditions did not provide quantitative extractions, the analyst could try increasing the extraction temperatures by 25

°C intervals at a time (if the analyte is thermally stable) until quantitative recoveries are attained. If the target analyte was thermally unstable, then slightly longer extraction times (e.g. 10 s at a time) at 150 °C could be next pursued until the extraction is complete.

7.2.3 µPLE Combined with an Ultra-Short GC Column Setup for Batch Drug Analyses

This thesis demonstrated the benefits of combining µPLE with an ultra-short GC column setup to very rapidly monitor ASA degradation in a single tablet. However, batch analyses of tablets were not yet tested using this approach. Monitoring API degradation in batch samples is interesting since it is more representative of typical pharmaceutical industry practice. In this regard, it would be interesting to further explore the benefits of combining µPLE with the ultra-short GC column setup to analyze batches of ASA tablets. This could be done by first obtaining representative samples (e.g. 20 tablet each) of different ASA batches and degrade these under conditions of controlled humidity and temperature. Next, these samples could be homogenized in a similar manner to routine pharmaceutical procedures and small portions then used for µPLE and subsequent ultra-short GC column analyses.

Finally, and in order to fully examine if this combination further addresses pharmaceutical industry needs, it would be interesting to use it for various other pharmaceutical applications. For example, it could be used for monitoring degradation in different drug substances and products at various stages of development. In this way, µPLE could become a very useful sample preparation tool in the future.

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APPENDIX A: GC-FID Chromatograms of 1) A Standard Solution Containing The 16 EPA PAHs and 2) A Bio-Char Amended Soil Extract

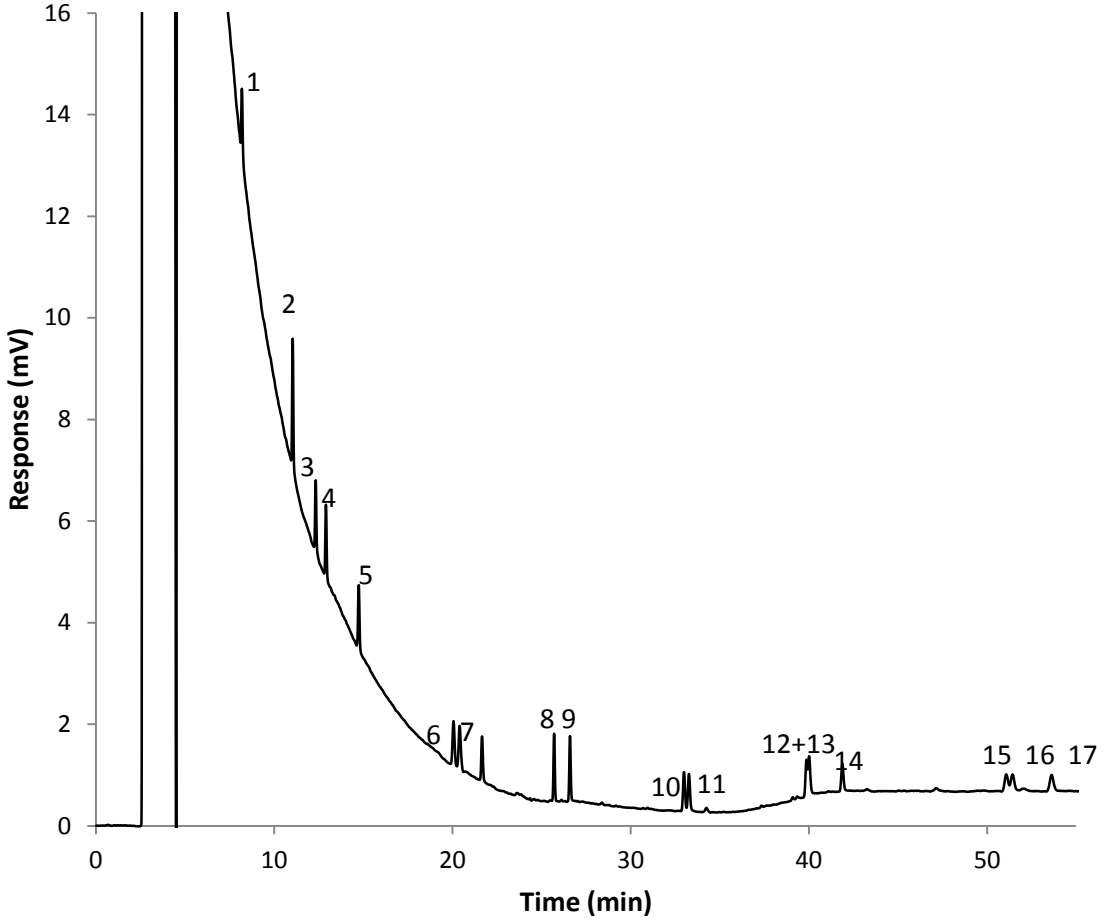


Figure A.1: GC-FID chromatogram of a standard solution containing the 16 EPA PAHs and C_{14} as an internal standard. 1- Naphthalene, 2- C_{14}, 3-Acenaphthene, 4-Acenaphthylene, 5- Fluorene, 6-Phenantherene, 7-Anthracene, 8- Fluoranthene, 9-Pyrene, 10-Benzo[a]anthracene, 11-Chrysene,12+13-Benzo[b]/[k]fluoranthene, 14-Benzo[a]pyrene, 15-Indeno[1,2,3-c,d]pyrene, 16-Dibenz[a,h]anthracene, 17-Benzo[g,h,i]perylene

