5-2016

# High intensity ultrasound assisted extraction of oak compounds for accelerated aging of wines and whiskies

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To the Graduate Council:

I am submitting herewith a thesis written by Lindsay Elizabeth Rogerson entitled "High intensity ultrasound assisted extraction of oak compounds for accelerated aging of wines and whiskies." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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# High intensity ultrasound assisted extraction of oak compounds for accelerated aging of wines and whiskies

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Lindsay Elizabeth Rogerson
May 2016

# ACKNOWLEDGEMENTS

I would first like to thank The University of Tennessee Institute of Agriculture for the UTIA Innovation Grant- “Production of oak wood extracts for use in wines and whiskeys” which funded the beginning stages of this research.

I would like to thank my advisor, Dr. Mark Morgan, for many hours of guidance towards my thesis and who has helped me overcome many hurdles during this process. I would also like to thank my committee member, Dr. Tim Young, who became an invaluable mentor to me in the last year and who has given me countless opportunities to network and learn outside of my graduate program and who has also given me the encouragement I needed to persevere. I would also like to thank Dr. Shawn Campagna for lending his time and expertise to help me refine and strengthen my thesis.

I would like to thank Dr. Svetlana Zivanovic for being my first mentor, welcoming me into her lab as a lost undergraduate student looking for a new research experience, and inviting me to the Food Science Graduate Program. It was in her Food Chemistry class where I found true excitement about a subject and the desire to pursue Food Science as a career.

I would also like to thank Anton Astner and Philipus Pangloli for their help in instrumentation and sample preparations and my family, fellow graduate students, and friends for their countless encouragement and support throughout this process.

# ABSTRACT

Aging of wines and whiskies in oak barrels is a timely and expensive process which could be reduced by acceleration. The purpose of this study is to identify if the use of high intensity ultrasound (HIUS) assisted extraction as an alternative, accelerated aging method could be utilized in the production of an oak extract to be used in wine and whiskies. HIUS will also be compared to reflux and room-temperature control extraction treatments as other accelerated aging methods. Secondary objectives of this study were to compare the heat treatment of charred and toasted staves donated by an anonymous donor, their individual layers, and whether time had an effect on the extraction of color, total soluble phenolics, pH level, and oak compounds identified by gas and liquid chromatography from extraction treatments, heat treated oak staves, and individual oak stave layers. Analysis of variance and Tukey HSD showed that reflux extraction treatment extracted more color, total soluble phenolics, and ellagic acid than sonication or control extraction treatments; however, after 3 months, there was no significant difference among extraction treatments for extraction of color or total soluble phenolics. Results also showed that toasted staves had a higher availability of oak compounds than charred oak staves, but were similar for color, total soluble phenolics, pH, and various oak compounds after 3 months. Similarly, individual layers had a similar amount of each compound extracted over time, but had significant differences between layers such as charred and toasted inner-layers compared to charred and toasted outer layers. HIUS was found to be a viable and controllable accelerated, extraction method; however, reflux was shown to be more effective during this study. If future studies are

done on the desirability of oak compounds, determination as to which method is a more effective method for production of an oak extract and the reduction of variability could change based on what compounds to include. A prepared oak extract utilizing desirable oak compounds would benefit the flavor and quality of aged wines and whiskies by reducing the time and cost of the aging process.

# TABLE OF CONTENTS

1. Literature Review ..... 1
2. Materials and Methods ..... 21
3. Results and Discussion ..... 29
4. Conclusions and Recommendations ..... 137
List of References ..... 143
Appendices ..... 153
Vita……..150

# LIST OF TABLES

Table 1. Solvent Gradient for Reversed-Phase HPLC Analysis of Polyphenolics……...27
Table 2. Stave width and length measurements prior to shaving……153
Table 3. Amount of oak shavings…..……..153
Table 4. Randomization of layers for each extraction treatment……..154
Table 5. Total Soluble Phenolics standard curve……...155
Table 6. HPLC Standard retention times……....156
Table 7. HPLC Gallic Acid standard curve……...156
Table 8. HPLC Protocatechuic Acid standard curve……..157
Table 9. HPLC Protocatechuic Aldehyde standard curve……158
Table 10. HPLC Methyl Gallate standard curve……..159
Table 11. HPLC Vanillic Acid standard curve……..160
Table 12. HPLC Syringealdehyde standard curve ……161
Table 13. HPLC Scopoletin standard curve ….....……....162
Table 14. HPLC Ellagic Acid standard curve……...163
Table 15. HPLC Sinapaldehyde standard curve ...……164
Table 16. GC Standard retention times……....165
Table 17. GC Furaldehyde standard curve……166
Table 18. GC Vanillin standard curve……..166
Table 19. Weight of vials……168
Table 20. Color intensity of each extracted sample over time……..169
Table 21. Amount of total soluble phenolics of each extracted sample over time…….170
Table 22. PH level of each extracted sample over time……171

# LIST OF FIGURES

Figure 1. Color extracted from charred and toasted staves……...30
Figure 2. Color extracted from each toasted layer……...31
Figure 3. Color extracted from each charred layer……...31
Figure 4. Color after each extraction treatment……33
Figure 5. Color of extracted samples over time……34
Figure 6. Color extracted by each extraction treatment within each heat treatment…...35
Figure 7. Color extracted by each extraction treatment over time ……36
Figure 8. Color extracted from each heat treatment over time ...……...37
Figure 9. Color extracted from each layer within each heat treatment……..38
Figure 10. Color extracted from each layer over time within each heat treatment……..39
Figure 11. Total soluble phenolics extracted from charred and toasted staves……...40
Figure 12. Total soluble phenolics extracted from each toasted layer……..41
Figure 13. Total soluble phenolics extracted from each charred layer……..…42
Figure 14. Total soluble phenolics extracted from each extraction treatment……..42
Figure 15. Total soluble phenolics extracted over time……43
Figure 16. Total soluble phenolics extracted by each extraction treatment within each heat treatment……..……45
Figure 17. Total soluble phenolics extracted by each extraction treatment over time…46
Figure 18. Total soluble phenolics extracted from each heat treatment over time……..47
Figure 19. Total soluble phenolics extracted from each layer within each heat treatment……..48
Figure 20. Total soluble phenolics extracted from each layer over time……...49
Figure 21. Level of pH in charred and toasted staves……..50
Figure 22. Level of pH after extraction from each toasted layer……52
Figure 23. Level of pF after extraction from each charred layer……52
Figure 24. Level of pH after each extraction treatment……53
Figure 25. Level of pH over time……..54
Figure 26. Level of pH extracted by each extraction treatment within each heat treatment……..55
Figure 27. Level of pH extracted by each extraction treatment over time……56
Figure 28. Level of pH extracted by each heat treatment over time……..57
Figure 29. Level of pH extracted from each layer within each heat treatment……58
Figure 30. Level of pH extracted from each layer over time……...59
Figure 31. Furaldehyde extracted from charred and toasted staves……60
Figure 32. Furaldehyde extracted from each toasted layer……..……62
Figure 33. Furaldehyde extracted from each charred layer……...63
Figure 34. Furaldehyde extracted by each extraction treatment……64
Figure 35. Furaldehyde extracted over time……..……65
Figure 36. Furaldehyde extracted by each extraction treatment within each heat treatment……..……66
Figure 37. Furaldehyde extracted by each extraction treatment over time……...67
Figure 38. Furaldehyde extracted by each extraction treatment over time……...68

