Abstract

STABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS AND BENZOIC ACID DERIVATIVES UNDER SUBCRITICAL WATER CONDITIONS

By

Edward J. Lindquist

March 2011

Chair: Dr. Rickey P. Hicks

Major Department: Chemistry

The development of green environmental remediation, chromatography, and extraction techniques using subcritical water is the focus of our research group. The polarity of subcritical water can be manipulated by increasing its temperature in the range of 25 to 374 ºC while keeping it in the liquid state under moderate pressure. At elevated temperatures liquid water becomes less polar and behaves more like an organic solvent. The goal of this research is to determine the conditions under which certain polycyclic aromatic hydrocarbons (PAHs) and benzoic acid derivatives degrade in subcritical water. The stability of two PAHs (pyrene and naphthalene) and benzoic acid and three of its derivatives (anthranilic acid, syringic acid, and salicylic acid) under subcritical water conditions was investigated and the results are discussed in this thesis.

PAHs are pollutants widely formed as a result of incomplete combustion of organic materials. The effects of temperatures ranging from 200 to 350 ºC and heating times of 30 and 300 min on the degradation of pyrene and naphthalene in solutions of water and 3% hydrogen peroxide were determined. Our results show that PAHs can be degraded under subcritical water

conditions, and thus, this technique may be applied to the environmental remediation of these pollutants.

Benzoic acid and its derivatives are found in medicinal herbs and other plants. While the extraction of these active ingredients from herbs using subcritical water is non-toxic and preferred, the decomposition of these compounds under subcritical water conditions has to be examined. The stability studies of this group of analytes were carried out at temperatures ranging from 50 to 250 ºC with heating times of 10 and 30 min. The degradation of the benzoic acid derivatives increased with rising temperature and additional heating time. The degradation products of benzoic acid and the three derivatives were identified and quantified by high-performance liquid chromatography (HPLC) and confirmed by gas chromatography/mass spectrometry (GC/MS). Under subcritical water conditions anthranilic acid, syringic acid, salicylic acid, and benzoic acid underwent decarboxylation to form aniline, syringol, phenol, and benzene, respectively.

STABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS AND BENZOIC ACID DERIVATIVES UNDER SUBCRITICAL WATER CONDITIONS

A Thesis

Presented To

The Faculty of the Department of Chemistry

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Edward J. Lindquist

March 2011

STABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS AND

BENZOIC ACID DERIVATIVES UNDER SUBCRITICAL WATER CONDITIONS

By

Edward J. Lindquist

APPROVED BY:

DIRECTOR OF THESIS: _____

Yu Yang, PhD

COMMITTEE MEMBER: _____

Colin S. Burns, PhD

COMMITTEE MEMBER: _____

Anthony Kennedy, PhD

COMMITTEE MEMBER: _____

Cindy Putnam-Evans, PhD

CHAIR OF THE
DEPARTMENT OF CHEMISTRY: _____

Rickey P. Hicks, PhD

DEAN OF THE
GRADUATE SCHOOL: _____

Paul J. Gemperline, PhD

DEDICATION

This thesis is dedicated to my wife, Cher Lindquist.

Without her love, support, and patience I would not have been able to succeed in this endeavor.

ACKNOWLEDGMENTS

I would like to thank my family for their support and encouragement over the years it has taken me to reach this goal.

I am grateful for Dr. Yu Yang's guidance throughout the course of my research. His enthusiasm kept me moving forward even when things got difficult.

I would like to express my thanks to Dr. Colin Burns, Dr. Anthony Kennedy, and Dr. Cindy Putnam-Evans for being on my thesis committee and challenging me to better understand the results of my experiments.

I appreciate the assistance given by the members of our research group: Zack Strickland, Leena Gujjar, Ann Marie Toebes, Marina Rybnikova, Bryan Pate, Chris Baker, Heather Anderson, Chris Jones, and Brett Weed. Their friendship and the camaraderie of my fellow graduate students made the late nights in the lab bearable.

I would like to thank Dr. Rickey Hicks and Dr. Brian Love for answering questions about my research that were in their areas of expertise.

I am thankful for the metal work done by Jim Saupe in the Department of Physics and the wooden vial trays made by Steve Simonds.

I appreciate the advice on HPLC instrumentation and method development from Phil Hodges, Jennifer Warren, Nicole Wilkerson, Kevin Jones, Elizabeth Boswell, Fallon O'Neal, Kym Rispress, and John Arnold of Metrics, Inc.

I also thank Dr. Arpie Korkin of Phenomenex, Inc. for her suggestions on HPLC column selection.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

Å	Angstrom
atm	Atmosphere
CO_2	Carbon dioxide
ºC	Degree Celsius
EI	Electron impact
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
HPLC	High-performance liquid chromatography
HTLC	High-temperature liquid chromatography
H_2O_2	Hydrogen peroxide
-OH	Hydroxyl functional group
ITE	*Isatis tinctoria*
MSD	Mass selective detector
m/z	Mass-to-charge ratio
MΩ-cm	Megaohm centimeter
min	Minute
M	Molar concentration
n	Number of replicate experiments
NSAID	Nonsteroidal anti-inflammatory drug
ppm	Parts-per-million
PCB	Polychlorinated biphenyl
PAH	Polycyclic aromatic hydrocarbon

$-NH_2$	Primary amine
% RSD	Relative standard deviation
SBWC	Subcritical water chromatography
SBWE	Subcritical water extraction
TLC	Thin layer chromatography
TCM	Traditional Chinese medicine
vol%	Volume percentage
wt%	Weight percentage
% w/v	Weight/volume percentage solution

CHAPTER 1: INTRODUCTION

1.1 Chemistry Using Subcritical Water

At ambient temperature and pressure (25 ºC, 1 atm) water is a polar solvent. Its high dielectric constant is a result of intermolecular hydrogen bonding. The solubility of nonpolar organic compounds in water at ambient temperature and pressure is usually poor. By increasing the temperature of water in the range of 25 to 374 ºC under moderate pressure (to keep the water in a liquid state) subcritical water is obtained. At elevated temperatures the intermolecular hydrogen bonds of water are weakened causing its dielectric constant to decrease. Water thus becomes less polar and behaves more like an organic solvent [1-3]. In addition to its dielectric constant, the surface tension and viscosity of water similarly decrease with increasing temperature. These properties of subcritical water have led to its use in a number of different applications, one of which is subcritical water extraction.

1.1.1 Subcritical Water Extraction

The increased solvating ability of subcritical water allows its use as an extraction fluid for many classes of organic compounds. Subcritical water extraction (SBWE) has been used to extract organic compounds from a variety of sample matrices including environmental, food, pharmaceutical, and plants [1, 2, 4-25]. SBWE has been used to extract organic pollutants including PAHs, polychlorinated biphenyls (PCBs), and pesticides from contaminated soil [1, 26-28]. Extraction of active ingredients from plant material has also been achieved using subcritical water [21, 23, 29-32].

1.1.2 Subcritical Water Chromatography

Another application of subcritical water is in subcritical water chromatography (SBWC) where subcritical water is used as the sole component in the mobile phase to mimic organic solvent programming to achieve reversed-phase separation [5, 33-48]. This green chromatography technique has been investigated in our laboratory for over a decade [3, 5, 34-40]. SBWC has also been found to be promising by other researchers [41-48]. The main advantage of SBWE and SBWC techniques is the elimination of the hazardous organic solvents that are typically required in traditional extraction and liquid chromatography separation processes. SBWE and SBWC methods also lead to reduced time and waste.

1.1.3 Chemical Reactions in Subcritical Water

In addition to SBWE and SBWC, organic synthesis can also be carried out in subcritical water [49-51]. The enhanced solubility of organic compounds in water is what makes this possible. It has been reported that oxidation of phenol, benzoic acid, and aniline occurs in subcritical water when hydrogen peroxide (H_2O_2) is used as an oxidant [26]. Oxidation of alkyl aromatic compounds to aldehydes, ketones, and acids in subcritical water by molecular oxygen mediated by transition metal catalysts has also been shown [49]. Other chemical reactions in subcritical water that have been investigated can be found in the literature [52-57].

1.2 Organic Degradation in Subcritical Water

While SBWE and SBWC are promising techniques, the thermal stability of the organic compounds under subcritical water conditions must be taken into consideration. A balance must be found where the conditions will allow the extraction of the analytes of interest without significant degradation or conversion into toxic compounds. Stability studies of the analytes under subcritical water conditions are necessary to ensure the accuracy of the data obtained in SBWE and SBWC experiments [17, 58].

The potential to inadvertently degrade the desired organic compounds during an extraction using subcritical water is definitely a disadvantage, but there are some instances when it could be beneficial. Significant increases in the solubility of liquid organics and active ingredients from plant material in water at subcritical temperatures have been reported in the literature [59]. Complete destruction of pollutants can occur at the high temperatures and pressures of subcritical water which has lead to its application in environmental remediation.

Benzoic acid and its derivatives are found in medicinal herbs and other plants [60]. In herbal medicine these compounds are typically extracted using boiling water to make a tea-like liquid extract for patient use. Since the solubility of organic molecules often increases as the temperature rises, subcritical water has the potential to be a better solvent for extracting benzoic acid and its derivatives. Previous work has been performed to determine the solubility of benzoic acid and salicylic acid in subcritical water [61]. While the extraction of these active ingredients using subcritical water is non-toxic and preferred, the decomposition of these compounds under subcritical water conditions has to be examined.

1.3 The Goal of This Research

The goal of this research is to determine the conditions under which certain polycyclic aromatic hydrocarbons and benzoic acid derivatives degrade in subcritical water. The stability of two PAHs (pyrene and naphthalene) and benzoic acid and three of its derivatives (anthranilic acid, syringic acid, and salicylic acid) under subcritical water conditions was investigated and the results are discussed in this thesis.

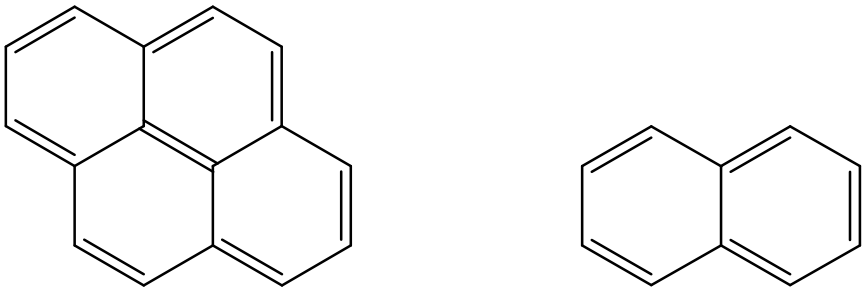
The effects of temperatures ranging from 200 to 350 ºC and heating times of 30 and 300 min on the degradation of pyrene and naphthalene in solutions of water and 3% hydrogen peroxide were investigated. If PAHs can be degraded under subcritical water conditions, this technique may be applied to the environmental remediation of these pollutants.

The effects of temperatures ranging from 50 to 250 ºC and heating times of 10 and 30 min on the degradation of benzoic acid, salicylic acid, syringic acid, and anthranilic acid in solutions of degassed water were investigated. From these experiments, the conditions under which the benzoic acid derivatives degrade in subcritical water were determined, and the degradation products were identified and quantified by HPLC and confirmed by GC/MS. The results of this stability study will facilitate future SBWE and SBWC projects.

CHAPTER 2: BACKGROUND

2.1 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons consist of fused aromatic rings and do not contain heteroatoms or carry substituents. Two examples, pyrene and naphthalene, are shown in Figure 1. PAHs are pollutants widely found in our environment, and significant amounts of them can be found in crude oil and coal deposits. They are also produced as byproducts of fossil fuel burning and by incomplete combustion of carbon containing fuels. PAHs are relatively resistant to combustion, and their hydrophobicity hinders their degradation in liquid media. The larger PAHs are even less water soluble [62].



a) **b)**

Figure 1. Structures of a) Pyrene and b) Naphthalene.

Polycyclic aromatic hydrocarbons are robust molecules that are also found in the interstellar media. The spectral signatures of anthracene and pyrene have been found in the light emitted by the Red Rectangle Nebula [63]. On Earth, PAHs are found primarily in soil, sediment, and oily substances. They are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic. PAH toxicity is very structurally

dependent, with isomers varying from being nontoxic to extremely toxic. The PAHs benzo[a]pyrene, and benz[a]anthracene are suspected mutagens and carcinogens [64].

Currently, there are several different remediation techniques that can be used to clean PAHs from the environment. The two main categories are “dig and dump” and “pump and treat”. Ex-situ methods involve excavation of affected soils and subsequent treatment at the surface while in-situ methods seek to treat the contamination without removing the soils. Over time, even water at ambient conditions can vaporize PAHs from contaminated sediments [65]. It can take around two weeks up to a few years for PAHs to decay naturally in the environment. For example, the half-life of naphthalene ranges from 16 days to 48 days and the half-life of phenanthrene from 16 days to 200 days. The minimum environmental half-life of Pyrene is 210 days and its maximum 5.2 years [66].

In an effort to speed up the process of degradation and achieve total destruction of PAHs, researchers have applied several different methods. They include composting (~50 days), phytochemicals (~40 days), microbial degradation with fungi (~10 days) or bacteria (~7 days), sonochemical degradation (~90 min), and photolysis (~7 min) [67-71]. There are also different extraction techniques that can be used to remove PAHs from environmental samples at the bench scale. These include soxhlet extraction, microwave assisted extraction, pressurized hot water extraction, and supercritical fluid extraction [72]. A procedure called flameless supercritical water incineration has also been developed [73]. If PAHs can be degraded under subcritical water conditions, then subcritical water may be applied to environmental remediation.

2.2 Benzoic Acid and Derivatives

Benzoic acid is mainly used as a preservative in food, cosmetics, and other commercial products. Benzoic acid and derivatives such as anthranilic acid, syringic acid, and salicylic acid, are found in Chinese medicinal herbs and other plants [60]. As mentioned previously, subcritical water extraction has been recently applied to the extraction of herbs. Benzoic acid, salicylic acid, syringic acid, and anthranilic acid, shown in Figure 2, were chosen for a stability study using subcritical water conditions.

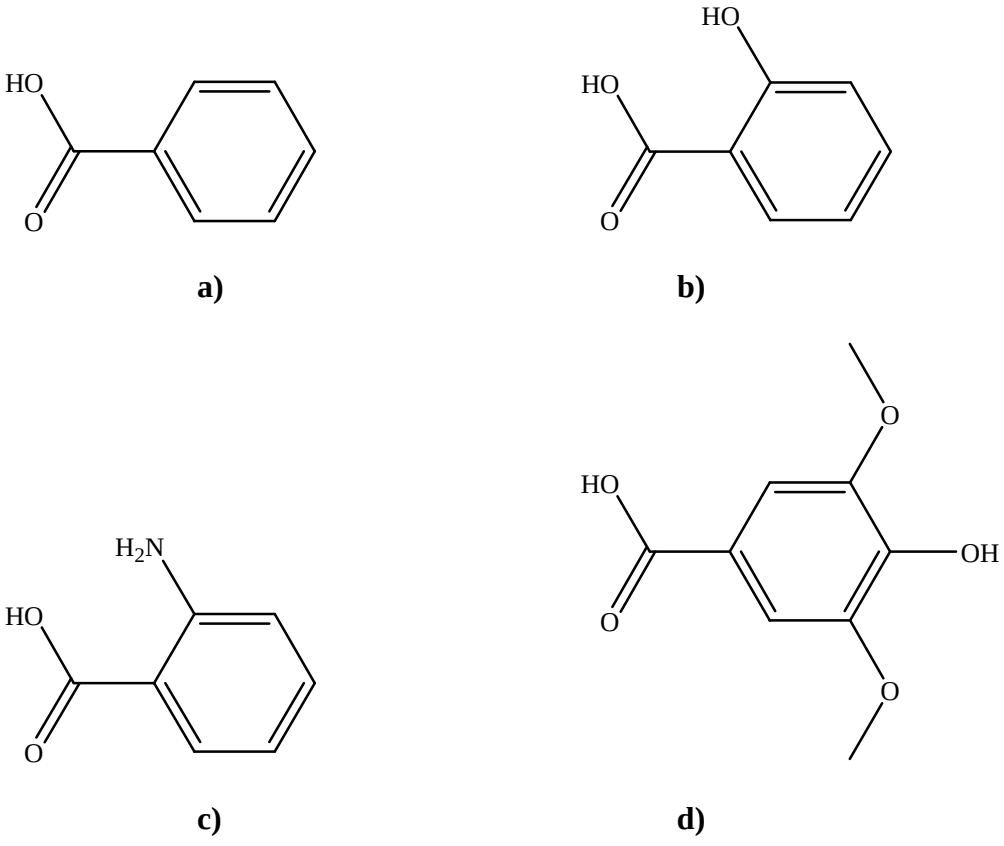


Figure 2. Structures of a) Benzoic Acid, b) Salicylic Acid, c) Anthranilic Acid, and d) Syringic Acid.