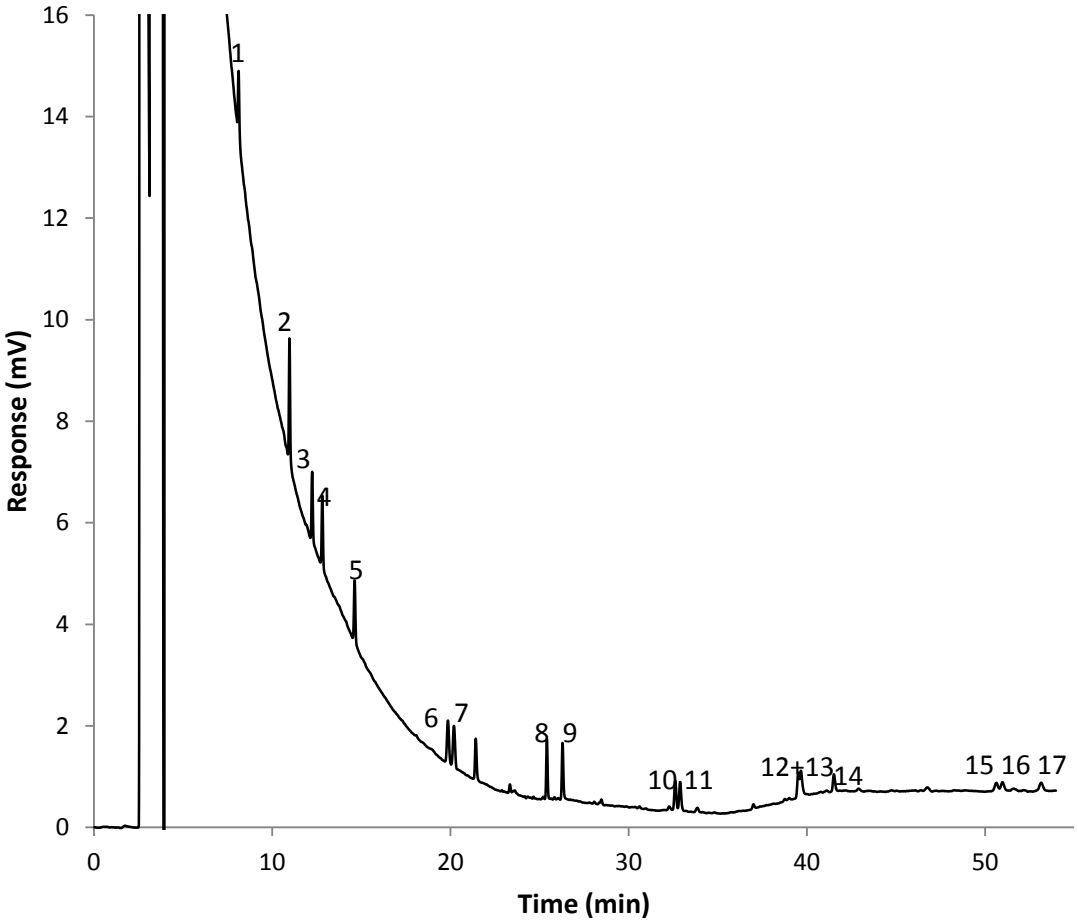


Figure A.2: GC-FID chromatogram of a biochar-amended soil extract. 1-Naphthalene, 2- C_{14},3-Acenaphthene, 4- Acenaphthylene, 5- Fluorene, 6-Phenantherene, 7-Anthracene, 8- Fluoranthene, 9-Pyrene, 10-Benzo[a]anthracene, 11-Chrysene,12+13-Benzo[b]/[k]fluoranthene, 14-Benzo[a]pyrene, 15-Indeno[1,2,3-c,d]pyrene, 16-Dibenz[a,h]anthracene, 17-Benzo[g,h,i]perylene

APPENDIX B: Sample Calculation of % Extraction Recovery of Naphthalene from a Biochar-Amended Soil Sample

Response factors were calculated for each PAH according to:

$$RF = \frac{\left(\frac{X_{std}}{C_{std}}\right)}{\left(\frac{X_{is}}{C_{is}}\right)}$$

Where X is the peak area of the standard and C is the concentration of the same standard inside the extracting vessel.