Figure 39. Furaldehyde extracted from each layer within each heat treatment……69
Figure 40. Furaldehyde extracted from each layer over time……...70
Figure 41. Vanillin extracted from charred and toasted staves ……73
Figure 42. Vanillin extracted from each toasted layer……..…74
Figure 43. Vanillin extracted from each charred layer……...75
Figure 44. Vanillin by each extraction treatment……...76
Figure 45. Vanillin extracted over time……..……77
Figure 46. Vanillin extracted by each extraction treatment within each heat treatment……..77
Figure 47. Vanillin extracted by each extraction treatment over time……..…..78
Figure 48. Vanillin extracted from each heat treatment over time……..……...79
Figure 49. Vanillin extracted by each layer within each heat treatment……....80
Figure 50. Vanillin extracted from each layer over time……..…….82
Figure 51. Average amount of oak compounds found in each layer……....83
Figure 52. Sinapaldehyde extracted from charred and toasted staves……..…...86
Figure 53. Sinapaldehyde extracted from each toasted layer……..….....86
Figure 54. Sinapaldehyde extracted from each charred layer……..…....87
Figure 55. Sinapaldehyde extracted by each extraction treatment….....88
Figure 56. Amount of sinapaldehyde extracted over time……..89
Figure 57. Sinapaldehyde extracted by each extraction treatment within each heat treatment……....90
Figure 58. Sinapaldehyde extracted by each extraction treatment over time……...91
Figure 59. Sinapaldehyde extracted from each heat treatment over time……..…..92
Figure 60. Sinapaldehyde extracted from each layer within each heat treatment……93
Figure 61. Sinapaldehyde extracted from each layer over time……....94
Figure 62. Protocatechuic acid extracted from charred and toasted staves.....……...97
Figure 63. Protocatechuic acid extracted from each toasted layer……..……...98
Figure 64. Protocatechuic acid extracted from each charred layer……..……....99
Figure 65. Protocatechuic acid extracted by each extraction treatment .....100
Figure 66. Protocatechuic acid extracted over time……...101
Figure 67. Protocatechuic acid extracted by each extraction treatment within each heat treatment……..……102
Figure 68. Protocatechuic acid extracted by each extraction treatment over time……103
Figure 69. Protocatechuic acid extracted from each heat treatment over time……103
Figure 70. Protocatechuic acid extracted from each layer within each heat treatment……..104
Figure 71. Protocatechuic acid extracted from each layer over time……..……105
Figure 72. Ellagic acid extracted from charred and toasted staves……....108
Figure 73. Ellagic acid extracted from each toasted layer……..…..108

Figure 74. Ellagic acid extracted from each charred layer……..…….109
Figure 75. Ellagic acid extracted by each extraction treatment……..…….110
Figure 76. Ellagic acid extracted over time…..……..…….111
Figure 77. Ellagic acid extracted by each extraction treatment within each heat treatment.....111
Figure 78. Ellagic acid extracted by each extraction treatment over time……..112
Figure 79. Ellagic acid extracted from each heat treatment over time…….....114
Figure 80. Ellagic acid extracted from each layer within each heat treatment……...114
Figure 81. Ellagic acid extracted from each layer over time……..……..115
Figure 82. Vanillic acid from charred and toasted staves……..……...117
Figure 83. Vanillic acid extracted from each toasted layer……..……117
Figure 84. Vanillic acid extracted from each charred layer……..……118
Figure 85. Vanillic acid extracted by each extraction treatment……..……119
Figure 86. Vanillic acid extracted over time……..……..120
Figure 87. Vanillic acid extracted by each extraction treatment within each heat treatment……121
Figure 88. Vanillic acid extracted by each extraction treatment over time…….....121
Figure 89. Vanillic acid extracted from each heat treatment over time……...122
Figure 90. Vanillic acid extracted from each layer within each heat treatment……...123
Figure 91. Vanillic acid extracted from each layer over time……..….124
Figure 92. Syringealdehyde extracted from charred and toasted staves……127
Figure 93. Syringealdehyde extracted from each toasted layer……..……..127
Figure 94. Syringealdehyde extracted from each charred layer……..….128
Figure 95. Syringealdehyde extracted by each extraction treatment.….....129
Figure 96. Syringealdehyde extracted over time……..……..130
Figure 97. Syringealdehyde extracted by each extraction treatment within each heat treatment……..……..……..131
Figure 98. Syringealdehyde extracted by each extraction treatment over time……131
Figure 99. Syringealdehyde extracted from each heat treatment over time……..132
Figure 100. Syringealdehyde extracted from each layer within each heat treatment……..……133
Figure 101. Syringealdehyde extracted from each layer over time……....135
Figure 102. Standard curve of gallic acid (Total Soluble Phenolics)……..……....155
Figure 103. Standard curve of gallic acid (HPLC)……..157
Figure 104. Standard curve of protocatechuic acid (HPLC)……158
Figure 105. Standard curve of protocatechuic aldehyde (HPLC)……159
Figure 106. Standard curve of methyl gallate (HPLC)……...160
Figure 107. Standard curve of vanillic acid (HPLC)……...161
Figure 108. Standard curve of syringealdehyde (HPLC)……..162

Figure 109. Standard curve of scopoletin (HPLC)……..163
Figure 110. Standard curve of ellagic acid (HPLC)……164
Figure 111. Standard curve of sinapaldehyde (HPLC)…….....165
Figure 112. Standard curve of furaldehyde (GC)……166
Figure 113. Standard curve of vanillin (GC)…….167
Figure 114-239. HPLC Chromatograms time 0……...172
Figure 240-369. HPLC Chromatograms after 3 months……204

# 1. LITERATURE REVIEW

## 1.1. Introduction

Oak barrel use has become a vital component in the aging process of wine and spirit. While traditionally used for transfer and storage, barrels are now recognized for the color, taste, and aroma they provide to the finished product (Perez-Prieto et al., 2002). Distillers purposefully select trees to form their barrels based on tree-type and geographical location to ensure they produce high-quality products (Doussot et al., 2002). However, aging in oak barrels is a time-intensive and expensive process which is encouraging distillers to look for new extraction methods to reduce time and production costs (Mosedale and Puech, 1998). Current alternative methods to traditional barrel aging include using oak chips or oak staves in stainless steel barrels with micro-oxygenation (Pizarro et al., 2014), soxhlet extraction (Kulkarni and Rathod, 2014), ultrasound irradiation (Tao et al., 2014), and electric field treatment (Zhang et al., 2014), to name a few.