Traditional Chinese medicines (TCMs) have been used for thousands of years to prevent and cure human diseases. At the present time they are attracting increased attention in several fields. Studies have shown that the compounds found in TCMs can possess significant pharmacological actions such as immunomodulatory activity and antioxidant effects. TCMs are usually composed of several herbs, each of them having multiple constituents. The complexity of these mixtures presents a challenge for pharmaceutical analysis [74]. Most research focuses on the determination of the active components in herbs with thin layer chromatography (TLC), HPLC, gas chromatography (GC), and electrophoresis [75-80].

Benzoic acid occurs naturally in many plants and is an intermediate in the formation of other compounds. Benzoic acid is primarily used as a preservative in many products. Significant amounts of benzoic acid can be found in fruits such as apple, cherry, cranberry, bilberry, grape, papaya, raspberry, blackberry, strawberry, and tomato [81]. As a food preservative, benzoic acid inhibits the growth of molds and yeast. It is also an active ingredient in at least 17 medicinal herbs. The Chinese proprietary medicine Xiao Yao Wan contains several active components, benzoic acid being one of them [74]. Benzoic acid and salicylic acid are two components of Zuguangsan, a traditional Chinese patent medicine used for treating bacterial diseases of the hands and feet [82].

Anthranilic acid is a chemical intermediate used in the synthesis of pharmaceuticals, perfumes, and dyes [83]. Certain derivatives of anthranilic acid constitute an important group of nonsteroidal anti-inflammatory drugs (NSAIDs) [84, 85]. Examples of these NSAIDs include diclofenac sodium, enfenamic acid, and mefenamic acid [86]. Anthranilic acid can be found in

Jasminum sambac, a species of jasmine that is native to Asia. The flower can be processed and combined with green tea to make jasmine tea.

Syringic acid shares some similarities with salicylic acid as they are both natural ingredients found in red wines and are intermediates for organic synthesis. During the breakdown of Malvidin, a primary plant pigment, syringic acid is released. Syringic acid can be found in *Ailanthus altissima* which is commonly known as tree of heaven. It is a deciduous tree native to China and has become an important weed in both Europe and North America due to its well-known stress tolerance [87]. In Chinese traditional medicine it is one of the medicinal plants that have been used in the treatment of different tumors [88]. *Ailanthus altissima* is also used as a bitter aromatic drug and in the treatment of colds and gastric diseases [89].

Salicylic acid affects a number of physiological processes in plants, ranging from promotion of flowering in some species to inhibition of stomatal closure and ion uptake in others. Some plants respond to infection by pathogens with both localized and systemic resistance responses. Increases in a plant's salicylic acid levels act as a signal for the activation of some of these induced defenses [90]. Salicylic acid can be found in Ban lan gen (*Isatis tinctoria*). Woad is the common name of the flowering plant and it has been used since antiquity as a source for indigo production. The solid dose, Banlangen Keli, is popular throughout China. The plant has been used medicinally as an astringent and for treating skin inflammation and ulcers. Extracts from *Isatis tinctoria* (ITE) have shown anti-inflammatory activity in several studies [91]. In one clinical pilot study, the anti-inflammatory activity of topically administered ITE was confirmed in skin irritation models [92]. It has been suggested that ITE may also be useful in allergic airway inflammations.

2.3 Organic Degradation in Subcritical Water and Its Mechanism

With any new technique there are advantages and disadvantages. Subcritical water has the ability to extract organic compounds in a way that can save time and reduce waste but some organic compounds are not thermally stable at the high temperatures required for SBWE. This can be a disadvantage when subcritical water is used in herbal extraction methods as compounds such as benzoic acid and its derivatives may degrade. The high temperatures required for SBWE can be advantageous though if the goal is to degrade certain compounds as in the case of pollutants like PAHs. This was the case with early applications of SBWE which involved the extraction of organic pollutants such as PAHs, PCBs, phenols, pesticides, herbicides, phenolic compounds, and others from solid environmental samples such as sediment, soil, and air particulates [21]. Research has been carried out using the abilities of subcritical water to extract advantageous compounds and degrade undesirable pollutants [50].

Before using subcritical water for extraction, chromatography, or organic synthesis, the stability of the compounds of interest under such conditions should be investigated. In order for SBWE, SBWC, or high-temperature liquid chromatography (HTLC) to be accurate analytes must be stable at the temperatures used. If the analytes were to degrade during SBWE, SBWC, or HTLC experimentation then the data obtained could be misleading. Understanding the mechanism by which the compounds of interest decompose is helpful when assessing the results of stability studies.

It has been shown that PAHs such as pyrene, phenanthrene, and naphthalene undergo oxidative decomposition under both subcritical and supercritical water conditions [93, 94]. In these types of experiments compounds are typically reacted in water with hydrogen peroxide

used as an oxidant. At subcritical temperatures and pressures PAH degradation requires longer exposure times and higher concentrations of hydrogen peroxide. Up to 99.9 wt% destruction of PAHs occurs at supercritical conditions [73]. The main processes that are suspected to take place are thermal cracking and carbonization at lower temperatures and oxidation at higher temperatures and pressures. The final combustion products are carbon dioxide (CO_2) and water.

Onwudili and Williams used a supercritical water oxidation reactor to demonstrate how PAHs could be incinerated using subcritical and supercritical water [73]. Supercritical water is obtained by increasing the temperature of water to above 374 ºC while keeping it at a pressure of at least 218 atm to keep the water in a liquid state. The reactor was constructed of stainless steel with a capacity of 500-mL, maximum pressure rating of 340 atm, and maximum temperature rating of 500 ºC. A sampling system allowed for collection of gas and liquid samples containing reaction products from the reactor throughout the experiments.

The compounds investigated by Onwudili and Williams were pyrene, phenanthrene, naphthalene, fluorene, and biphenyl [73]. Several different reaction matrices were used in these experiments, and they included water phase, spiked sand samples, and spiked clay soil. Parameters affecting PAH decomposition were temperature, pressure, subcritical and supercritical conditions, and reaction time. Table 1 shows the results for the decomposition of naphthalene in water under both subcritical and supercritical water oxidation conditions. At supercritical conditions, there was a high percentage of naphthalene decomposition which increased with increasing amount of hydrogen peroxide in the water. Under subcritical conditions, it was clear that the hydrogen peroxide was needed in order to cause the destruction of naphthalene. The mass of the naphthalene did not appear to influence the efficiency of

destruction. Although maximum oxidation of naphthalene occurs under supercritical water incineration conditions, similar oxidation properties are observed at subcritical water conditions.

Table 1. Decomposition of Naphthalene (Reaction time 60 min, modified from reference 73)

Temperature (°C)	Pressure (atm)	H_2O_2 (vol%)	Sample Size (g)	Decomposition (wt%)
350	171	0.0	0.1	6.6
350	171	1.5	0.1	33.9
350	171	3.0	0.1	57.5
350	171	6.0	0.1	71.2
380	221	0.0	0.1	24.4
380	221	1.5	0.1	79.5
380	222	3.0	0.1	85.2
380	222	3.0	0.05	89.7
380	221	6.0	0.1	98.4
380	221	6.0	0.05	98.8
380	222	6.0	0.15	99.2

The decomposition of phenanthrene was similarly dependent on the presence of an oxidant in the reaction system as shown in Table 2. Most of the destruction occurred in a short reaction time; however, extended reaction times produced a small increase in the destruction of phenanthrene. There was significant formation of naphthalene from the destruction of phenanthrene. With the addition of hydrogen peroxide at a concentration of 6.0 vol% under supercritical water incineration conditions, both phenanthrene and naphthalene were essentially destroyed.

Table 2. Decomposition of Phenanthrene (Sample size 0.1 g, modified from reference 73)

Temperature (°C)	Pressure (atm)	H_2O_2 (vol%)	Reaction Time (min)	Phenanthrene Decomposition (wt%)	Naphthalene Produced (wt%)
350	171	0.0	60	22.0	19.2
350	171	3.0	60	61.2	20.5
380	222	0.0	60	31.6	11.9
380	221	3.0	60	96.1	1.2
380	221	6.0	0	97.2	0.9
380	222	6.0	30	98.5	0.7
380	222	6.0	60	99.7	0.9
380	222	6.0	120	99.4	0.4

Table 3 shows the results for the decomposition of pyrene in water at a 6.0 vol% hydrogen peroxide concentration under subcritical and supercritical water conditions. At subcritical conditions as the temperature and pressure increased, there was a progressive destruction of pyrene. As the reaction time increased, the percentage destruction of pyrene increased. Reaction products from the subcritical water oxidation of pyrene included phenanthrene and naphthalene in low concentrations.

Table 3. Decomposition of Pyrene (Sample size of 0.1 g, 6 vol% H_2O_2, modified from reference 73)

Temperature (°C)	Pressure (atm)	Reaction Time (min)	Pyrene Decomposition (wt%)	Phenanthrene Produced (wt%)	Naphthalene Produced (wt%)
250	45	0	13.9	0.0	0.0
280	73	0	30.4	0.8	0.0
300	92	0	50.0	0.7	< 0.1
350	172	0	69.7	0.9	1.0
350	172	30	82.2	0.7	0.9
350	172	60	82.7	0.4	1.1
350	172	120	89.1	0.3	1.4
370	202	0	81.2	4.9	0.7
370	202	30	87.5	3.1	0.9
370	202	60	88.7	3.0	0.9
370	202	120	93.1	1.6	0.5
380	222	0	94.5	< 0.1	< 0.1
380	222	30	97.3	< 0.1	< 0.1
380	222	60	96.6	< 0.1	< 0.1
380	222	120	98.3	< 0.1	< 0.1

The conclusions reached by Onwudili and Williams showed that at subcritical temperature and pressure PAH degradation required longer reaction times and higher concentrations of hydrogen peroxide [73]. The high temperatures and pressures of the supercritical water incineration process allows high solubility and complete miscibility of organic compounds, rapid reaction rates, and complete decomposition of organic compounds. Up to 99.9 wt% destruction of PAHs occurs at supercritical conditions and the final combustion products are carbon dioxide and water.

The stability of phenanthrene in subcritical water was investigated by Yang and Hildebrand [26]. They believed that the decreased recovery in some subcritical water extractions at higher temperatures might be an indication that chemical degradation of the analytes was occurring. Extra peaks were also observed in chromatographic separations at higher

temperatures which also suggest chemical transformations may occur under certain subcritical water conditions.

In their experiments two different size stainless steel vessels were used as high-temperature reactors. The small reactors (3.02-mL volume, 6-cm length, 0.8-cm internal diameter) contained a high concentration of phenanthrene and the large reactors (7.07-mL volume, 9-cm length, 1-cm internal diameter) contained a low concentration of phenanthrene. Both deionized water and water with 3% hydrogen peroxide were used to determine the degradation and oxidation of phenanthrene in subcritical water. The effects of temperature on phenanthrene degradation in deionized water are shown in Table 4. The percentage of degraded phenanthrene for reactions in the small reactors (high concentration of phenanthrene) was not significant. However, the percentages increased considerably for reactions in the large reactors (low concentration of phenanthrene). The mass of phenanthrene degraded per milliliter of water in both size reactors was similar which indicated that the capacity of deionized water to degrade phenanthrene is the determining factor in phenanthrene degradation efficiency. It was also thought that the dissolved oxygen in water might be a contributor to the oxidizing power of subcritical water in this reaction system. It was hypothesized that significant degradation of phenanthrene was not observed in the literature during subcritical water extraction processes since the water used in most of these works was degassed before use so that a large portion of dissolved oxygen was removed from the water, thus weakening the oxidizing power of the water. The concentration of organic matter in most literature work was also much higher than the phenanthrene concentration in these experiments so the quantity of oxygen available in the degassed water could only degrade a small fraction of the organic matter present in the extraction vessel.

Table 4. Temperature Effect on Phenanthrene Degradation in Deionized Water (Modified from reference 26)

	High Concentration (3.68 mg/mL)		Low Concentration (0.393 mg/mL)	
Temperature (ºC)	**Degraded (mg/mL)**	**Degraded (%)**	**Degraded (mg/mL)**	**Degraded (%)**
100	0	0	0	0
150	0.009 ± 0.006	0.2	0.006 ± 0.003	1.5
200	0.029 ± 0.013	0.8	0.022 ± 0.006	5.6
250	0.077 ± 0.028	2.1	0.073 ± 0.011	18.5
300	0.140 ± 0.049	3.8	0.160 ± 0.040	40.6
350	0.206 ± 0.070	5.6	0.243 ± 0.034	61.9

Table 5 shows the results for the effect of temperature on phenanthrene degradation in a 3% hydrogen peroxide-97% water mixture. As was seen with the degradation of phenanthrene in deionized water, temperature had a significant effect on phenanthrene oxidation in the hydrogen peroxide-water system. These results show that both temperature and analyte concentration play roles in the oxidation of phenanthrene under subcritical water conditions.

Table 5. Temperature Effect on Phenanthrene Degradation in 3% Hydrogen Peroxide-97% Water Mixture (Modified from reference 26)

	High Concentration (3.68 mg/mL)		Low Concentration (0.393 mg/mL)	
Temperature (ºC)	**Degraded (mg/mL)**	**Degraded (%)**	**Degraded (mg/mL)**	**Degraded (%)**
100	0	0	0	0
150	0.195 ± 0.049	5.3	0.209 ± 0.042	53.2
200	0.596 ± 0.042	16.2	0.390 ± 0.031	99.2
250	3.12 ± 0.374	84.8	0.393 ± 0	100
300	3.58 ± 0.107	97.3	0.393 ± 0	100
350	3.68 ± 0	100	0.393 ± 0	100

Several degradation products of phenanthrene in subcritical water were identified by GC/MS. They included phenol, naphthalene, and 1-indanone at 300 to 350 ºC with 9-fluorenone, 4-hydroxy-9-fluorenone, 9,10-anthracenedione, and 9,10-phenanthrenedione observed at lower temperatures. Except naphthalene, all these oxidation products of phenanthrene were also found in the hydrogen peroxide-water system of the small reactors. Additionally, benzoic acid, phthalic anhydride, *ortho*-phthalaldehydic acid, and 1,8-napthalic anhydride were found in the reactions with hydrogen peroxide. It should be noted that organic acids including benzoic acid are frequently found as degradation products of PAHs. It was believed that no products were detected in the hydrogen peroxide-water system of the large reactors due to their conversion into carbon dioxide and water since gas escaped from the reactors when they were opened after high-temperature reactions.

High-temperature water extraction of herbs like basil and oregano is of interest because the cooking process may be mimicked by water extraction at temperatures around 100 ºC. What is found in the water extract after high-temperature water extraction may reflect the identity and quantity of the active ingredient found in a soup cooked with basil or oregano leaves. Yang, Kayan, Bozer, Pate, Baker, and Gizir, investigated the stability of five terpenes (α-pinene, limonene, camphor, citronellol, and carvacrol) often found in basil and oregano leaves under subcritical water conditions [17]. Stainless steel vessels with a 7.07-mL volume, a 9-cm length, and 1-cm internal diameter were used as high-temperature reactors for this study. Experiments were performed over a temperature range of 100 to 250 ºC and at heating times of 30 and 300 min. There was a clear temperature effect on terpene stability in subcritical water with α-pinene and limonene being the least stable. Terpene stability decreased with increasing temperature and when the heating time was extended to 300 min the percent degradation increased.

After optimizing conditions based on the degradation and recovery studies, basil and oregano leaves were extracted using water at both 100 and 150 ºC. The subcritical water extraction system that was used consisted of a Keystone extraction vessel (50-mm length, 4.6-mm internal diameter), an oven, two syringe pumps and several shutoff valves. The SBWE was performed dynamically with the sample placed in the extraction vessel, through which the extraction fluid flowed. The concentration of carvacrol in the oregano-water extract was found to be as high as 4270 µg of carvacrol/g of oregano. The concentrations of the other four terpenes found in both the basil-water extract and oregano-water extract ranged from only trace amounts up to 65 µg/g herb.

At the temperatures used for the subcritical water extraction of organic pollutants, bioactive compounds, like benzoic acid and derivatives, which are present in botanicals and medicinal plants could be degraded. Extraction methods must take into account the stability of the botanical extracts since the whole analytical process can be wasted if a severely degraded sample is produced. Parameters that can affect the subcritical water extraction efficiencies include temperature, extraction time, and the addition of a small percentage of organic solvent.

CHAPTER 3: EXPERIMENTAL

3.1 Chemicals and Materials

Pyrene (purity > 98%), naphthalene (purity > 99%), pentadecane (purity > 99+%), syringic acid (purity > 98%), and aniline (purity > 99.5+%) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Certified A.C.S. grade methylene chloride, benzoic acid, salicylic acid, benzene, sodium phosphate monobasic, and o-phosphoric acid 85%, USP grade hydrogen peroxide 3% w/v, reagent A.C.S. grade phenol, and HPLC grade acetonitrile were all acquired from Fisher Scientific (Fair Lawn, NJ, USA). Methylene chloride (purity > 99.9%), syringol (purity > 99%), and m-cresol (purity > 97%) were purchased from Acros Organics (Geel, Belgium). Anthranilic acid (practical) from Eastman Chemical Company (Kingsport, TN, USA), HPLC grade methanol from Burdick & Jackson (Morristown, NJ, USA), and methylene chloride (reagent grade ACS) from Pharmco Products Inc. (Brookfield, CT, USA) were also used. Deionized water (18 MΩ-cm) was prepared in our laboratory and purged using UHP Grade compressed helium from Machine and Welding Supply Company (Dunn, NC, USA) to remove dissolved oxygen when required. Acetone (meets ACS specifications) was obtained from VWR International, LLC (West Chester, PA, USA).