The concentrations of unknown analytes inside the extraction vessels were then calculated according to:

$$C_{unk} = \frac{X_{unk}}{RF \times \frac{X_{is}}{C_{is}}}$$

Where X_{unk} is the peak area of the unknown analyte and C_{unk} is the concentration of the unknown analyte in the extraction vessel. The recovery for an analyte was then determined according to:

$$\%\ Recovery\ = \frac{C_{unk}}{C_{std}} \times 100\%$$

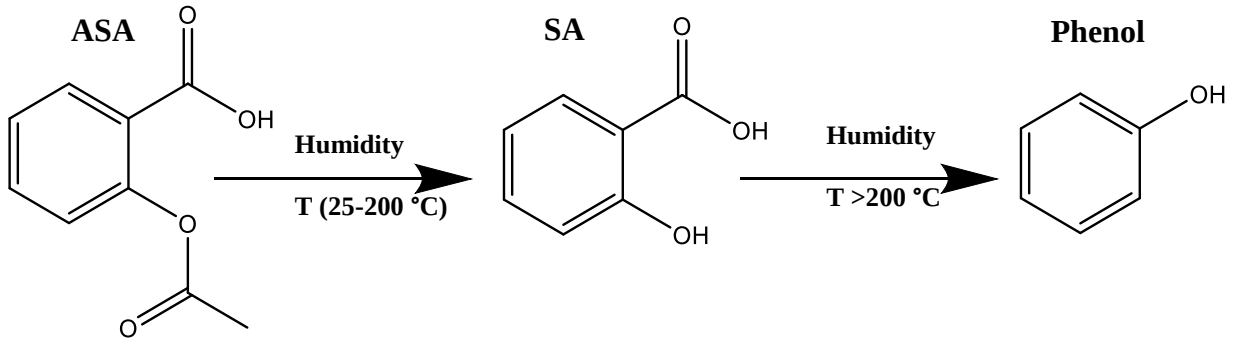
Sample calculation of the % Recovery of Naphthalene from a Biochar-amended Soil sample:

RF Naphthalene=1.49125238, $X_{Naphthalene(unk)}$= 6.812, $X_{i.s}$=12.3405, $C_{i.s}$= 0.016124031 mg/mL

$$C_{Naphthalene} = \frac{6.812}{1.49125238 \times \frac{12.3405}{0.016124031}} = 0.00614860$$

$$\%\ Recovery\ (Naphthalene) = \frac{0.00614860}{0.00620155} \times 100\% = 99.1\%$$

APPENDIX C: Products of ASA Degradation under Conditions of Humidity and High Temperatures



APPENDIX D: LC-MS Chromatogram of a pure room temperature methanol extraction of Fluoxetine Hydrochloride from a Prozac® capsule

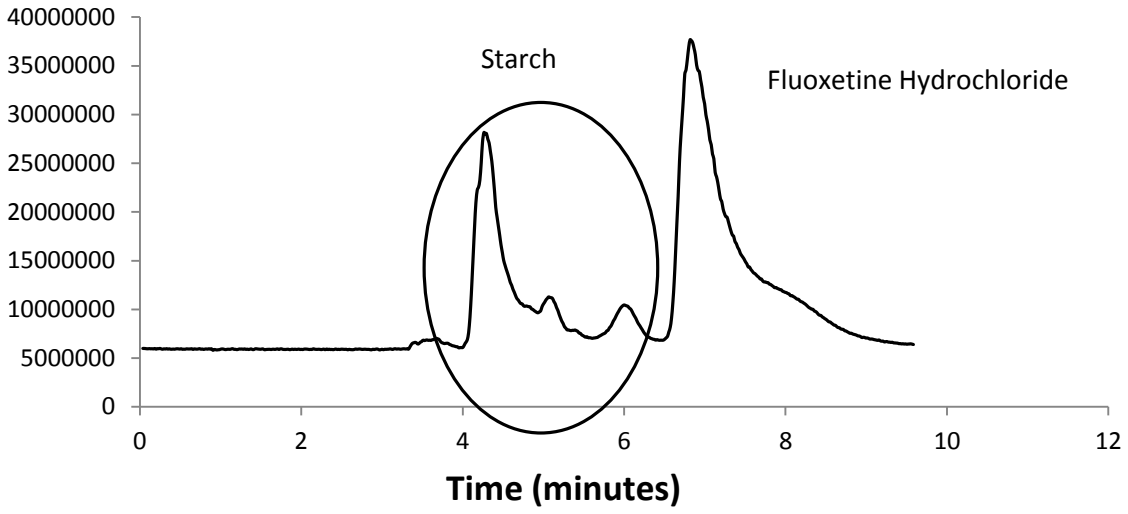


Figure D: HPLC-MS Chromatogram of Prozac® capsule extracts obtained by 50 mL of pure room temperature methanol.