The purpose of this research is to identify if the use of high intensity ultrasound (HIUS) assisted extraction is an effective accelerated aging method which could be utilized in the production of an oak extract to be used in wine and whiskies. Whiskey will remain the focus of study as it has higher alcohol content and working methods could easily be applied to wines which have lower alcohol content. Along with HIUS assisted extraction, thermal/soxhlet extraction (hereafter referred to as reflux) and a room-temperature controlled extraction were also compared as potential accelerated aging methods. For extraction method evaluation, two types of heat treated oak staves

donated by an anonymous donor, charred and toasted, were shaven into individual 1-2 millimeter layers to compare oak compounds extracted by each method. After evaluation of methods, extracted oak compounds were compared among layers, between stave types, and were identified using standards.

## 1.2. Whiskey

Originating from Irish monks in Scotland during the 1400s, whisky, or whiskey in countries other than Scotland and Japan, has gained popularity throughout the years. There are currently five large international whisky distilling countries, Scotland, Ireland, the United States, Canada, and Japan. Each country hosts their own legal definitions, distillation procedures, ingredient choices, and marketing strategies (Russell, 2003). Producers are actively trying to perfect their products while reducing cost and time which has led to research of the aging process using both taste experts and compositional analysis. However, the elements which contribute to the aging of whiskies are so broad and complex that it is difficult to isolate a specific factor that is key in transforming distillate into a mature product (Sonderegger et al., 2015).

According to the Scotch Whisky Association, Scotland is the current leading producer of whisky with 99 million cases exported last year (2015). To be labeled as Scotch, whisky must be produced and distilled in Scotland and allowed to mature in oak casks for a minimum of three years with a minimum alcohol by volume of 40 percent (Jackson, 2004; Piggott and Conner, 2003; Russell, 2003). There are two main types of Scotch whisky, malt and grain, which can be classified into the following categories: malt whisky, single malt whisky, single cask, vatted malt, pure malt, blended Scotch

whisky, grain whisky, and single grain whisky. The proportions of malt and grains differ in each category (Jackson, 2004). Scotch whisky is further classified by region—Lowland, Highland, Speyside, Island, and Campbeltown— as water, soil, climate, temperature, and air quality vary across Scotland changing mature whisky composition (Jackson, 2004; Lapointe and Legendre, 1994).

Irish whiskey follows the same standards and procedures as Scotch whisky; however, unlike Scotch, Irish whiskey is distilled three times instead of twice and does not use peated barley during malting (Russell, 2003; Locke, 2015). Because of this, Irish whiskey flavor is characterized as light, delicate, smooth, and natural (González-Arjona et al., 1998).

North American whiskies can be classified by their cereal composition into the three following categories: Bourbon, Tennessee, and Rye whiskies—Bourbon is made up of 70% corn, 15% rye, and 15% malted barley; Tennessee is made up of 80% corn, 10% rye, and 10% malted barley; Rye is made up of 39% corn, 51% rye, and 10% malted barley (Russell, 2003). According to US regulations, whiskey must be distilled at less than 190° proof, matured in brand new charred oak barrels, and matured for two or more years (Piggott and Conner, 2003). To be labeled Tennessee whiskey, whiskey must be manufactured in the state of Tennessee, made of at least 51% corn, distilled to no more than 160° proof, matured in new charred oak barrels, filtered through maple charcoal prior to aging, placed in a barrel with no more than 125° proof, and not bottled at less than 80° proof (H. 1084, 2013). Whereas, bourbon and rye regulations are less specific and are as follows: must be made in the US to be labeled as bourbon, made of

no less than 51% corn, rye, wheat, malted barley, or malted rye, does not exceed 160° proof following fermentation, not stored at less than 125° proof, and is matured in new charred oak barrels (Alcohol, Tobacco Products and Firearms, 2015). Bourbon whiskey has become synonymous with any corn-based whiskey; however, corn whiskey differs from bourbon in that is can be matured in either used or new uncharred oak barrels (Russell, 2003).

Canadian whiskey is mostly made of rye as it was abundant at the start of distillation in 1858. Canadian whiskey must be made of mashed cereal grains, distilled, and aged for a minimum of three years in Canada and may additionally have caramel color and flavor (Russell, 2003). Distillation in Canada is similar to Scotch and American distillation, but flavorings from wines and/or other spirits are allowed to remain or be added (Piggott and Conner, 2003).

Crafted in the style of Scotch malt whisky, Japanese whisky has recently gained popularity following several international taste awards (Jackson, 2004). Japanese whisky, first established by Suntory in 1929, was originally made up of locally grown barley and was slowly matured in Japanese oak barrels. However, increased demand drove Suntory to import malted barley and other cereal grains and to switch from Japanese oak to American oak barrels (Russell, 2003). Recently, Suntory's Yamazaki Single Malt Sherry Cask 2013 was named the best in the world by Jim Murray's Whiskey Bible 2015 edition (Gibson, 2014).

Whiskey is one of the most consumed spirits around the world. Product recognition is key to determining the market for individualized brands. Compared to

American, Canadian, and Japanese whiskies, Scotch whisky requires minimal promotion as it has traditionally been the leader in production and sales. However, Scotland will need to keep up with the distillation and maturation improvements other countries are implementing to maintain Scotch whisky's international exportation lead.

## 1.3. Production

Whiskey is made using a five stage process—malting, mashing, fermentation, distillation, and maturation. Water, cereal grain, and yeast used during the whiskey process can affect the final flavor indirectly and directly as their acids, esters, and phenols interact during the maturation process (Russell, 2003).