3.2 Reaction and Analytical Systems

Research on the stability of pyrene under subcritical water conditions was initially investigated using a large, dynamic reaction vessel. However, due to difficulties encountered during the course of experimentation, the research focus was shifted to using several smaller, static reaction vessels instead.

Experimentation was performed to determine the degradation of pyrene at 150 ºC using the large reaction vessel diagramed in Figure 3 and the system illustrated in Figure 4. The large reaction vessel was made of stainless steel and had an aluminum support collar. The reaction vessel was rinsed with acetone and allowed to dry prior to analysis. Approximately 6 mg of pyrene was weighed into a glass container, 150 mL of deionized water added, and the container placed inside the reaction vessel. The reaction vessel was sealed using a Teflon gasket and placed inside the oven of a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA, USA) to provide temperature control. After connecting the reactor's carbon dioxide inlet line and sampling outlet line it was heated for 1 hour at a pressure of 30 atm until a temperature of 150 ºC was reached. At the 60 min timepoint an attempt to withdraw a 5-mL aliquot of sample was unsuccessful. The plan was to determine the exact weight of the aliquot and repeat the sampling procedure every 30 min for the next 3 hours.

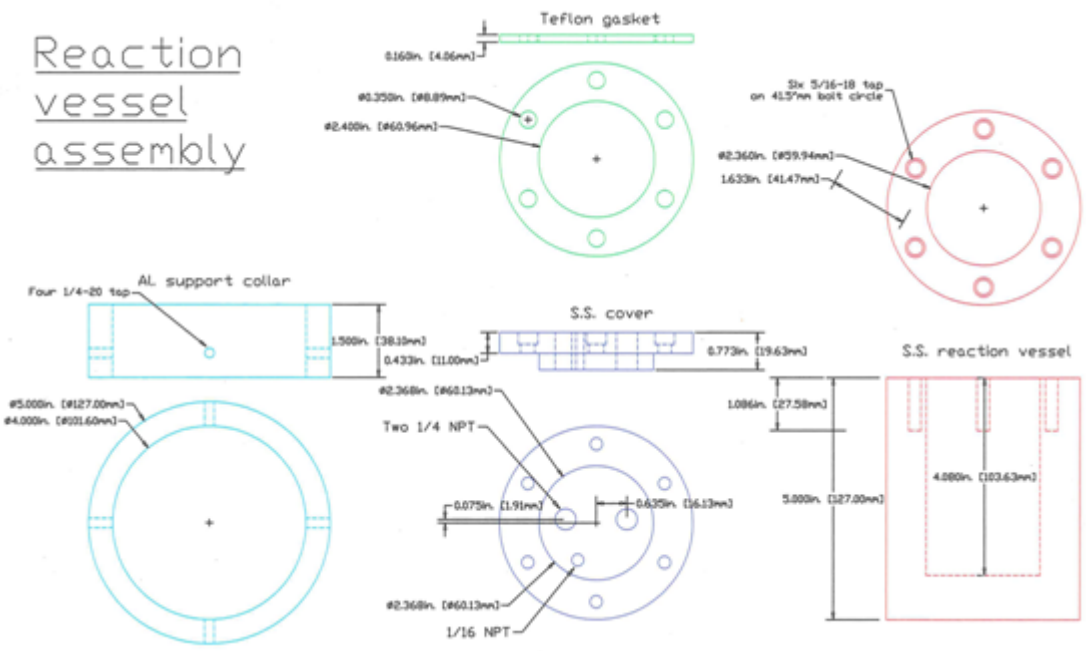


Figure 3. Dynamic Reaction Vessel.

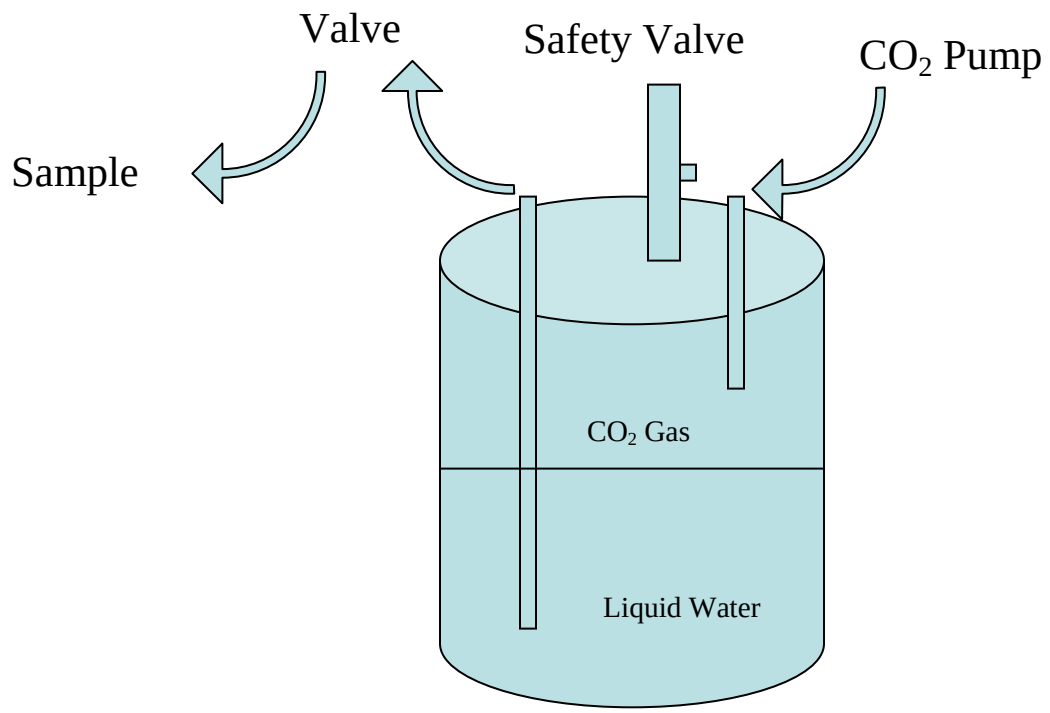


Figure 4. Dynamic Reaction Vessel System.

While troubleshooting the problem it was discovered that the reaction vessel was not sealing properly which allowed evaporation of water to occur. New polyimide gaskets and additional Teflon gaskets were manufactured. The reaction vessel was disassembled, all connections cleaned, and reassembled. The reaction vessel sealed properly but there were still difficulties withdrawing sample aliquots.

Due to the problems encountered using the large reaction vessel, the research focus was shifted to using static vessels, shown in Figure 5 for heating the mixtures of pyrene (or any other given analyte) and water. Stainless steel vessels with a 7.07-mL volume, a 9-cm length, and 1-cm internal diameter from Raleigh Valve and Fitting Company were used as high-temperature reactors. The vessels were machined by Jim Saupe in the Department of Physics so that a 10 x 75 mm borosilicate glass disposable culture tube could be placed inside each reaction vessel if desired.

Figure 5. Stainless Steel Vessel Used as a Static High-Temperature Reactor (Modified from Swagelok.com).

3.3 Experimental Procedures

3.3.1 Pyrene

Gas chromatography was used to quantify the amount of degradation for pyrene. An internal standard and four calibration standards were prepared for use in chromatographic analysis. The internal standard used was 10% pentadecane in methylene chloride. It was prepared by transferring 0.5 mL of pentadecane and 4.5 mL of methylene chloride by pipet into a glass vial and mixing well. A stock calibration standard was prepared by transferring 0.0205 g of pyrene into a glass vial and then adding 20 mL of methylene chloride by pipet and mixing well. This calibration standard had a concentration of 1025 ppm and was labeled Pyrene Standard A (PSA). Serial dilutions were made from the stock calibration standard to produce calibration standards with concentrations of 205 ppm, 51 ppm, 21 ppm, and 5 ppm. After each desired concentration was achieved, an appropriate volume of internal standard was added to produce a pentadecane concentration equivalent to the extraction sample concentration of 5 µL/mL.

Stainless steel vessels with a 7.07-mL volume, a 9-cm length, and 1-cm internal diameter were used as high-temperature reactors. The reaction vessels were rinsed with acetone and allowed to dry prior to analysis. Both ends of each reaction vessel were wrapped with Teflon tape and one end was sealed tightly with an end cap. Approximately 1 to 3 mg of pyrene was accurately weighed into each reaction vessel. Either 7 mL of deionized water or 7 mL of 3% w/v hydrogen peroxide was added to each vessel. The remaining volume of each reaction vessel was left as void for safety during the high-temperature reactions. The open end of each reaction vessel was sealed tightly with another end cap. The reaction vessels were placed inside the oven

of a Hewlett-Packard 5890 gas chromatograph to provide temperature control. The effect of temperature on pyrene degradation was determined in quadruplicate at temperatures of 200, 250, 300, and 350 ºC. The effect of time on pyrene degradation was determined at 30 and 300 min at each temperature.

The reaction vessels were heated from an initial temperature of 30 ºC to the desired temperature at a rate of 10 ºC/min. Once the reaction vessels reached the set temperature, they were held there for the desired amount of time. After the appropriate amount of time, heating was stopped and the reaction vessels were allowed to cool to room temperature. The reaction vessels were kept in the upright position, taken out of the oven, and one end cap removed. The water reaction mixture in each reaction vessel was transferred into a 10-mL glass vial. To the empty reaction vessel 1 mL of methylene chloride was added by pipet to rinse down the sides and remove any residue. The methylene chloride was then transferred into the glass vial containing the water reaction mixture. Using a syringe, 10 µL of the internal standard solution was added to the glass vial and a liquid-liquid extraction was performed. The water-methylene chloride extraction was performed by gently swirling the glass vial by hand. After mixing the two layers and allowing them to settle, the methylene chloride layer remained on the bottom of the glass vial. The methylene chloride layer was removed using a plastic transfer pipet and transferred into another glass vial. Another 1 mL of methylene chloride was added by pipet to the empty reaction vessel and then transferred into the glass vial containing the water reaction mixture. A liquid-liquid extraction was performed again. The methylene chloride layer was removed and combined with the first methylene chloride fraction in the glass vial. Both the water and methylene chloride layers were saved and stored in a refrigerator until they were analyzed.

3.2.2 Naphthalene

Gas chromatography was used to quantify the amount of degradation for naphthalene. An internal standard and five calibration standards were prepared for use in chromatographic analysis. The internal standard used was a neat solution of pentadecane. A stock calibration standard was prepared by transferring 0.0117 g of naphthalene into a glass vial and then adding 10 mL of methylene chloride by pipet and mixing well. This calibration standard had a concentration of 1170 ppm and was labeled Naphthalene Standard A (NSA). Serial dilutions were made from the stock calibration standard to produce calibration standards with concentrations of 213 ppm, 47 ppm, 27 ppm, and 6 ppm. After each desired concentration was achieved, an appropriate volume of internal standard was added to produce a pentadecane concentration equivalent to the extraction sample concentration of 1.25 µL/mL.

Stainless steel vessels with a 7.07-mL volume, a 9-cm length, and 1-cm internal diameter were used as high-temperature reactors. The reaction vessels were rinsed with acetone and allowed to dry prior to analysis. Both ends of each reaction vessel were wrapped with Teflon tape and one end was sealed tightly with an end cap. A 10 x 75 mm borosilicate glass disposable culture tube was placed inside each reaction vessel. Approximately 2 to 3 mg of naphthalene was accurately weighed into the glass culture tube of each reaction vessel. Either 3 mL of deionized water, 3 mL of degassed deionized water, or 3 mL of 3% w/v hydrogen peroxide was added to each reaction vessel. The remaining volume of each reaction vessel was left as void for safety during the high-temperature reactions. The open end of each reaction vessel was sealed tightly with another end cap. The reaction vessels were placed inside the oven of a Hewlett-Packard 5890 gas chromatograph to provide temperature control. The effect of

temperature on naphthalene degradation was determined in triplicate at temperatures of 150, 200, 250, 300, and 350 ºC. The effect of time on naphthalene degradation was determined at 30 and 300 min at each temperature.

The reaction vessels were heated from an initial temperature of 30 ºC to the desired temperature at a rate of 10 ºC/min. Once the reaction vessels reached the set temperature, they were held there for the desired amount of time. After the appropriate amount of time, heating was stopped and the reaction vessels were allowed to cool to room temperature. The reaction vessels were kept in the upright position, taken out of the oven, and one end cap removed. The glass tube was removed from each reaction vessel. The water reaction mixture in each glass tube was transferred into a glass vial. To the empty glass tube 2 mL of methylene chloride was added by pipet to rinse down the sides and remove any residue. The methylene chloride was then transferred into the glass vial containing the water reaction mixture. Using a syringe, 5 µL of the internal standard solution was added to the glass vial and a liquid-liquid extraction was performed. The water-methylene chloride extraction was performed by gently swirling the glass vial by hand. After mixing the two layers and allowing them to settle, the methylene chloride layer remained on the bottom of the glass vial. The methylene chloride layer was removed using a plastic transfer pipet and transferred into another glass vial. Another 2 mL of methylene chloride was added by pipet to the empty glass tube and then transferred into the glass vial containing the water reaction mixture. A liquid-liquid extraction was performed again. The methylene chloride layer was removed and combined with the first methylene chloride fraction in the glass vial. Both the water and methylene chloride layers were saved and stored in a refrigerator until they were analyzed.

3.3.3 Benzoic Acid and Derivatives

High performance liquid chromatography was used to quantify the amount of degradation for benzoic acid and the three chosen derivatives, identify the main degradation product of each acid, and quantify the amount of these degradation products. The mobile phases used were 0.02 *M* sodium phosphate buffer, pH 2.7 (mobile phase A) and methanol (mobile phase B). Mobile phase A was prepared by transferring approximately 2.76 g of sodium phosphate, monobasic, monohydrate, into 1000 mL of high purity water and mixed well to dissolve. The pH was adjusted to 2.7 using 21 to 22 drops of 85% phosphoric acid and mixed well. Two different internal standards and five calibration standards were prepared for use in chromatographic analysis. Anthranilic acid dissolved in methanol at a concentration of 0.500 mg/mL and syringic acid dissolved in methanol at a concentration of 0.500 mg/mL were used as internal standards. A stock calibration standard (to quantify the amount of degradation for each acid) was prepared by transferring 0.0100 g each of anthranilic acid, syringic acid, salicylic acid, and benzoic acid into a glass vial and then adding 10 mL of methanol by pipet and mixing well. This calibration standard had a concentration of 1.00 mg/mL for each acid and was labeled Acid Standard A (ASA). Serial dilutions were made from the stock calibration standard to produce calibration standards with concentrations of 0.182 mg/mL (ASB), 0.040 mg/mL (ASC), 0.023 mg/mL (ASD), and 0.005 mg/mL (ASE) for each acid. A second stock calibration standard (to quantify the amount of main degradation product for each acid) was prepared by transferring 0.0100 g each of aniline, phenol, anthranilic acid, syringic acid, syringol, and benzene into a glass vial and then adding 10 mL of methanol by pipet and mixing well. This calibration standard had a concentration of 1.00 mg/mL for each compound and was labeled Degradant Standard A (DSA). Serial dilutions were made from the stock calibration standard to produce calibration standards

with concentrations of 0.182 mg/mL (DSB), 0.040 mg/mL (DSC), 0.023 mg/mL (DSD), and 0.005 mg/mL (DSE) for each compound. Individual marker solutions of each acid and degradation product were prepared by transferring approximately 10 mg of each analyte into a glass vial and then adding 10 mL of methanol by pipet and mixing well. Marker solutions for the degradation products that were liquids at standard state were prepared by transferring approximately 10 mg of each analyte dropwise via syringe into a glass vial containing 10 mL of methanol and mixing well.

Stainless steel vessels with a 7.07-mL volume, a 9-cm length, and 1-cm internal diameter from Raleigh Valve and Fitting Company were used as high-temperature reactors. The reaction vessels were rinsed with acetone and allowed to dry prior to analysis. Both ends of each reaction vessel were wrapped with Teflon tape and one end was sealed tightly with an end cap. Approximately 5 mg of either anthranilic acid, syringic acid, salicylic acid, or benzoic acid was accurately weighed into each reaction vessel. 5 mL of degassed deionized water was added to each reaction vessel. The remaining volume of each reaction vessel was left as void for safety during the high-temperature reactions. The open end of each reaction vessel was then sealed tightly with another end cap. The reaction vessels were then placed inside a Fisher Scientific Isotemp Oven (Fair Lawn, NJ, USA) to provide temperature control. The effect of temperature on organic acid degradation was determined in triplicate at temperatures of 50, 100, 150, 200, and 250 ºC. The effect of time on organic acid degradation was determined at 10 and 30 min at each temperature.