Water selection is a main driving component for distillery location as it is used in the malting, mashing, and distillation stages of whiskey production. No two water sources are the same and may differ in appearance, potability, mineral content, microbiological standards, and supply reliability. Flavor of the final whiskey product may be affected by water selection due to its ability to add minerals which can influence pH and provide nutrients such as calcium, magnesium, and zinc for yeast metabolism during fermentation (Russell, 2003).

Cereal grain choice affects levels of esters, alcohols, acids, and precursors to flavor compounds by their differences in "pH, amino acid concentration, and insoluble material". These changes can make a difference in the final product's flavor (Piggott and Conner, 2003). Cereal grain, such as barley, corn, wheat, and rye, are chosen for their high starch content which produces a higher spirit yield (Piggott and Conner, 2003). In order to produce alcohol, starch must be gelatinized to release amylose and

amylopectin structures for starch-degrading enzymes, α- and β-amylase, to break down into the fermentable sugars yeast use to convert to alcohol (Russell, 2003).

A single culture strain of yeast is preferred during fermentation to prevent off-flavors and to maximize sugar conversion (Campbell, 2003). Strains from *Saccharomyces cerevisiae* are selected for differences in flavor, fermentation rate, sugar profile consumption, alcohol production level, and survivability in anaerobic conditions (White and Zainasheff, 2010). Genetically different strains produce varying flavors due to a difference in fermentation response to temperature and oxygen and amino acid content of wort (Russell, 2003).

### 1.3.1. The Five Stages

In the malting stage, cereal grain is coarsely milled and then alternately soaked and dried until germinated (Piggott and Conner, 2003). Germination is an important sign of enzyme activity and is the beginning stage of starch hydrolysis. Malting activates enzymes which “break down starch and proteins into smaller, soluble fractions” and also activates α-amylase and β-amylase, the enzymes used in mashing to break down starches into sugars (White and Zainasheff, 2010). Germination ends when the cereal grain is dried in a kiln and allowed to rest for several weeks. Proper malting and kilning can maximize the availability of enzymes for further degradation of complex sugars in mashing and fermentation steps, increasing the amount of fermentable sugars for yeast to metabolize (Russell, 2003). A high amount of complex sugars is harder for yeast to ferment which can negatively affect the amount of alcohol produced (White and Zainasheff, 2010).

Prior to mashing, the germinated, cereal grain is milled into “grist”, a 20% husk, 70% grit, and 10% flour mixture, which is placed into a mash tun filled with hot water. The milling of germinated, cereal grain into grist allows efficient “gelatinization, enzymolysis, and dissolution” of sugars to form “wort” (Russell, 2003). Wort must be cooled and placed into washbacks, tanks either made of wood or stainless steel, prior to the addition of yeast for fermentation (Piggott and Conner, 2003).

Wort must not be cooled below 68°C as lower temperatures can slow and stall fermentation. However, while increased temperature increases yeast activity, yeast are susceptible to high temperatures as the heat from the energy of metabolism during fermentation can raise the wort temperature causing yeast to die from extreme heat, create off-flavors, or mutate (White and Zainasheff, 2010). At the ideal temperature, yeast will ferment wort by converting sugar into alcohol called “wash” which is 8% alcohol by volume ABV. However, if the malting step is not done appropriately, yeast fermentation will be limited due to the lack of soluble amino nitrogen in wort for growth and rapid fermentation and a lack of unsaturated fatty acids, sterols, and vitamins which aid yeast to function in anaerobic conditions (Russell, 2003).

Distillation is the process of heating spirit so that it vaporizes and condenses in a copper still. Stills are made of copper because it is a good heat conductor, resists wear, and removes sulfurous compounds. Stills have three main components—a still pot containing the wash, swan neck, and lyne arm which may vary in size and shape. Typically, a short, fat still will produce a fuller, richer spirit whereas a tall, long-neck still will produce a lighter, finer spirit. When distilleries replace stills, they even replace the

dents found on the old stills as they may be a factor in the composition of the final product (Piggott and Conner, 2003).

First distillation of wash produces a product referred to as “low wines” which is 21-23% ABV while second distillation produces 65-75% ABV. The spirit is then passed through a spirit safe where it is split into foreshots, heart, and feints (Piggott and Conner, 2003). Foreshots are considered unusable as they contain high unfavorable volatiles and have a milky, turbid color due to long chain fatty acids and esters (Russell, 2003). The middle-cut, or heart, portion is what is used for the maturation process (Piggott and Conner, 2003). The heart portion is clear and is collected for the maturation process while the feint portion, or final cut point, is removed as it contains heavy oils and esters which are unfavorable in a final product (Russell, 2003).

During the maturation stage, distillate is placed into oak barrels and allowed to age (Piggott and Conner, 2003). Aging times differ depending on country regulations and what is desired by the distiller. Maturation produces compositional chemical changes in the distillate to impart characteristic whiskey color, flavors, and aromas to the final product (Russell, 2003). Change is also made to alcohol content and volume as they decrease during maturation helping to further mellow the final product by removing astringency (Mosedale and Puech, 1998; Jarauta et al., 2005).

## 1.4. Aging

Traditionally used for storage and transport, whiskey barrels are now known to positively change the chemical composition of distillate over time (Mosedale and Puech, 1998; Russell, 2003). The sensory improvements in aroma, taste, and color mellow

fresh distillate into a desirable product for consumers to enjoy (Jarauta et al., 2005; Clyne et al., 1993). According to Mosedale and Puech, the chemical changes are due to "direct extraction of wood compounds, decomposition of wood macromolecules and extraction of their products into the distillate, reactions between wood components and the constituents of the raw distillate, reactions involving only wood extractives, reactions involving only the distillate components, and evaporation of volatile compounds" (1998). All chemical changes are influenced by uncontrollable and controllable factors such as oak geographical origin, species, climate, temperature, moisture content, wood treatment, degradation, and aging time (Sonderegger et al., 2015; Prida and Puech, 2006; Caldeira et al., 2010; Sanza and Domínguez, 2005; Moreno et al., 2007). These factors create whiskey variability causing many distillers to invest in ways which provide a more uniform product (Russell, 2003). The cost of barrel production and maturation time of distillate has recently encouraged more scientific studies on wood composition and extractives in order to find more economical methods for aging whiskey (Rodríguez-Bencomo et al., 2009).