The reaction vessels were heated from an initial temperature of 25 ºC to the desired temperature and held there for the desired amount of time. Counting of the heating time began

when the oven temperature reached the desired value. After the experimental conditions had been met, heating was stopped and the reaction vessels were allowed to cool to room temperature. The reaction vessels were kept in the upright position, taken out of the oven, and one end cap removed. The water reaction mixture in each reaction vessel was transferred into a 10-mL glass vial. Either anthranilic acid or syringic acid dissolved in methanol was used as an internal standard. Anthranilic acid was used if syringic acid, salicylic acid, or benzoic acid was the analyte and syringic acid was used if anthranilic acid was the analyte. To the empty reaction vessel 1 mL of internal standard solution was added by pipet to rinse down the sides of the reaction vessel and remove any residue. The internal standard solution was then transferred into the glass vial containing the water reaction mixture. Another 1 mL of internal standard solution was added by pipet to the empty reaction vessel and then transferred into the glass vial containing the water reaction mixture. The sample solution was mixed by gently swirling the glass vial by hand.

For selected experimental conditions, gas chromatography/mass spectrometry was used to confirm the identification of each main degradation product. Experiments were repeated as described above up to the point where the reaction vessels were allowed to cool to room temperature. The reaction vessels were taken out of the oven and one end cap removed. The water reaction mixture in each reaction vessels was transferred into a 10-mL glass vial. To the reaction vessel 2 mL of methylene chloride was added by pipet to rinse down the sides of the reaction vessel and remove any residue. The methylene chloride was then transferred into the glass vial containing the water reaction mixture. Using a syringe, 5 µL of an internal standard solution (a neat solution of m-cresol) was added to the glass vial and a liquid-liquid extraction was performed. The water-methylene chloride extraction was performed by gently swirling the

glass vial by hand. After mixing the two layers and allowing them to settle, the methylene chloride layer remained on the bottom of the glass vial. The methylene chloride layer was removed using a plastic transfer pipet and transferred into another glass vial. Another 2 mL of methylene chloride was added by pipet to the empty reaction vessel and then transferred into the glass vial containing the water reaction mixture. A liquid-liquid extraction was performed again. The methylene chloride layer was removed and combined with the first methylene chloride fraction in the glass vial.

3.4 Experimental Conditions

3.4.1 GC Analysis for Pyrene

A Hewlett-Packard 6890 Series Gas Chromatograph System with a flame ionization detector was used to analyze the pyrene samples using the following instrumental conditions. Approximately 1 µL of each pyrene sample was injected into the GC. The injection mode was splitless and the injector temperature was set at 300 ºC. The GC capillary column used was a HP-5 (Crosslinked 5% PH ME Siloxane) having a 30-m length and 0.25-µm film thickness. The initial oven temperature was 70 ºC with a 12 ºC increase per min until a final temperature of 300 ºC was reached. The final temperature was held for 2 min giving a total run time of 21.17 min. The flame ionization detector was set at a temperature of 300 ºC. HP GC ChemStation software Rev. A.07.01 was used to acquire the data. An injection of methylene chloride was run as a blank injection in order to bake the column. Four calibration standards were injected in order from least to most concentrated and a calibration curve was obtained which was used to calculate

the pyrene sample concentrations. After all pyrene samples were run another injection of methylene chloride was run as a blank injection.

3.4.2 GC Analysis for Naphthalene

An Agilent Technologies 6890N Network Gas Chromatograph System with a flame ionization detector was used to analyze the naphthalene samples using the following instrumental conditions. The injection volume was 1.0 µL for each naphthalene sample. The injection mode was split and the injector temperature was set at 300 ºC. The GC capillary column used was an Agilent Technologies HP-5 (5% Phenyl Methyl Siloxane) having a 30-m length and 0.250-mm film thickness. The initial oven temperature was 40 ºC with a 25 ºC increase per min up to a temperature of 70 ºC then the oven temperature was increased 10 ºC/min until a final temperature of 260 ºC was reached. The total run time was 20.20 min. The flame ionization detector was set at a temperature of 300 ºC. Agilent Technologies GC ChemStation software Rev. A.10.02 was used to acquire the data. An injection of methylene chloride was run as a blank injection in order to bake the column. Five calibration standards were injected in order from least to most concentrated and a calibration curve was obtained which was used to calculate the naphthalene sample concentrations. After all naphthalene samples were run another injection of methylene chloride was run as a blank injection.

3.4.3 HPLC Analysis for Benzoic Acid and Derivatives

A Waters gradient HPLC system equipped with a UV detector was used to quantify the organic acid samples. Separation was achieved using a Phenomenex Synergi Polar-RP column (80-Å, 100-mm x 4.60-mm, 4-micron) maintained at a temperature of 25 ºC with a hot pocket column heater. The mobile phases used were 0.02 *M* sodium phosphate buffer, pH 2.7 (mobile phase A) and methanol (mobile phase B). The gradient program was as follows: 75% A from 0-10 min, 70% A from 10-11 min, and 75% A from 11-15 min (re-equilibration step). The flow rate was set at 1.50 mL/min and injections were made using an autosampler with an injection volume of 10 µL for each sample. UV detection occurred at 254 nm and Waters Breeze Version 3.30 SPA was used to acquire the data. A calibration curve obtained from the ASA, ASB, ASC, ASD, and ASE calibration standards was used to calculate the amount of degradation that the organic acid samples experienced under each set of experimental conditions. There was one main degradation product observed for each organic acid. In order to calculate the amount of each degradation product present in the organic acid samples a calibration curve was obtained from the DSA, DSB, DSC, DSD, and DSE calibration standards.

3.4.4 GC/MS Analysis for Benzoic Acid and Derivatives

An Agilent Technologies 6890N Network GC System coupled with a JEOL Ltd. JMS-GCmate II Mass Selective Detector (MSD) was used to confirm the identity of the organic acid degradants. The carrier gas was helium and the column flow was 1 mL/min. The injection volume was 1 µL for each acid sample. The injection mode was split and the injector temperature was set at 250 ºC. The GC capillary column used was an Agilent HP-5MS (5%-Phenyl)-methylpolysiloxane (30-m x 0.250-mm, 0.25-µm film thickness). The oven

temperature profile was as follows: 30 ºC (hold 5.00 min) ramp at 20 ºC/min to 250 ºC (hold 5.00 min). The GC interface was set at a temperature of 250 ºC. The MSD had an electron impact (EI) source and the ion chamber was set at 200 ºC. The solvent delay time was 3 min and total run time was 21 min. MS scan parameters were from 12 m/z (low mass) to 522 m/z (high mass). Shrader Analytical and Consulting Laboratories, Inc. TSSPro Version 3.0 was used to acquire the data.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Polycyclic Aromatic Hydrocarbons

Results for the degradation of pyrene in water and 3% hydrogen peroxide at 30 min are shown in Table 6. With 30 min of heating in pure water, pyrene underwent greater degradation with increasing temperature. The relative standard deviation (% RSD) decreased with higher temperatures. An average percent degradation of 91.1% was achieved for heating pyrene in pure water at 350 ºC for 30 min. All results obtained for pyrene oxidation in 3% hydrogen peroxide at 30 min were very similar. An average percent degradation of greater than 99.1% was achieved for heating pyrene in 3% hydrogen peroxide at and above 250 ºC for 30 min.

Table 6. Summary of Pyrene Degradation after 30-min Heating

	Water		**3% H_2O_2**	
Temperature (ºC)	**Average % Degradation**	**% RSD n=4**	**Average % Degradation**	**% RSD n=4**
200	52.4	32.5	95.0	9.1
250	72.4	20.0	99.6	0.1
300	76.3	18.6	99.3	0.5
350	91.1	8.0	99.1	0.7

Results for the degradation of pyrene in water and 3% hydrogen peroxide with heating for 300 min is shown in Table 7. Although there was variability in the results for the study with 300 min of heating in pure water, in general, pyrene underwent greater degradation with

increasing temperature. The % RSD varied in an inconsistent manner at higher temperatures. There was no experimental explanation for these inconsistencies. An average percent degradation of 83.6% was achieved for heating pyrene in pure water at 350 ºC for 300 min. All results obtained for pyrene oxidation in 3% hydrogen peroxide at 300 min were very similar. An average percent degradation of greater than 99.1% was achieved for heating pyrene in 3% hydrogen peroxide at and above 200 ºC for 300 min.

Table 7. Summary of Pyrene Degradation after 300-min Heating

	Water		3% H_2O_2	
Temperature (ºC)	**Average % Degradation**	**% RSD n=4**	**Average % Degradation**	**% RSD n=4**
200	84.4	8.8	99.5	0.5
250	60.3	20.5	99.7	0.2
300	61.3	14.6	99.1	0.4
350	83.6	5.7	99.3	0.6

Results for the degradation of naphthalene in degassed water, water, and 3% hydrogen peroxide at 30 min are shown in Table 8. With 30 min of heating in either degassed water or pure water, naphthalene underwent greater degradation with increasing temperature. The % RSD decreased with higher temperatures. An average percent degradation of greater than 83.0% was achieved for heating naphthalene in either degassed water or pure water at 350 ºC for 30 min. All results obtained for naphthalene oxidation in 3% hydrogen peroxide at 30 min were

very similar. An average percent degradation of greater than 97.0% was achieved for heating naphthalene in 3% hydrogen peroxide at and above 150 ºC for 30 min.

Table 8. Summary of Naphthalene Degradation after 30-min Heating

	Degassed Water		Water		3% H_2O_2	
Temperature (ºC)	**Average % Degradation**	**% RSD n=5**	**Average % Degradation**	**% RSD n=5**	**Average % Degradation**	**% RSD n=3**
150	68.5	33.7	69.6	33.4	99.0	1.4
200	74.9	26.0	71.7	35.4	99.8	0.4
250	78.9	18.9	84.0	15.1	99.6	0.6
300	82.3	20.0	82.1	18.3	100.0	0.0
350	84.4	21.4	83.0	23.5	97.0	4.6

Results for the degradation of naphthalene in degassed water, water, and 3% hydrogen peroxide at 300 min is shown in Table 9. With 30 min of heating in either degassed water or pure water, naphthalene underwent greater degradation with increasing temperature. The % RSD decreased with higher temperatures. An average percent degradation of greater than 84.6% was achieved for heating naphthalene in either degassed water or pure water at 350 ºC for 300 min. An average percent degradation of greater than 99.9% was achieved for heating naphthalene in 3% hydrogen peroxide at and above 150 ºC for 300 min.

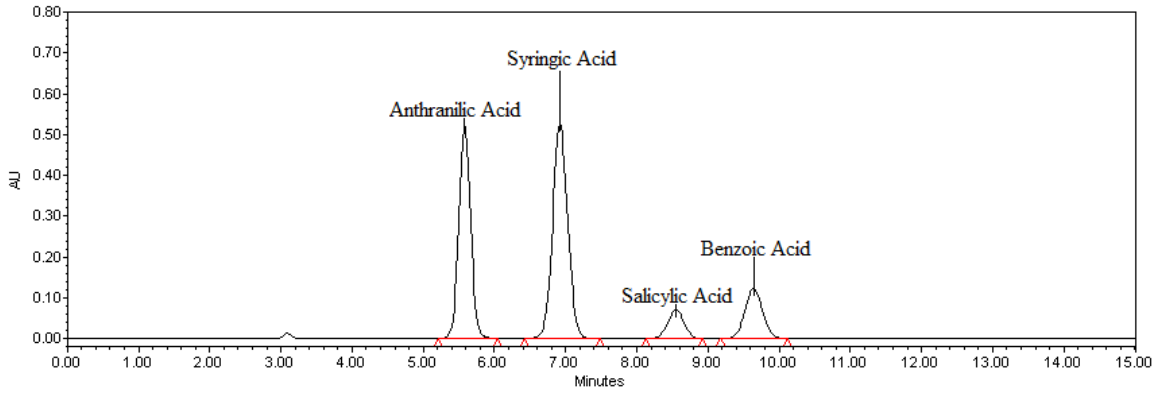
Table 9. Summary of Naphthalene Degradation after 300-min Heating

	Degassed Water		Water		3% H_2O_2	
Temperature (°C)	Average % Degradation	% RSD n=5	Average % Degradation	% RSD n=5	Average % Degradation	% RSD n=3
150	82.3	24.3	61.8	11.7	100.0	0.0
200	78.2	13.2	77.9	10.9	99.9	0.2
250	77.9	11.7	75.0	13.1	99.9	0.2
300	76.3	28.8	79.0	18.3	99.9	0.1
350	88.0	9.9	84.6	9.3	99.9	0.1

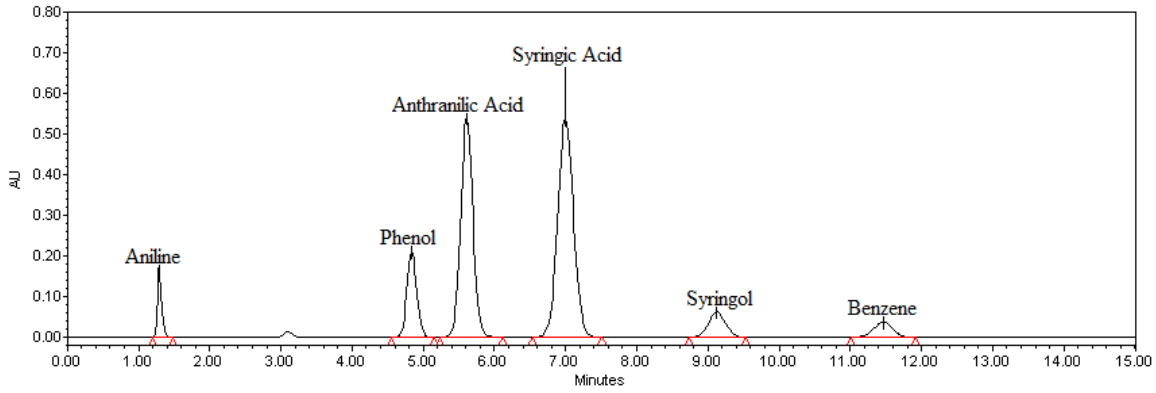
Pyrene and naphthalene were approximately 75% degraded after heating at 250 °C for 30 min in subcritical water. Although there was some variability in the results obtained for PAH degradation in water and degassed water, the reactions carried out using 3% hydrogen peroxide were very reproducible. The additional oxygen contained in the hydrogen peroxide solution has a positive effect on the degradation of PAHs. These results show that PAHs can be degraded under subcritical water conditions, and thus, this technique may be applied to the environmental remediation of these pollutants.

4.2 Benzoic Acid and Derivatives

High performance liquid chromatography was used to quantify the amount of degradation for each of the four acids, identify the main degradation product of each acid, and quantify the amount of these degradation products. Example HPLC chromatograms of the calibration standards Acid Standard A and Degradant Standard A are shown in Figure 6.



a)



b)

Figure 6. HPLC Chromatograms of a) Acid Standard A and b) Degradant Standard A.

4.2.1 Effects of Temperature and Heating Time on Degradation

Results for the degradation of anthranilic acid, syringic acid, salicylic acid, and benzoic acid in degassed water at 10 and 30 min are shown in Tables 10 and 11, respectively. Figure 7 illustrates these results graphically. All four acids remained stable at temperatures up to 100 ºC. Anthranilic acid, syringic acid, and salicylic acid showed minor degradation at 150 ºC. These three benzoic acid derivatives showed significant degradation at 200 ºC while benzoic acid remained stable. Anthranilic acid was the least stable followed by salicylic acid, syringic acid, and benzoic acid. All three benzoic acid derivatives were almost completely degraded at 250 ºC.

Benzoic acid was exceptionally stable throughout the course of the study, showing no degradation at 250 ºC.

At a given temperature, a longer exposure time causes greater acid degradation. For example, 56% of anthranilic acid was degraded after heating at 200 °C for 10 min. By lengthening the heating time to 30 min at the same temperature, anthranilic acid degradation increased to 91%. Similar trends were observed for salicylic acid and syringic acid. Additional experiments were performed to investigate the stability of benzoic acid at higher temperatures and longer heating times. As shown in Table 12, benzoic acid degradation at 350 °C was intensified from 4% to 46% by increasing the heating time from 10 to 630 min.

Table 10. Temperature Effect on Organic Acid Degradation in Degassed Water after 10-min Heating

	Anthranilic Acid		Syringic Acid		Salicylic Acid		Benzoic Acid	
Temperature (ºC)	Average % Degradation	% RSD n=3	Average % Degradation	% RSD n=3	Average % Degradation	% RSD n=3	Average % Degradation	% RSD n=3
50	4	1	0	N/A	5	4	0	N/A
100	0	N/A	0	N/A	0	N/A	0	N/A
150	0	N/A	1	2	3	2	5	2
200	56	5	16	2	26	3	6	2
250	100	1	95	1	98	2	0	N/A

Table 11. Temperature Effect on Organic Acid Degradation in Degassed Water after 30-min Heating

Temperature (°C)	Anthranilic Acid		Syringic Acid		Salicylic Acid		Benzoic Acid	
	Average % Degradation	% RSD n=3	Average % Degradation	% RSD n=3	Average % Degradation	% RSD n=3	Average % Degradation	% RSD n=3
50	0	N/A	0	N/A	0	N/A	0	N/A
100	0	N/A	0	N/A	0	N/A	0	N/A
150	9	1	4	2	6	4	3	2
200	91	2	44	1	69	4	7	5
250	100	1	99	1	98	3	0	N/A

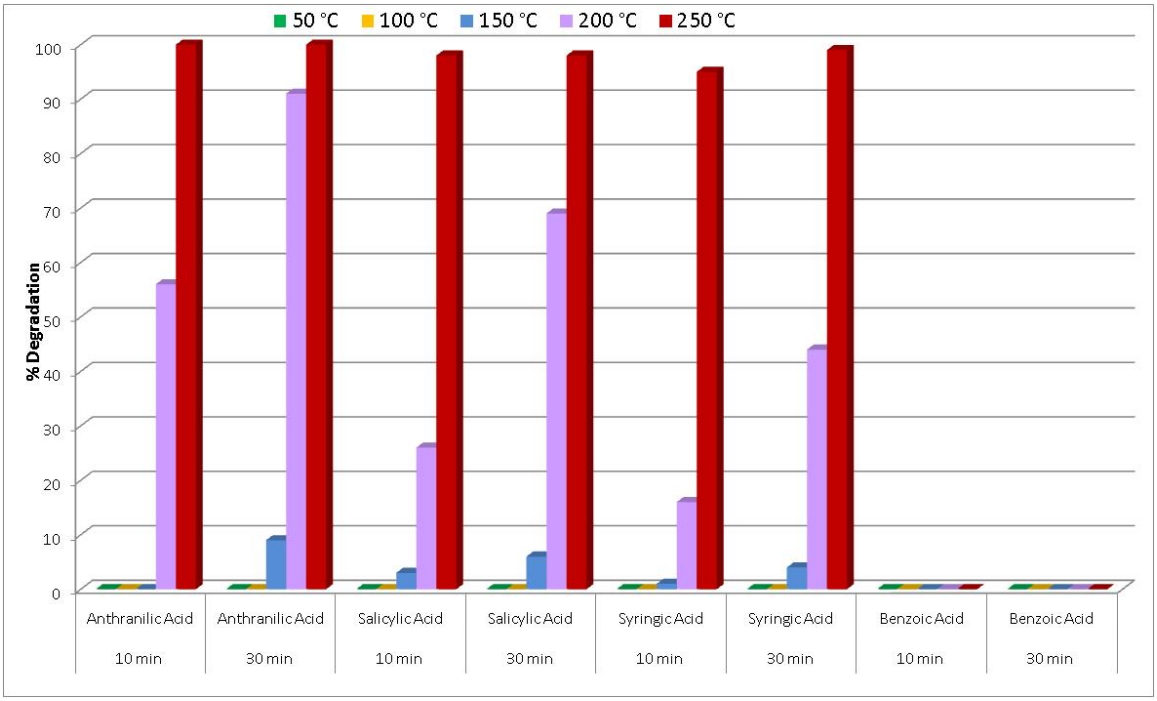
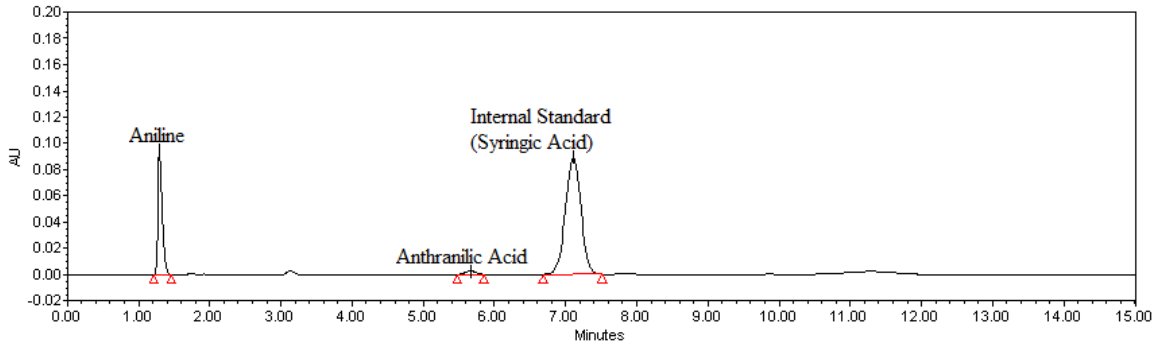


Figure 7. Effects of Temperature and Heating Time on the Stability of Benzoic Acid and Derivatives in Subcritical Water.

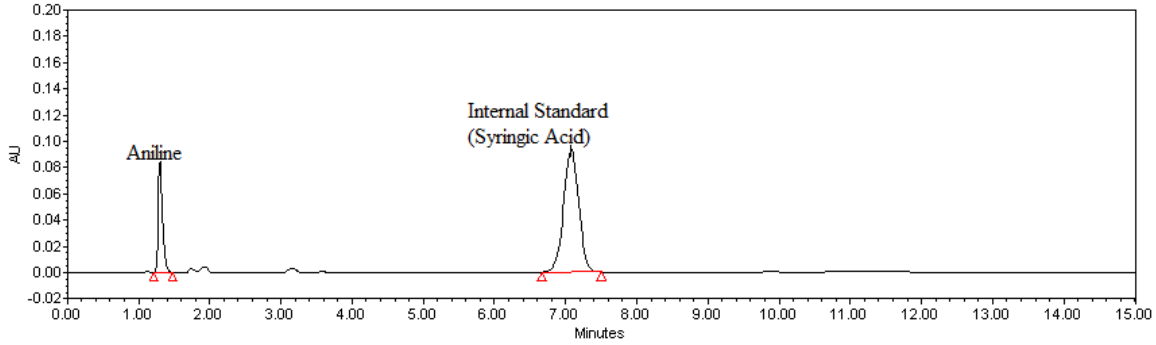
Table 12. Effects of Temperature and Heating Time on Benzoic Acid Degradation in Degassed Water

	Average % Degradation	% RSD n=3
250 °C / 60 min	0	N/A
250 °C / 90 min	1	1
250 °C / 120 min	7	14
250 °C / 150 min	0	N/A
250 °C / 180 min	3	2
250 °C / 210 min	8	20
250 °C / 240 min	1	5
250 °C / 270 min	3	1
250 °C / 300 min	0	N/A
300 °C / 10 min	0	N/A
300 °C / 30 min	3	0.8
350 °C / 10 min	4	5.5
350 °C / 30 min	7	5.7
350 °C / 90 min	16	1.2
350 °C / 150 min	21	7.6
350 °C / 210 min	24	3.3
350 °C / 270 min	33	3.1
350 °C / 330 min	36	5.6
350 °C / 390 min	37	1.0
350 °C / 450 min	35	5.4
350 °C / 510 min	40	8.8
350 °C / 570 min	43	6.1
350 °C / 630 min	46	8.8

Chromatograms of anthranilic acid, syringic acid, salicylic acid, and benzoic acid at different temperatures and times can be seen in Figures 8 through 11. The HPLC chromatograms of salicylic acid in Figure 10 clearly show that when the temperature is increased from 200 to 250 °C the area of the salicylic acid peak decreases while the area of the phenol peak increases. Thus, it can be concluded that salicylic acid degrades into phenol under subcritical water conditions. The same trend was observed for each acid/degradant pair showing that as each acid degrades under subcritical water conditions one main degradation product is formed.

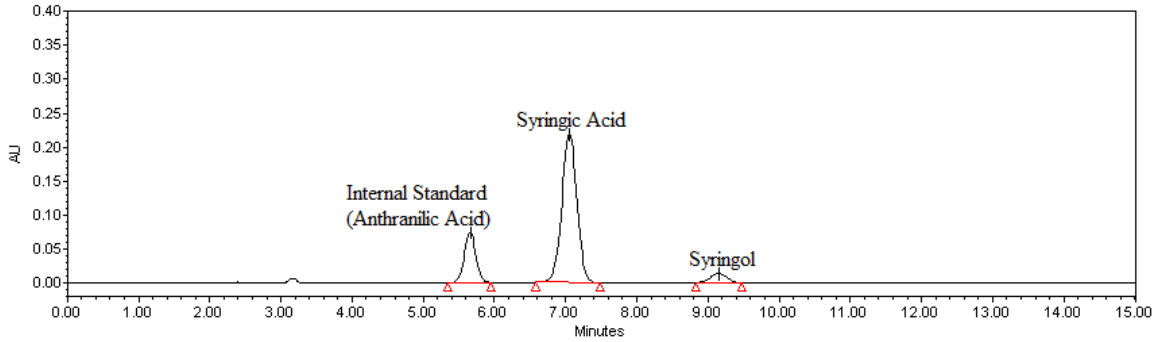


a)

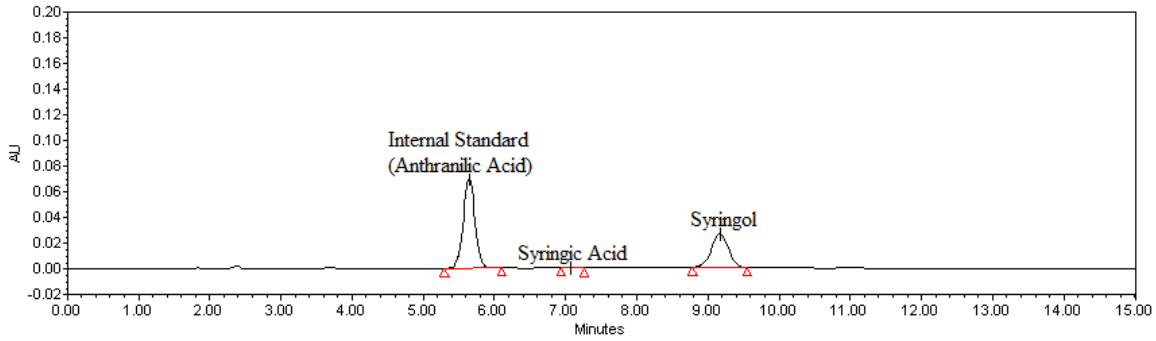


b)

Figure 8. HPLC Chromatograms of Anthranilic Acid at a) 200 °C for 30 min and b) 250 °C for 30 min.

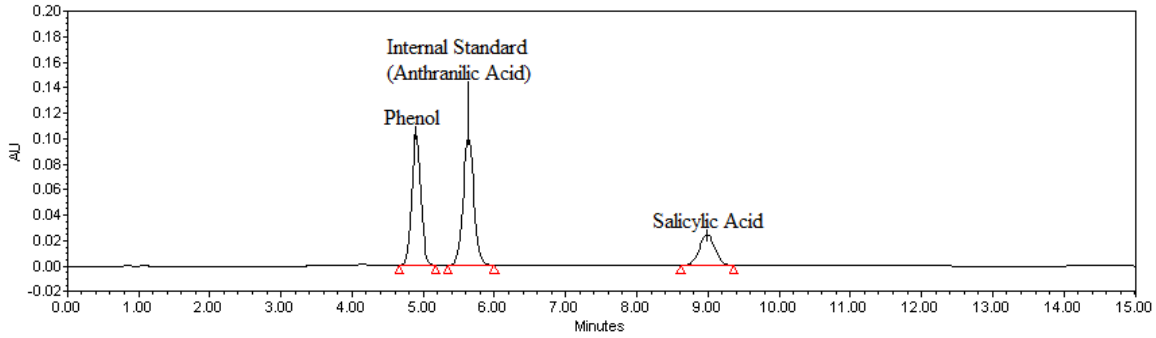


a)

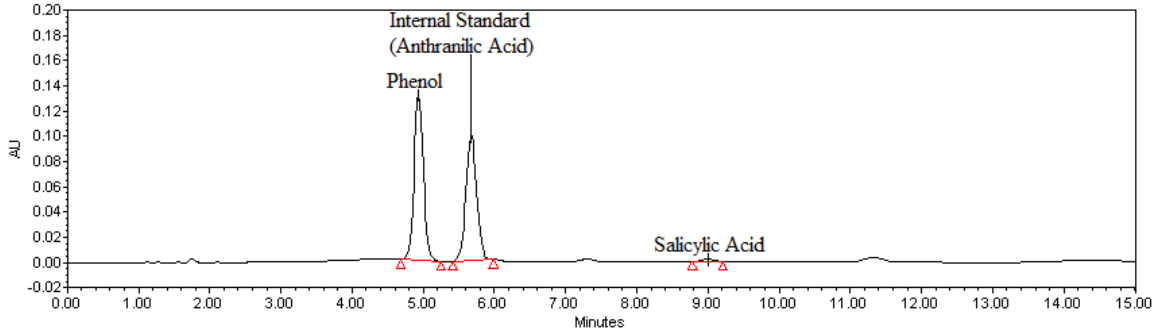


b)

Figure 9. HPLC Chromatograms of Syringic Acid at a) 200 °C for 30 min and b) 250 °C for 30 min.

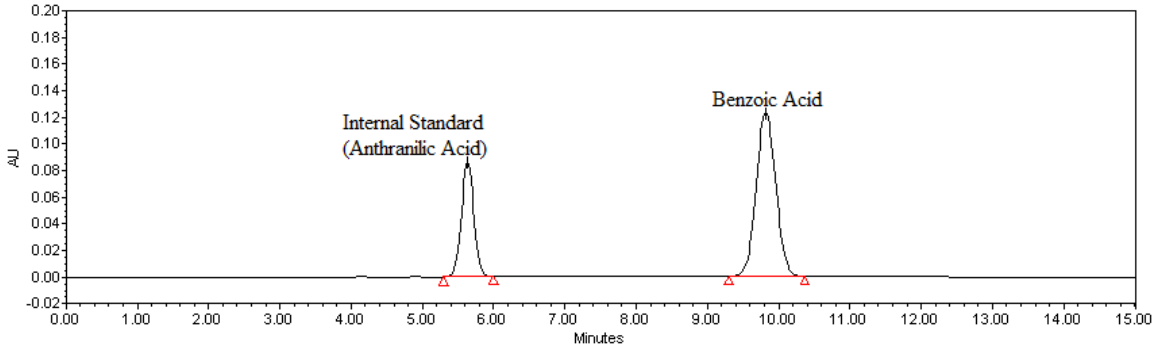


a)

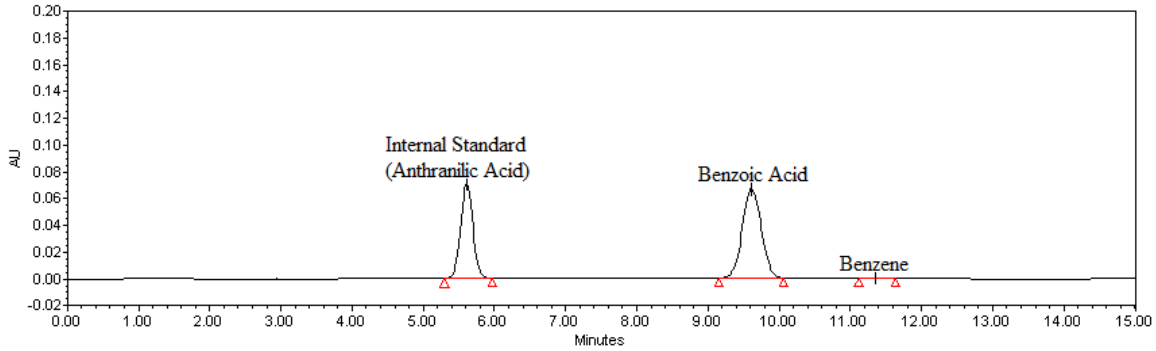


b)

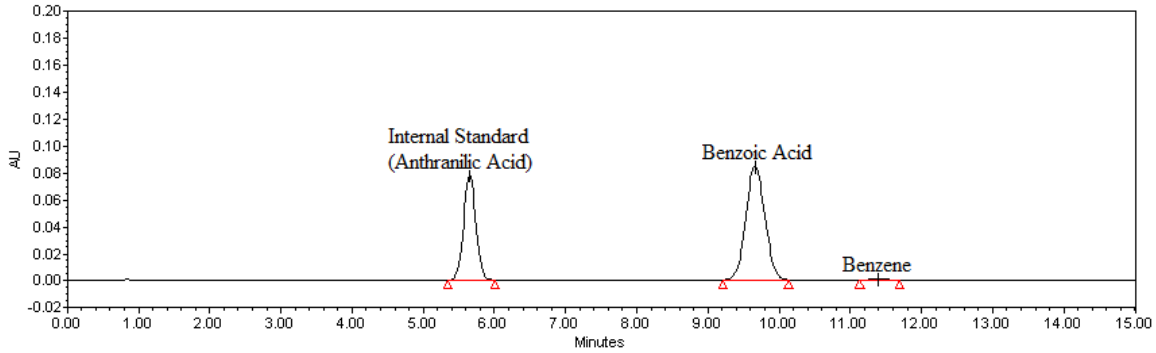
Figure 10. HPLC Chromatograms of Salicylic Acid at a) 200 °C for 30 min and b) 250 °C for 30 min.