### 1.4.1. Oak Wood

In whiskey maturation, white oak is typically used in barrel formation and for spirit maturation due to structural components which prevent it from leaking (Prida and Puech, 2006; Waterhouse and Towey, 1994). White oak's structural components, medullary rays and tyloses, make staves to be used in cooperaging flexible and strong, impervious to distillate loss, and sealable at stave ends (Russell, 2003). There are many species of oak, but it is mostly American oak, *Quercus alba*, and European oak, *Q.*

*petraea* and *Q. robur*, which are used for whiskey maturation (Mosedale and Puech, 1998; Glabasnia and Hofmann, 2006; Jarauta et al., 2005; Russell, 2003). There are differences between these species which allow the distiller to select barrels based on what components are more desirable in their final products. For example, American oak is known to provide a higher level of elligitannins, such as whiskey lactone, and lower overall polyphenols when compared to European oak (Prida and Puech, 2006; Glabasnia and Hofmann, 2006). The US predominately uses new American oak while other countries such as Scotland, Ireland and Canada, use either European or used American oak barrels to mature distillate due to a limited supply of new oak (Russell, 2003).

White oak tissue is made up of three insoluble fibers, 45-50% cellulose, 22-25% hemicelluloses, 23-32% lignins, and a group of 3-10% extractable components made up of acids, carbohydrates, and phenols (Glabasnia and Hofmann, 2006; Delgado de la Torre et al., 2013). The main oak component, cellulose, is a uniform chain structure made of anhydroglucopyranose bound by β-(1-4)-glycosidic linkages and adjacent hydroxyl groups which form cell wall layers. Inside the cell walls, formed by cellulose, lie hemicelluloses made up of xylose and other sugar components such as pentoses, hexoses, hexuronic acids, and deoxy-hexoses. Cell walls are bound by lignin, a highly branched structure, made from phenylpropane groups substituted with hydroxyl and methoxyl groups that are chemically bonded to almost all hemicellulose components which eventually form significant compounds necessary for whiskey maturation following degradation (Russell, 2003; Conner et al., 1999; Mosedale and Puech, 1998).

These structural insoluble fibers are important for chemical interactions during maturation because they contain electron bonding sites. For example, cellulose and hemicelluloses donate oxygen electrons from hydroxyl groups to lignin phenyl rings (Barrera-García et al., 2008).

Extractable components fall into three categories, phenols with hydrolyzed ellagitannin derived polyphenols, fatty acids, and other extractives made up of lactones, alcohols, hydrocarbons, norisoprenoids, and inorganic substances (Mosedale and Puech, 1998; Doussot et al., 2002). While extractable components are important for the maturation and development of distillate, they are not vital to oak wood structure (Russell, 2003). Oak variability within species and geographical origin contributes to the extractability and complexity of these compounds (Delgado de la Torre et al., 2013; Russell, 2003; Prida and Puech, 2006). Of the extractable components, cis-oak lactone, linolenic, and acetic acid dominate (Russell, 2003). Linolenic and acetic acid degrade into aldehydes and alcohols such as guaiacol, eugenol, vanillin, syringaldehyde, and furanic aldehydes, to name a few (Natali et al., 2006). Phenols can be either increased or decreased during the heating treatment of oak during barrel formation (Russell, 2003).

### 1.4.2. Cooperage

An average European barrel costs 2 to 4 times more than an American barrel which costs about 360-500 U.S. dollars (Waterhouse and Towey, 1994; Puckette and Hammack, 2013). The cost difference is due to European oak availability, inconsistency of grain structure, and use of a harder cooperage technique, split instead of sawn,

which is necessary because of high wood porosity (Waterhouse and Towey, 1994). Sawn staves are produced by slicing logs into quarters, removing the flat surface parallel to the tree's radius to cut the first stave, turning the log 90°, and repeating the process for the sequential staves until not enough wood is left (Russell, 2003).

The staves are then put through a seasoning process. During seasoning, oak is either dried outdoors or kiln-dried (Mosedale and Puech, 1998). In the U.S., staves are kiln-dried for up to a month in order to reduce moisture content. Seasoning serves two purposes: to remove humidity, thus preventing splits and cracks in stave ends, and to induce chemical aging due to weather and biological activity (Russell, 2003; Doussot et al., 2002). After seasoning, staves are shaped and formed with wide middles compared to stave ends and with smooth, angled edges (Russell, 2003). Staves are bent into barrel shape either using a windlass or bending machine while internally treating with heat ranging from 230-260°C for up to fifteen minutes and externally steaming for twenty minutes at 95°C to soften wood (Mosedale and Puech, 1998; Russell, 2003).

Following barrel formation, the barrel goes through a toasting or charring heat treatment process in order to degrade wood polymers for readily extractable flavor compounds and destruction of unpleasant aromas (Russell, 2003). To meet U.S. regulations, American barrels must be charred (Alcohol, Tobacco Products and Firearms, 2015). Char levels on American barrels range from light to heavy and are charred using a gas burner for 15 to 45 seconds. European barrels are put through a toasting heat treatment using a wood-fired brazier. Toasting treatment level can be light, 5 to 10 minutes, medium, 10 to 15 minutes, and heavy, 15 to 20 minutes (Mosedale and

Puech, 1998). American barrel companies prefer charring to toasting because it is more rapid and produces a layer of carbon on the inside of the barrel which removes undesirable sulfurous compounds (Russell, 2003).

The degree of heat treatment produces varying levels of degradation during cooperaging which affects the amount of volatile compounds available during maturation (Mosedale and Puech, 1998; Doussot et al., 2002; Russell, 2003). The structural components, cellulose, hemicelluloses, and lignins are affected physically, chemically, and biochemically helping in extraction and production of phenolic compounds and their derivatives (Jarauta et al., 2005; Delgado de la Torre et al., 2014). Degradation of cellulose and hemicelluloses produce furanic aldehydes and ketones which are known only to have a minor affect on final aroma and flavor (Natali et al., 2006). Compounds from cellulose and hemicelluloses, formed by the pyrolysis of sugars, provide a sweet, caramel, almond, and toasted aroma and flavor to whiskey after maturation (Russell, 2003). Lignin degradation, however, produces smoked, spiced, and vanilla aromas and flavors due to the formation of methoxylated volatile phenols, phenolic ketones, and phenolic aldehydes (Natali et al., 2006). Lignin compounds include the following: vanillin, syringealdehyde, coniferaldehyde, sinapaldehyde which can oxidize further producing vanillic and syringic acids; gallic and ellagic acids, eugenol, guaiacol, and whiskey lactone (Delgado de la Torre et al., 2013; Russell, 2003).