Internal Standard
(Anthranilic Acid)
Benzoic Acid
AU
Minutes

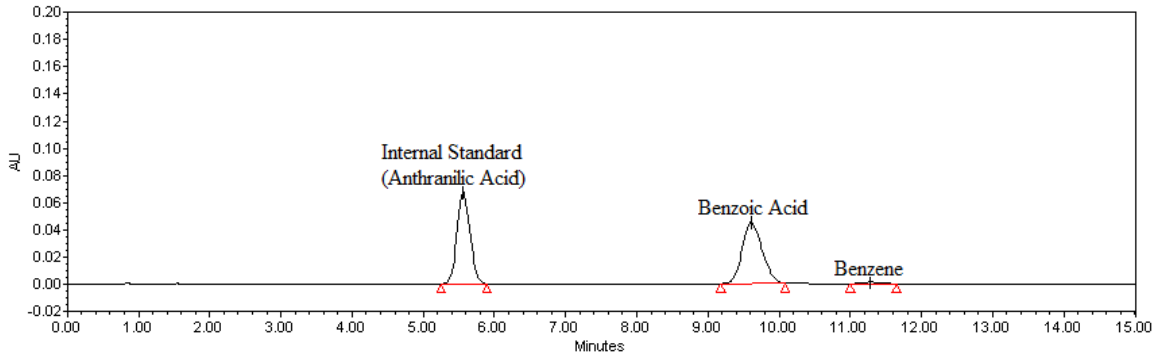
a)


Internal Standard
(Anthranilic Acid)
Benzoic Acid
Benzene
AU
Minutes

b)


Internal Standard
(Anthranilic Acid)
Benzoic Acid
Benzene
AU
Minutes

c)

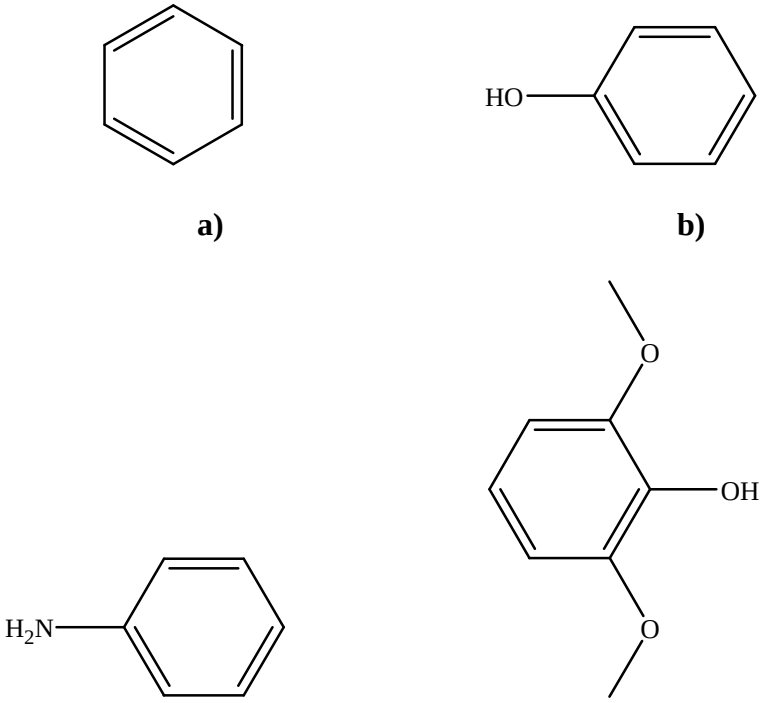


d)

Figure 11. HPLC Chromatograms of Benzoic Acid at a) 350 °C for 30 min, b) 350 °C for 150 min, c) 350 °C for 210 min, and d) 350 °C for 630 min.

4.2.2 Degradation Products and Mechanism

In order to characterize the degradation products of the acids, experiments were repeated at 200 and 250 ºC and the main degradation product for each acid was identified and quantified by HPLC. It was found that under subcritical water conditions anthranilic acid, syringic acid, salicylic acid, and benzoic acid undergo decarboxylation to form aniline, syringol, phenol, and benzene, respectively. The structures of these degradation products are shown in Figure 12.



a) **b)** **c)** **d)**

Figure 12. Structures of a) Benzene, b) Phenol, c) Aniline, and d) Syringol.

Calculations based on a 1:1 stoichiometry for each acid/degradant pair were performed and the results are shown in Tables 13 and 14. It is clear that for each acid the percent yield of the degradant is correlated with the percent degradation of the acid. As the degradation of each acid increases, a greater yield of the corresponding degradation product is seen.

Based on the 1:1 (acid:degradant) stoichiometry, the percent yield of the degradant should equal the percent degradation of the acid if there is only one degradation product for each acid. The data indicates that phenol is the only degradation product for salicylic acid since the percent degradation of salicylic acid is similar to the percent yield of phenol. The results for heating salicylic acid at 200 ºC for 30 min show an average percent yield of the degradant that is

greater than the average percent degradation of the acid. If the range of both results are taken into consideration (degradation high of 73%, yield low of 71%) this pair of data points fall into trend with the rest of the data obtained. The data for salicylic acid and phenol indicate that there is no degradation of phenol in subcritical water at 200 and 250 ºC. Previous studies performed in our laboratory show that phenol is stable in water at temperatures up to 250 ºC [95].

It should be noted that the percent yield of aniline and syringol is lower than the percent degradation for anthranilic acid and syringic acid, respectively. There are two possibilities for the lower than expected percent yield. One is that there may potentially be other degradation products. The other possibility is that the degradants are themselves degrading in subcritical water, causing the lower percent yield. Since the acid degradation mechanism is the same for all four acids that were studied and only one degradation product (phenol) was obtained from salicylic acid degradation, it is believed that degradation of the degradants in subcritical water is the reason for the lower yield of the degradation products.

Figure 13 illustrates the correlation of benzoic acid degradation and the formation of benzene in subcritical water at 350 °C. As with the other benzoic acid derivatives, there is a good correlation between benzoic acid degradation and benzene formation. It was also noted that the percent yield of benzene is lower than the percent degradation for benzoic acid. This is believed to be the result of benzene degradation in subcritical water as was seen with aniline and syringol.

Table 13. Quantification of Degradation Products for Anthranilic Acid, Syringic Acid, and Salicylic Acid

	Average % Degradation	% RSD n=3	Average Yield (%)	% RSD n=3
Anthranilic Acid 200 °C / 30 min	95	4.5	73% Aniline	25.2
Anthranilic Acid 250 °C / 30 min	100	0.1	81% Aniline	9.0
Syringic Acid 200 °C / 30 min	53	6.3	41% Syringol	2.3
Syringic Acid 250 °C / 30 min	100	0.1	67% Syringol	5.0
Salicylic Acid 200 °C / 30 min	70	4.6	78% Phenol	9.3
Salicylic Acid 250 °C / 30 min	99	1.5	97% Phenol	5.3

Table 14. Quantification of Degradation Products for Benzoic Acid

	Average % Degradation	% RSD n=3	Average Yield (% Benzene)	% RSD n=3
350 °C / 10 min	4	5.5	0	N/A
350 °C / 30 min	7	5.7	1	0.1
350 °C / 90 min	16	1.2	3	0.7
350 °C / 150 min	21	7.6	4	0.4
350 °C / 210 min	24	3.3	5	1.1
350 °C / 270 min	33	3.1	7	1.3
350 °C / 330 min	36	5.6	8	0.9
350 °C / 390 min	37	1.0	7	0.4
350 °C / 450 min	35	5.4	10	1.7
350 °C / 510 min	40	8.8	9	1.2
350 °C / 570 min	43	6.1	14	1.1
350 °C / 630 min	46	8.8	11	1.8

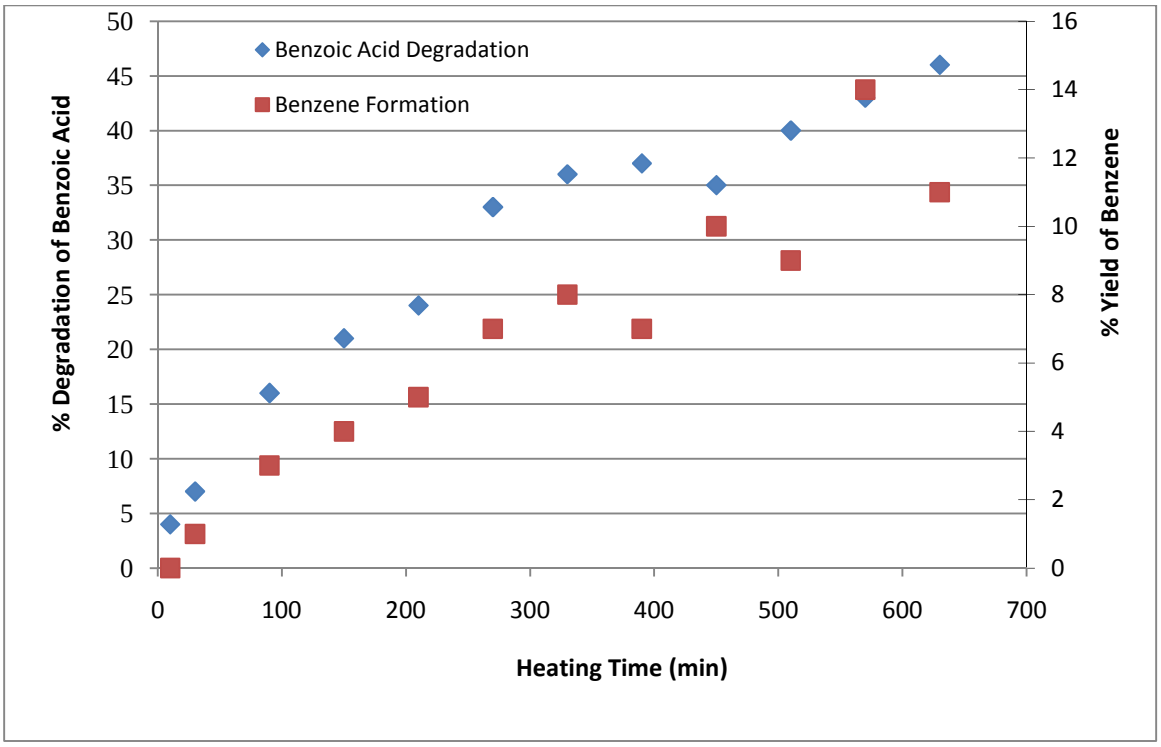
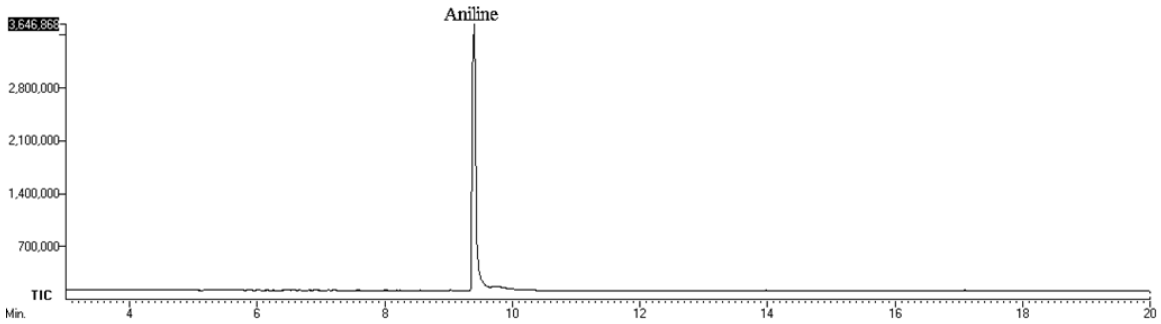
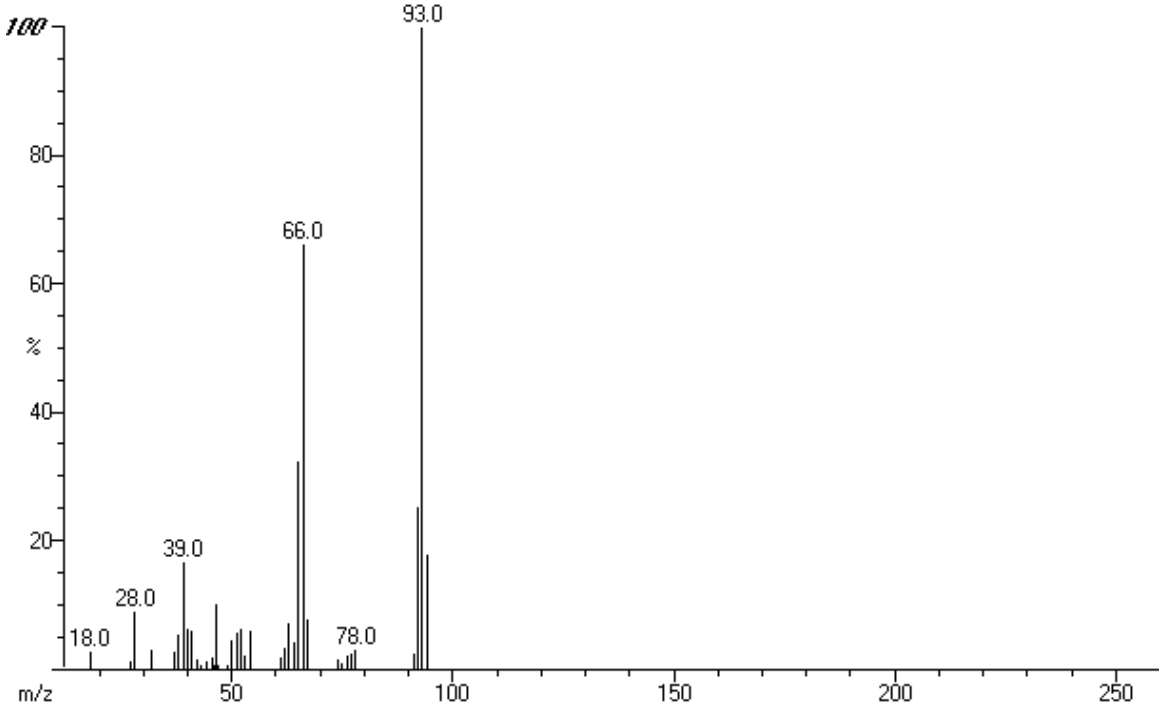


Figure 13. Effects of Heating Time on the Degradation of Benzoic Acid and the Yield of Benzene Formation in Subcritical Water at 350 °C.

GC/MS was used to verify the identification of the degradation product of each acid. Figures 14 through 17 contain the GC/MS chromatograms of each acid at selected experimental conditions and the corresponding mass spectrums of their degradants. The degradation products determined by HPLC were confirmed by GC/MS based on the retention time and mass spectra of the degradants.

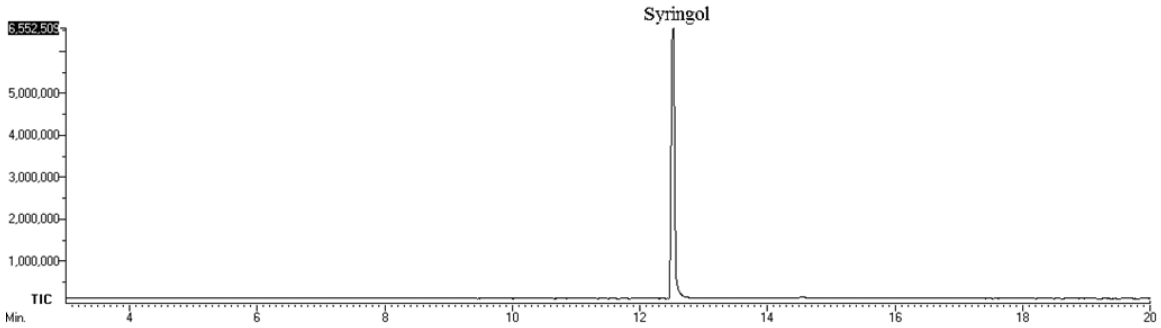


a)

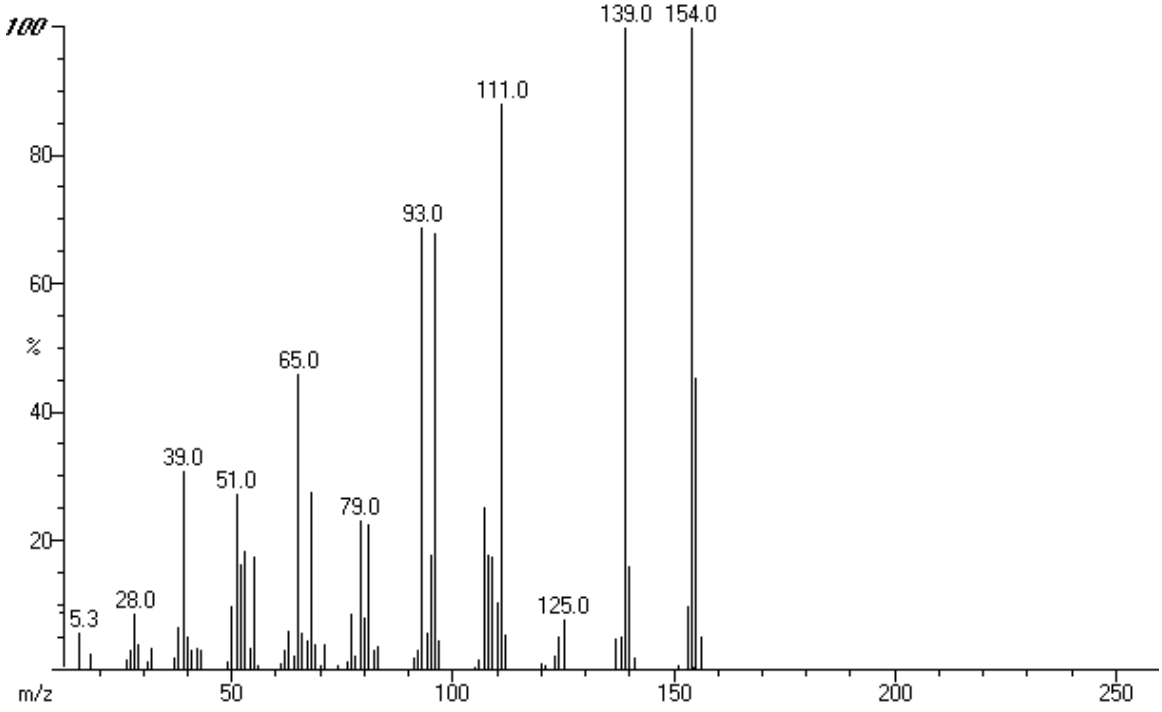


b)

Figure 14. a) GC/MS Chromatogram of Anthranilic Acid at 250 °C for 30 min and b) Aniline Mass Spectrum.

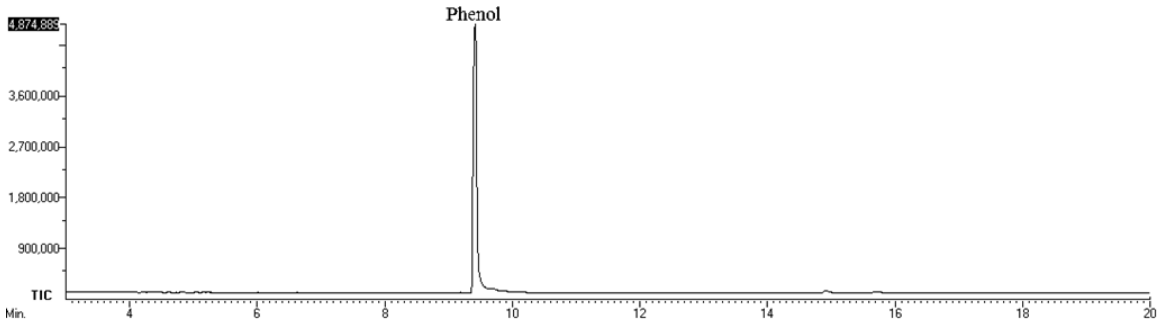


a)

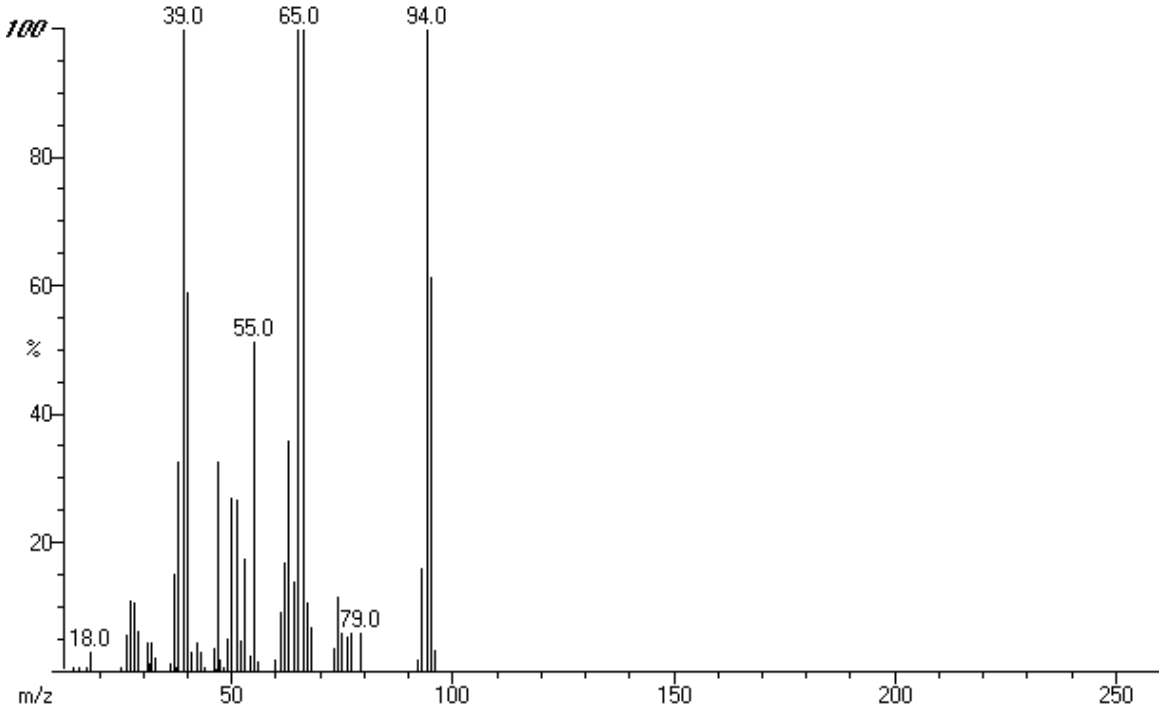


b)

Figure 15. a) GC/MS Chromatogram of Syringic Acid at 250 °C for 30 min and b) Syringol Mass Spectrum.

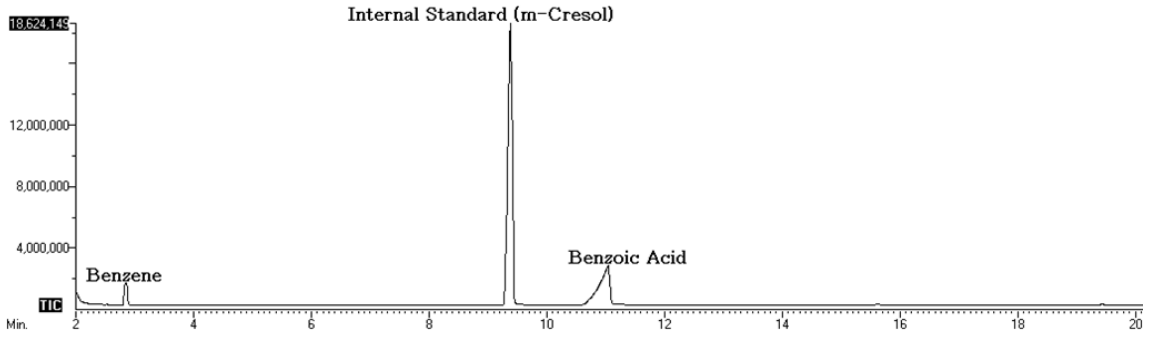


a)

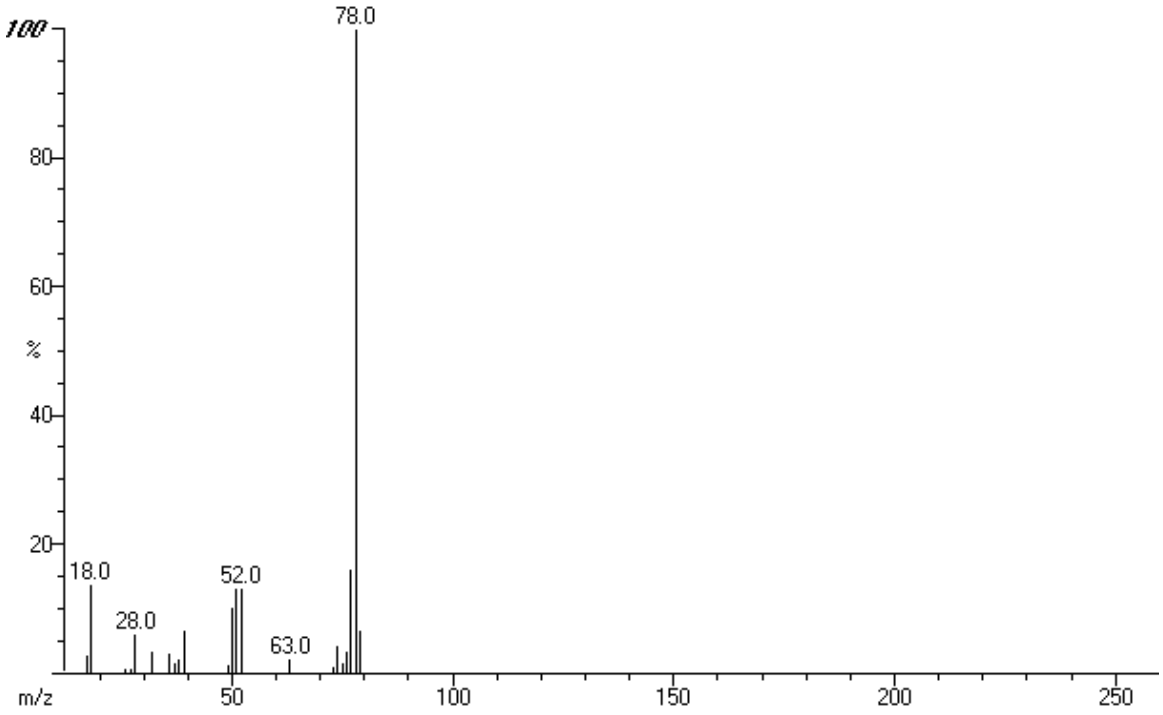


b)

Figure 16. a) GC/MS Chromatogram of Salicylic Acid at 250 °C for 30 min and b) Phenol Mass Spectrum.



a)



b)

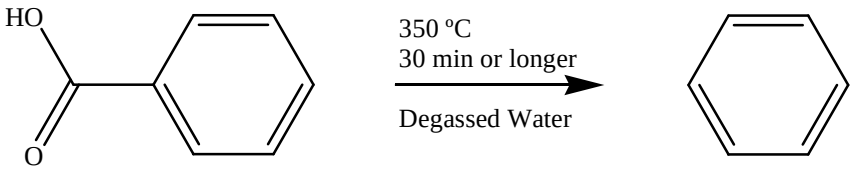
Figure 17. a) GC/MS Chromatogram of Benzoic Acid at 350 °C for 210 min and b) Benzene Mass Spectrum.

The first degradation product of each acid is the result of the loss of carbon dioxide from the acid. The results from the HPLC and GC/MS experiments support the decarboxylation reactions shown in Figure 18.

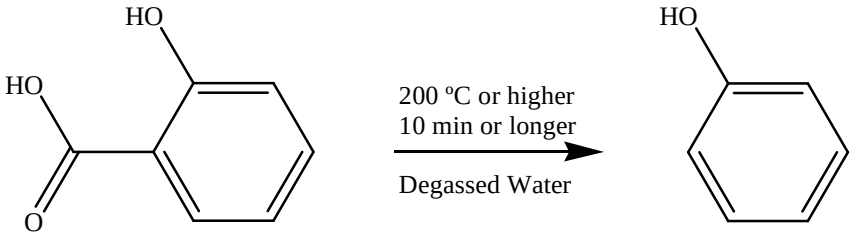
Chuchev and Belbruno proposed reaction mechanisms for the decarboxylation of *ortho*-substituted benzoic acids under neutral conditions [96]. Their computational studies of anthranilic acid involved a water molecule bridging the hydroxyl and amino groups to create a transition state containing a six-membered ring. As shown in Figure 19, the reaction begins with a lengthening of the carbon-carbon distance, followed by migration of the carboxyl hydrogen to the water. The reaction ends with a transfer of the water hydrogen to the benzene ring which results in a loss of carbon dioxide.

The benzoic acid derivatives have substituents that interact with the π-electrons in the benzene ring and have a stabilizing effect on the decarboxylation reactions. Additionally, if these substituents are in the *ortho*-position, physically close to the reaction site, they may participate more actively in stabilizing the transition state. Electron withdrawing substituents, such as a hydroxyl functional group (-OH), can reduce the hydrothermal stability of benzoic acid and make decarboxylation easier [97]. Hydroxyl substitution on the ring lowers the decarboxylation barrier of aromatic acids since the electron withdrawing nature of the hydroxyl substituent stabilizes the developing negative charge during the course of the reaction [96]. Electron donating substituents like a primary amine ($-NH_2$) can increase the electron density on the carbon atom next to the carboxy group and facilitate the approach of a proton which can also make decarboxylation easier [97]. The hydroxyl group in salicylic acid and the amino group in anthranilic acid also have the ability to contribute to hydrogen bond formation.

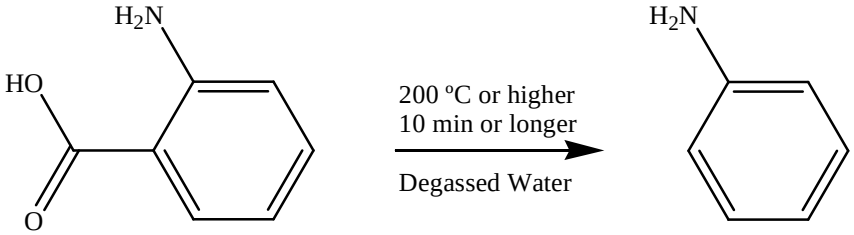
The decarboxylation results obtained from our experiments agree with the previously mentioned computational reaction model. The amino, methoxy, and hydroxyl groups of anthranilic acid, syringic acid, and salicylic acid, respectively, are activating groups. These activating groups direct the thermal decarboxylation of the *ortho*-carboxyl group for each benzoic acid derivative causing the degradation in subcritical water. Benzoic acid lacks an activating group which may account for its exceptional stability.



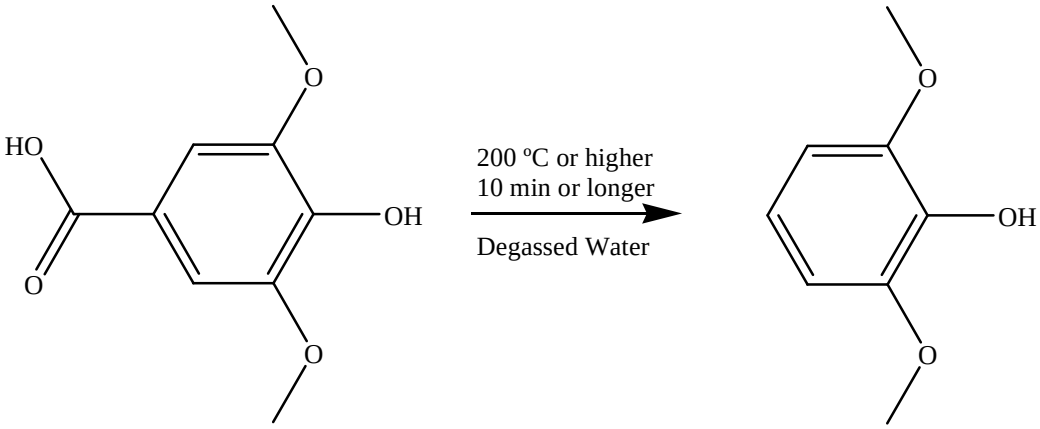
a)



b)



c)



d)

Figure 18. Degradation Reactions of a) Benzoic Acid, b) Salicylic Acid, c) Anthranilic Acid, and d) Syringic Acid.