Studies of extracted compounds have been limited to low concentrated, low molecular weight compounds, such as vanillin and syringaldehyde, which are well-

known wood odorants and has not focused on other high molecular wood extractives (Viriot et al., 1993; Jarauta et al., 2005; Doussot et al., 2002). The limited information is due to the complexity and variation of extractable compounds in mature whiskey and because of lacking method validity when detecting, recovering, and quantifying fatty acids, volatile phenols, and other extractable components (Russell, 2003; Caldeira et al., 2004). There is also a lot of repeated information such as studying the same compounds using different techniques and no development of information regarding their biological role during maturation (Mosedale and Puech, 1998).

### 1.4.3. Variability

Many producers find a high rate of variability in their products because of the lack of understanding and information (Jackson, 2004; Russell, 2003). A recognizable method to control variability is to blend batches of whiskey to produce a more uniform product (Russell, 2003). The blending of whiskies improves sensory aspects and product inconsistencies (Jeffery, 2012). However, even blended whiskies vary from batch to batch (Russell, 2003).

### 1.4.4. Accelerated Methods

Oak chips and oak staves placed in stainless steel barrels have recently been studied as alternatives to oak barrel aging in order to reduce cost and time of whiskey aging (Mosedale and Puech, 1998; Tesfaye et al., 2004; Álamo-Sanza and Domínguez, 2005; Natali et al., 2006; Álamo et al., 2008, 2010; Rodríguez-Bencomo et al., 2009; Caldeira et al., 2010; Pizarro et al., 2014). Extraction methods using oak chips or staves to age distillate have been done using oak chips placed in stainless steel barrels in

corresponding ratio to distillate at room-temperature, soxhlet extraction, ultrasound and microwave irradiation, micro-oxygenation, electric field treatment, and bath ultrasound (Kulkarni and Rathod, 2014; Tao et al., 2014; Nevares and Álamo, 2008; Álamo et al., 2010; Pizarro et al., 2014; Zhang et al., 2013). While these techniques show promise in accelerating the aging process, they have not been implemented in industry because of lack of method validity and the need for further study. Ultrasound has the most potential for extraction as it limits time and heat compared to soxhlet method and is a “simple, efficient, and economical alternative when compared to other extraction” methods (Kulkarni and Rathod, 2014).

## 1.5. High Intensity Ultrasound

There are two types of ultrasound (sonication), low intensity and high intensity, which are used across many industries today. More commonly known, low-intensity ultrasound is used in the medical industry for scanning because of its ability to identify something not readily visible; whereas, high intensity, or power, ultrasound is used to alter a medium or promote a chemical reaction (Lee and Feng, 2011). As the food industry is mostly interested in alteration of materials and sanitation, high intensity ultrasound will be the primary focus of this section.

Although currently limited to laboratory settings, interest in high intensity sonication use has recently increased for food processing and sanitation procedures since sonication is a “simple, efficient, and economical alternative” to current methods (Santos et al., 2009; Vilkhu et al., 2007; Kulkarni and Rathod, 2014). According to laboratory tests, sonication would improve current food industry applications in drying,

cleaning, homogenizing, mixing, degassing, oxidating, nucleating, and extracting by reducing time and cost of procedures (Arzeni et al., 2012). However, industrial-scale productions are challenging to produce and slow to implement due to the feasibility of sonication use for unique applications and processes (Vilkhu, 2007). Furthermore, not all sonication devices perform equally nor can they be used for the same applications. Each food industry process has different requirements and ultrasound selection can make a direct difference to the final product (Santos et al., 2009).

### 1.5.1. How sonication works

Sonication works by using high intensity and high frequency sound waves to disturb a medium (Santos et al., 2009). Sound waves are mechanical vibrations which must pass through a medium such as a solid, liquid, or gas in order to produce energy (Luque-García, 2003). Sound wave speed is dependent on the medium; gases have the lowest speed range of 200-500 m/s, liquids have the middle range of 1,200-2,000 m/s, and gases have the highest range with 3,200-6,500 m/s. Ethanol, used for this project, has a speed of 1,207 m/s (Martini, 2013).

As sound waves propagate through the medium, they create voids called cavitations, or microbubbles, which oscillate in size (Santos et al., 2009). Oscillation is a series of expansion and compression cycles that pull apart and push together medium molecules (Luque-García, 2003). During oscillation, medium molecules are displaced around their equilibrium which creates high and low concentrations of molecules in the media. These high and low concentrations correspond to maximum and minimum sound wave amplitudes (Martini, 2013).

Eventually, the displacement of molecules creates a zone of negative pressure, cavities, which implode releasing both high pressure and temperatures (Luque-García, 2003; Santos et al., 2009). According to Suslick et al., pressure created by cavitation implosion can be estimated to be 1000 atm and the temperature created can be estimated at 5000°C; however, the heat and pressure produced by the cavitations do not alter the environment due to the small size of the cavitation bubbles (Luque-García, 2003).

Physical and chemical properties of materials are changed by the high pressure and temperature produced by cavitations (Bermúdez-Aguirre et al., 2011). For example, sonicated food materials may see changes in texture, color, flavor, and nutrients (Lee and Feng, 2011). Food processes with material changes due to sonication currently studied are as follows: extraction, emulsification, viscosity modifier, defoaming, pasteurization, sonocrystallization, fermentation, heat transfer, extrusion, filtration, degassing, depolymerization, cooking, and changing protein properties (Martini, 2013). For the purposes of this project, extraction is the main focus and will be the only form of material change discussed.

Extraction of cellular contents is a process which requires the breaking of cell walls due to heat or pressure. Sonication provides both increased heat and pressure which accelerates the release of cell contents, and furthermore, produces a higher extraction yield due to increased solvent penetration, mass transfer, and cell wall disruption (Bermúdez-Aguirre et al., 2011; Martini, 2013). During extraction, cavitations adjacent to cells implode breaking down cell walls and releasing cell content

(Bermúdez-Aguirre et al., 2011; Santos et al., 2009). Not only are rapid cavitations useful in creating shear forces which propel cell content release, but they also can generate free radicals and create turbulent fluid flow which can accelerate and drive many chemical reactions (Weiss et al., 2011).

### 1.5.2. Types of Sonicators

There are two types of sonicators, bath, which deliver power to the sample indirectly, and probe, which deliver power directly (Luque-García, 2003). Sonicator type is chosen based on application as both have advantages and disadvantages (Santos et al., 2009). Bath sonicators have three classes: classic, which works on one frequency; multifrequency unit, which has transducers using multiple frequencies; and modern, dual-frequency which allows the use to control frequency, power, intensity, operations, heat and time (Santos et al., 2009). While the bath is more widely used, it does not provide uniform energy distribution and loses power over time which leads to lack of repeatable and reproducible results (Luque-García, 2003). Probe sonicators deliver a higher intensity to a localized zone in samples due to direct power; however, direct power to a sample can lead to cross-contamination due to open containment and detachment of metal probe pieces over time (Luque-García, 2003; Santos et al., 2009).

Temperature must be controlled during sonication. While high temperatures produce faster extraction rates, they can disrupt constant ultrasound power thus creating lower cavitation efficiency (Santos et al., 2009). Lower temperatures provide a better extraction environment by increasing cavitation efficiency, and also help maintain a controlled environment in order to produce repeatable results (Santos et al., 2009;

Luque-García, 2003). To maintain control, ultrasonic probes and baths with heaters or water cooling recirculation systems are used (Santos et al., 2009).

Power delivery must also be controlled to ensure uniform energy distribution (Bucur, 2006). Baths and probe ultrasound systems consist of an electrical power generator, a transducer, and an emitter. An electrical generator is used to deliver energy to the transducer “indirectly through voltage (V) and current (I) settings” (Bermúdez-Aguirre et al., 2011). The energy is then converted by a transducer into sound energy by mechanical vibrations at a specified frequency. An emitter, bath or probe with sonotrode tip, emits the sound wave from the transducer into the medium delivering the energy necessary for physical and chemical changes (Bermúdez-Aguirre et al., 2011; Santos et al., 2009). The shape of either the reaction container or probe determines the amplification of power within a sample and should be considered depending upon application (Santos et al., 2009).

## 1.6. Objectives

Using high intensity ultrasound (HIUS) will accelerate the extraction of oak compounds from barrel staves which could be utilized in the production of a concentrated oak extract to be used in accelerated aging in wines and whiskies. The primary objective will be to compare extraction using HIUS to both reflux and room-temperature control methods by examining color, total soluble phenolics, pH, and concentration levels of individual oak compounds identified by gas and liquid chromatography. Secondary objectives include comparing concentrations of oak compounds extracted from donated charred and toasted staves, from individual layers

within charred and toasted staves, and from initial extraction treatments to extraction after 3 months.

# 2. MATERIALS AND METHODS

## 2.1. Samples

**Oak wood shavings** prepared at the UT Center for Renewable Carbon (Knoxville, TN) from five toasted and six charred oak staves donated by an anonymous donor.

## 2.2. Chemicals and Reagents

**Standards**: β-resorcylic acid (2,4- dihydroxybenzoic acid, 97%); β-resorcylic aldehyde (2,4-dihydroxybenzaldehyde, 98%); butyric acid (≥99%); caffeic acid (≥98.0%); coniferaldehyde (4-hydroxy-3-methoxycinnamaldehyde, 98.0%); ellagic acid (≥95.0%); ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid, ≥99.0%); 2-furaldehyde (99.0%); gallic acid (3,4, 5-trihydroxybenzoic acid, 97.5-102.5%); gallic aldehyde (3,4,5-trimethoxybenzaldehyde, 98.0%); methyl gallate (methyl 3,4,5-trihydroxybenzoate, 98.0%); octanoic acid (98.0%); p-coumaric acid (≥98.0%); protocatechuic acid (3,4-dihydroxybenzoic acid, ≥97.0%); protocatechuic aldehyde (3,4-dihydroxybenzaldehyde, ≥97.0%); scopoletin (≥99.0%); sinapic acid (≥99.0%); sinapaldehyde (trans-3,5-dimethoxy-4-hydroxycinnamaldehyde, 98.0%); syringic acid (≥95%); syringaldehyde (98.0%); vanillic acid (≥97.0%); vanillin (99.0%) were all purchased from Sigma-Aldrich (St. Louis, MO). Distilled water was prepared at the University of Tennessee (Knoxville, TN). Ethanol (200 proof) was purchased from Sigma-Aldrich (St. Louis, MO). Folin & Ciocalteu Phenol Reagent 2.0 N was purchased from MP Biomedical (LLC Solen, OH). Sodium carbonate, anhydrous was purchased from Sigma-Aldrich (≥99.5%, St. Louis, MO). Dichloromethane, anhydrous was purchased from Sigma-Aldrich (≥99.8%, contains 50-150 ppm amylene as stabilizer, St. Louis, MO). HPLC grade water,

methanol, and acetonitrile were purchased from Fisher-Scientific (Fairlawn, NJ). Ammonium hydroxide, reagent grade was purchased from Fisher Scientific Education (Nazareth, PA). O-phosphoric acid, 85% was purchased from Fisher-Scientific (Fairlawn, NJ).

## 2.3 Instruments

High Intensity Ultrasound 20 kHz with standard stainless-steel probe with removable ½ inch stainless-steel tip diameter (13 mm) was purchased from Sonics & Materials, INC. (VC-750, Newton, CT). Isotemp 3016D waterbath was purchased from Fisher-Scientific (Fairlawn, NJ). Ultrabasic (UB-10) pH meter was purchased from Denver Instruments (Bohemia, NY). Reciprocal Shaking bath (model 25) was purchased from Precision Scientific (Buffalo, NY). UV-Visible spectrophotometer was purchased from Thermo-Fisher Scientific, INC (Evolution 201/220, Waltham, MA). Stirrer mantel with controller 500 mL purchased from Thermo-Fisher Scientific, INC (Waltham, MA). Gas Chromatography-flame ionization detector was purchased from Agilent-Hewlett Packard, (Agilent HP 6890, St. Paul, MN) and a DB-WAX 52 DB, 30 m x 0.25 mm ID; 0.25 µm column was purchased from Agilent (J&W Scientific, Santa Clara, CA). High Performance Liquid Chromatography-photodiode array detector was purchased from Waters (Milford, PA) and 5-µmX250-mmX4.6-mm C18 reversed-phase column was purchased from Thermo-Fisher Scientific, INC (Waltham, MA) with a 4.0/4.6 mm ID3Thermo-Scientific Unifilter HPLC Column Protection System guard column. BD 5 mL syringe-tip with Econofilter 0.45 µm poly-tetrafluiriethylene (PTFE) was purchased from

Agilent (Santa Clara, CA). Nylon membrane filters 0.45 μm PTFE was purchased from Sigma-Aldrich (St. Louis, MO).