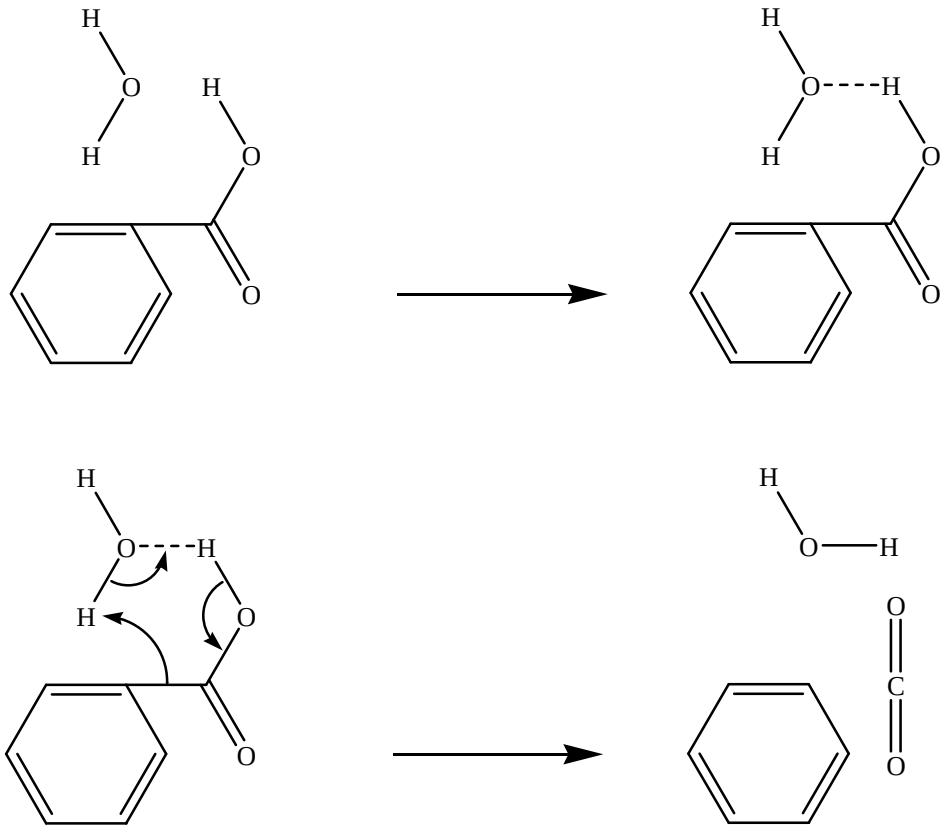


Figure 19. Benzoic Acid Decarboxylation Mechanism (Modified from reference 96).

CHAPTER 5: CONCLUSIONS

Pyrene and naphthalene were approximately 75% degraded after heating at 250 ºC for 30 min in subcritical water. Although there was some variability in the results obtained for PAH degradation in water and degassed water, the reactions carried out using 3% hydrogen peroxide were very reproducible. The additional oxygen contained in the hydrogen peroxide solution has a positive effect on the degradation of PAHs. These results show that PAHs can be degraded under subcritical water conditions, and thus, this technique may be applied to the environmental remediation of these pollutants.

The stability studies of anthranilic acid, syringic acid, salicylic acid, and benzoic acid under subcritical water conditions showed that all four acids remained stable in water at temperatures up to 100 ºC. The three benzoic acid derivatives showed mild degradation after heating in water at 150 ºC for 30 min. Severe degradation of the benzoic acid derivatives was observed at 200 ºC while their complete degradation occurred at 250 ºC. Benzoic acid remained stable at temperatures up to 300 ºC. The degradation products of each acid were identified and quantified by HPLC and further confirmed by GC/MS. Under subcritical water conditions anthranilic acid, syringic acid, salicylic acid, and benzoic acid undergo decarboxylation to form aniline, syringol, phenol, and benzene, respectively. The percent degradation of benzoic acid and its derivatives is in good correlation with the percent yield of their degradation products.

REFERENCES

1. S.B. Hawthorne, Y. Yang, D. Miller, *Analytical Chemistry* (1994) Vol. 66, 2912-2920
2. Y. Yang, M. Belghazi, A. Lagadec, D. Miller, S. Hawthorne, *Journal of Chromatography A* (1998) Vol. 810, 149-159
3. Y. Yang, *Journal of Separation Science* (2007) Vol. 30, 1131-1140
4. Y. Yang, B. Li, *Analytical Chemistry* (1999) Vol. 71, 1491-1495
5. L. Lamm, Y. Yang, *Analytical Chemistry* (2003) Vol. 75, 2237-2242
6. Y. Yang, S. Bowadt, S.B. Hawthorne, D.J. Miller, *Analytical Chemistry* (1995) Vol. 67, 4571-4576
7. Y. Yang, S.B. Hawthorne, D.J. Miller, *Environmental Science & Technology* (1997) Vol. 31, 430-437
8. T.M. Pawlowski, C.F. Poole, *Journal of Agricultural and Food Chemistry* (1998) Vol. 46, 3124-3132
9. M.D. Johnson, W. Huang, Z. Dang, W.J. Weber Jr., *Environmental Science & Technology* (1999) Vol. 33, 1657-1663
10. T. Andersson, K. Hartonen, T. Hyotylainen, M.-L. Riekkola, *Analytica Chimica Acta* (2002) Vol. 466, 93-100
11. R.M. Smith, *Journal of Chromatography A* (2002) Vol. 975, 31-46
12. J. Kronholm, K. Hartonen, M.-L. Riekkola, *Trends in Analytical Chemistry* (2007) Vol. 26, 396-412
13. M.S.S. Curren, J.W. King, *Journal of Chromatography A* (2002) Vol. 954, 41-49

14. A. Basile, M.M. Jimenez-Carmona, A.A. Clifford, *Journal of Agricultural and Food Chemistry* (1998) Vol. 46, 5205-5209
15. L. Gamiz-Gracia, M.D. Luque de Castro, *Talanta* (2000) Vol. 51, 1179-1185
16. M.Z. Ozel, F. Gogus, A.C. Lewis, et al., *Food Chemistry* (2003) Vol. 82, 381-386
17. Y. Yang, B. Kayan, N. Bozer, B. Pate, C. Baker, A. Gizir, *Journal of Chromatography A* (2007) Vol. 1152, 262-267
18. C. Deng, N. Li, X. Zhang, *Journal of Chromatography A* (2004) Vol. 1059, 149-155
19. J.-Y. Baek, J.-M. Lee, S.-C. Lee, *Separation and Purification Technology* (2008) Vol. 63, 661-664
20. O. Guclu-Ustundag, J. Balsevich, G. Mazza, *Journal of Food Engineering* (2007) Vol. 80, 619-630
21. E.S. Ong, J.S.H. Cheong, D. Goh, *Journal of Chromatography A* (2006) Vol. 1112, 92-102
22. W.-J. Kim, J. Kim, B. Veriansyah, J.-D. Kim, Y.-W. Lee, S.-G. Oh, R.R. Tjandrawinata, *The Journal of Supercritical Fluids* (2009) Vol. 48, 211-216
23. I. Rodriguez-Meizoso, L. Jaime, S. Santoyo, F.J. Senorans, A. Cifuentes, E. Ibanez, *Journal of Pharmaceutical and Biomedical Analysis* (2010) Vol. 51, 456-463
24. C. Deng, A. Wang, S. Shen, D. Fu, J. Chen, X. Zhang, *Journal of Pharmaceutical and Biomedical Analysis* (2005) Vol. 38, 326-331
25. C. Deng, N. Yao, A. Wang, X. Zhang, *Analytica Chimica Acta* (2005) Vol. 536, 237-244

26. Y. Yang, F. Hildebrand, *Analytica Chimica Acta* (2006) Vol. 555, 364-369

27. A.J.M. Lagadec, D.J. Miller, A.V. Lilke, S.B. Hawthorne, *Environmental Science & Technology* (2000) Vol. 34, 1542-1548

28. S.B. Hawthorne, C.B. Grabanski, E. Martin D.J. Miller, *Journal of Chromatography A* (2000) Vol. 892, 421-433

29. M. Khajenoori, A.H. Asl, F. Hormozi, *Chineese Journal of Chemical Engineering* (2009) Vol. 17, 359-365

30. A. Kubatova, D.J. Miller, S.B. Hawthorne, *Journal of Chromatography A* (2001) Vol. 923, 187-194

31. A. Kubatova, B. Jansen, J.-F. Vaudoisot, S.B. Hawthorne, *Journal of Chromatography A* (2002) Vol. 975, 175-188

32. E. Ibanez, A. Kubatova, F.J. Senorans, S. Cavero, G. Reglero, S.B. Hawthorne, *Journal of Agricultural and Food Chemistry* (2003) Vol. 51, 375-382

33. D.J. Miller, S.B. Hawthorne, *Analytical Chemistry* (1997) Vol. 69, 623-627

34. Y. Yang, *Analytica Chimica Acta* (2006) Vol. 558, 7-10

35. Y. Yang, T. Kondo, T.J. Kennedy, *Journal of Chromatographic Science* (2005) Vol. 43, 518-521

36. T. Kondo, Y. Yang, *Analytica Chimica Acta* (2003) Vol. 494, 157-166

37. P. He, Y. Yang, *Journal of Chromatography A* (2003) Vol. 989, 55-63

38. Y. Yang, L.J. Lamm, P. He, T. Kondo, *Journal of Chromatographic Science* (2002) Vol. 40, 107-112

39. Y. Yang, A.D. Jones, J.A. Mathis, M.A. Francis, *Journal of Chromatography A* (2002) Vol. 942, 231-236

40. Y. Yang, A.D. Jones, C.D. Eaton, *Analytical Chemistry* (1999) Vol. 71, 3808-3813
41. T.M. Pawlowski, C.F. Poole, *Analytical Communications* (1999) Vol. 36, 71-75
42. R.M. Smith, *Analytical and Bioanalytical Chemistry* (2006) Vol. 385, 419-421
43. J.W. Coym, J.G. Dorsey, *Analytical Letters* (2005) Vol. 37, 1013-1023
44. K. Hartonen, M.-L. Riekkola, *Trends in Analytical Chemistry* (2008) Vol. 27, 1-14
45. G. Vanhoenacker, P. Sandra, *Analytical and Bioanalytical Chemistry* (2008) Vol. 390, 245-248
46. C.V. McNeff, B. Yan, D.R. Stoll, R.A. Henry, *Journal of Separation Science* (2007) Vol. 30, 1672-1685
47. R.M. Smith, *Journal of Chromatography A* (2008) Vol. 1184, 441-455
48. H.A. Classens, M.A. van Straten, *Journal of Chromatography A* (2004) Vol. 1060, 23-41
49. R.L. Holliday, B.Y.M. Jong, J.W. Kolis, *The Journal of Supercritical Fluids* (1998) Vol. 12, 255-260
50. P.E. Savage, *Chemical Reviews* (1999) Vol. 99, 603-621
51. J.B. Dunn, M.L. Burns, S.E. Hunter, P.E. Savage, *The Journal of Supercritical Fluids* (2003) Vol. 27, 263-274
52. A. Kubatova, A.J.M. Lagadec, S.B. Hawthorne, *Environmental Science & Technology* (2002) Vol. 36, 1337-1343
53. R.L. Holiday, J.W. King, G.R. List, *Industrial & Engineering Chemistry Research* (1997) Vol. 36, 932-935

54. K. Chandler, F. Deng, A.K. Dillow, C.L. Liotta, C.A. Eckert, *Industrial & Engineering Chemistry Research* (1997) Vol. 36, 5175-5179

55. M. Siskin, A.R. Katritzky, *Journal of Analytical and Applied Pyrolysis* (2000) Vol. 54, 193-214

56. M. Siskin, A.R. Katritzky, *Chemical Reviews* (2001) Vol. 101, 825-835

57. A.R. Katritzky, D.A. Nichols, M. Siskin, R. Murugan, M. Balasubramanian, *Chemical Reviews* (2001) Vol. 101, 837-892

58. A.A. Galkin, V.V. Lunin, *Russian Chemical Reviews* (2005) Vol. 74, 21-35

59. D.J. Miller, S.B. Hawthorne, A.M. Gizir, A.A. Clifford, *Journal of Chemical Engineering Data* (1998) Vol. 43, 1043-1047

60. T.S.C. Li, *Chinese and Related North American Herbs: Phytopharmacology and Therapeutic Values*, CRC Press (2002)

61. B. Kayan, Y. Yang, E.J. Lindquist, A.M. Gizir, *Journal of Chemical & Engineering Data* (2010) Vol. 55, 2229-2232

62. Z. Khan, J. Troquet, C. Vachelard, *International Journal of Environmental Science and Technology* (2005) Vol. 2, 275-286

63. U.P. Vijh, A.N. Witt, K.D. Gordon, *The Astrophysical Journal* (2004) Vol. 606, L65-L68

64. J. Jacob, *Pure & Applied Chemistry* (1996) Vol. 68, 301-308

65. S.B. Hawthorne, C.B. Grabanski, *Environmental Science & Technology* (2000) Vol. 34, 4348-4353

66. P.H. Howard, R.S. Boethling, W.F. Jarvis, W.M. Meylan, E.M. Michalenko, *Handbook of Environmental Degradation Rates* (1991)

67. A.R. Johnsen, L.Y. Wick, H. Harms, *Environmental Pollution* (2005) Vol. 133, 71-84

68. X. Wang, Z. Gong, P. Li, L. Zhang, X. Hu, *Environmental Engineering Science* (2008) Vol. 25, 677-684

69. L. Hua, W. Wu, Y. Liu, Y. Chen, M.B. McBride, *Water Air & Soil Pollution* (2008) Vol. 193, 259-267

70. H. Yi, D.E. Crowley, *Environmental Science & Technology* (2007) Vol. 41, 4382-4388

71. B. David, *Ultrasonics Sonochemistry* (2009) Vol. 16, 260-265

72. J.S. Miller; D. Olejnik, *Water Research.* (2001) Vol. 35, 233-243

73. J. A. Onwudili, P.T. Williams, *International Journal of Energy Research* (2006) Vol. 30, 523-533

74. J. Xie, W. Wang, Y. Zhang, Y. Bai, Q. Yang, *Journal of Pharmaceutical and Biomedical Analysis* (2007) Vol. 45, 450-455

75. A. Endale, B. Kammerer, T. Gebre-Mariam, P.C. Schmidt, *Journal of Chromatography A* (2005) Vol. 1083, 32-41

76. N.Y. Qin, F.Q. Yang, Y.T. Wang S.P. Li, *Journal of Pharmaceutical and Biomedical Analysis* (2007) Vol. 43, 486-492

77. P. Chen, C. Li, S. Liang, G. Song, Y. Sun, Y. Shi, S. Xu, J. Zhang, S. Sheng, Y. Yang, M. Li, *Journal of Chromatography B* (2006) Vol. 843, 183-193

78. X. Yuan, H.-L. Koh, W.-K. Chui, *Journal of Pharmaceutical and Biomedical Analysis* (2005) Vol. 39, 697-704

79. N.G. Tayade, M.S. Nagarsenker, *Journal of Pharmaceutical and Biomedical Analysis* (2007) Vol. 43, 839-844

80. S.-C. Hsieh, M.-F. Huang, B.-S. Lin, H.-T. Chang, *Journal of Chromatography A* (2006) Vol. 1105, 127-134

81. Monographs on Fragrance Raw Materials: Benzoic Acid, *Food and Cosmetics Toxicology* (1979) Vol. 17, 715-722

82. L. Jiao, S. Gao, F. Zhang, H. Li, *Talanta* (2008) Vol. 75, 1061-1067

83. T.M. Schmitt, R.J. Ziegler, E.S. Muzher, R.J. Doyle, J.L. Freers, *Tahnta* (1986) Vol. 33, 657-660

84. S. Sharma, V.K. Srivastava, A. Kumar, *European Journal of Medicinal Chemistry* (2002) Vol. 37, 689-697

85. A. Kumar, D. Bansal, K. Bajaj, S. Sharma, A. Srivastava, V.K. Srivastava, *Bioorganic & Medicinal Chemistry* (2003) Vol. 11, 5281-5291

86. C.S.P. Sastry, A.S.R.P. Tipirneni, M.V. Suryanarayana, *Microchemical Journal* (1989) Vol. 39, 277-282

87. P. Trifilo, F. Raimondo, A. Nardini, M.A. Lo Gullo, S. Salleo, *Tree Physiology* (2004) Vol. 24, 107-114

88. V. De Feo, L. De Martino, A. Santoro, A. Leone, C. Pizza, S. Franceschelli, M. Pascale, *Phytotherapy Research* (2005) Vol. 19, 226-230

89. V. De Feo, L. De Martino, E. Quaranta, C. Pizza, *Journal of Agricultural and Food Chemistry* (2003) Vol. 51, 1177-1180

90. N. Yalpani, I. Raskin, *Trends in Microbiology* (1993) Vol. 1, 88-91

91. C. Oberthur, R. Jaggi, M. Hamburger, *Fitoterapia* (2005) Vol. 76, 324-332

92. A. Brattstrom, A. Schapowal, M.A. Kamal, I. Maillet, B. Ryffel, R. Moser, *Phytomedicine* (2010) Vol. 17, 551-556

93. J.A. Onwudili, P.T. Williams, *Fuel* (2006) Vol. 85, 75-83

94. J.A. Onwudili, P.T. Williams, *Journal of Supercritical Fluids* (2007) Vol. 39, 399-408

95. A.D. Jones, Subcritical Water Degradation and Extraction of Organochlorine Pollutants from Environmental Solids, Thesis, East Carolina University, 2002

96. K. Chuchev, J.J. Belbruno, *Journal of Molecular Structure: THEOCHEM* (2007) Vol. 807, 1-9

97. J. Fu, P.E. Savage, X. Lu, *Industrial & Engineering Chemistry Research* (2009) Vol. 48, 10467-1047