## 2.4 Sample Preparation

### 2.4.1. Staves

Oak staves either toasted (5 staves) or charred (6 staves) were shaven into 4 toasted and 7 charred individual layers using visual gradation with approximate depth between 1-2 mm. Toasted stave layers one through four and charred layers five through seven were shaven by wood planer (Gizzly Industrial, INC, Bellingham, WA). Charred layers one through four were shaven by handheld Ironton straight draw shave tool. Length and width of both toasted and charred staves were measured and each shaved layer was weighed (g) (Table 1). Each layer of shavings was sifted with 28 μm diameter mesh and then sifted again using a 0.63 μm diameter mesh to separate large, medium, and small shavings (Table 2). The middle portion (medium sized shavings) of each sifted layer was used for sample extraction. The middle portion of each layer was combined from all staves within each seasoning treatment—toasted or charred (e.g. all of layer 1 from each toasted stave 1 through 5 were combined and thoroughly mixed). Each layer was randomized by a number generator (random.org) and run in duplicates for control, reflux, and sonication extraction treatments (Table 3).

### 2.4.2. Standard curves

Folin-Ciocalteu method was used to determine total soluble phenolics (1927). A standard curve was prepared using gallic acid concentration 0.0, 0.0125, 0.025, 0.05, 0.075, and 0.1mg/mL (Fig.102).

Gas chromatography (GC) standards' concentrations were 0.01 to 0.5 mg/mL and were prepared using dichloromethane as a solvent. GC standards were run according to GC method (Section 2.5.6.).

Concentrations for high performance liquid chromatography (HPLC) standards were 0.005 to 0.5 mg/mL prepared using MeOH as a solvent and were filtered by 0.45 µm syringe-tip filters. Standards were run according to HPLC method (Section 2.5.7.).

## 2.5 Methods

### 2.5.1. Sonication

Five grams of oak shavings were placed in 250 mL beakers with 100 mL of 35 % EtOH added prior to sonication. Ethanol (35%) was prepared using distilled water and 200 proof ethanol. A high intensity ultrasound (HIUS) stainless steel probe was submerged ¼ way from top of sample solution and run for a total of 60 minutes of on time at 40 % amplification with 30 sec on/off pulse. Sample solution was held in 4.0 °C waterbath for duration of sonication to maintain room temperature of extraction sample. A pH measurement was taken before and after sonication. Immediately after the sonication, an 8 mL sample was placed in a centrifuge tube and centrifuged at 4,700 rpm in ambient temperature for 15 minutes. The supernatant portion of the sample was then filtered using 0.45 µm filters and stored in a glass tube with oxygen removed using compressed nitrogen, and placed in -40 °C freezer. The rest of the extract was placed in a 250 mL amber glass container, weighed, and placed at ambient temperature in a dark cabinet for three month storage.

### 2.5.2 Reflux

A 250 mL round bottom flask containing 5 g of oak wood shavings and 100 mL of 35% EtOH was placed with magnetic spinner onto stirrer mantel with attached condenser. The 60 minute reflux treatment began after the first sign of condensation in condenser. A pH measurement was taken before and after reflux. An 8 mL sample was then placed in a centrifuge tube and centrifuged at 4,700 rpm in ambient temperature for 15 minutes. The supernatant was then filtered using 0.45 µm filters and stored in a glass tube with oxygen removed using compressed nitrogen placed in -40 °C freezer. The rest of the extraction (~ 90 ml?) was placed in a 250 mL amber glass container, weighed, and placed at ambient temperature in a dark cabinet for three month storage with air in the headspace.

### 2.5.3. Control

In a 250 mL amber glass container, 100 mL of 35 % EtOH was added to 5 g oak shavings. The container was placed in a water bath at 22 °C and was shaken for 60 minutes at 125 rpm. An 8 mL sample was centrifuged at 4,700 rpm in ambient temperature for 15 minutes then supernatant was filtered using 0.45 µm filters and were stored in a glass tube with oxygen removed using compressed nitrogen placed in -40 °C freezer. The rest of the extraction was placed in a 250 mL amber glass container, weighed, and placed at ambient temperature in a dark cabinet for three month storage with air in the headspace.

### 2.5.4. Color

Each extraction sample was placed in a plastic cuvette and measured for absorbance at 420 nanometers in a UV-Spectrophotometer. Absorbance wavelength of 420 nm was chosen as it is complementary to the yellow color of the extracted samples (Reusch, 2013).

### 2.5.5. Total Soluble Phenolics

Total Soluble Phenolics (TSP) were measured by Folin-Ciocalteu method (1927) and expressed as gallic acid equivalents (mg GA eq/mL). Following this method, a 1 mL sample was prepared in a 1:10 (v/v) dilution extraction sample to EtOH and placed in a glass tube with 7 mL DI water added. The sample was vortexed and 1 mL of 1 % Folin-Ciocalteu reagent was added and vortexed. After three minutes, a 12.4 % sodium carbonate solution was added and vortexed. Samples were placed in a 40 °C water bath for 30 minutes and then measured at 725 nm absorbance using a UV-spectrophotometer. Concentration of TSP was calculated using a standard curve using gallic acid equivalents (mg/mL).

### 2.5.6. Gas Chromatography

A 1:1 (v/v) sample to dichloromethane (DCM) ratio was placed in a glass test tube and vortexed for 10 seconds, allowed to sit for 1 minute, then vortexed again for 10 seconds. Samples were allowed to sit until separation for 15 minutes and placed in the -40 °C freezer in a plastic test tube until analysis.

After thawing frozen a frozen samples at room temperature, the bottom portion of each separation was removed using glass pipettes, and placed in GC vials. Samples

were injected into a GC inlet set at 200 °C and were detected using a flame ionization detector (FID). Samples were held in a 60 °C oven for three minutes and were ramped 4 °C/min until oven temperature reached 230 °C and held for 30 min.

### 2.5.7. Liquid Chromatography

Oak compound determination using HPLC was based on a method established by Kim and Lee (2002). Samples were prepared by mixing equal amounts MeOH and extracted samples (1:1 v/v). Samples were vortexed and placed in HPLC amber glass vials. Three mobile phases, 50 mM $(NH_4)H_2PO_4$, pH 2.6 adjusted with orthophosphoric acid (Solvent A), 80:20(v/v) acetonitrile/50 mM $(NH_4)H_2PO_4$, pH 2.6 (Solvent B), and 200 mM $H_3PO_4$, pH 1.5 adjusted with $NH_4OH$ (Solvent C), were prepared and filtered before use. Ten µL of each sample was injected and run at a flow rate of 1 mL/min at ambient temperature. An attached photodiode array detector (PDA) was set at 280, 320, 370, and 420 nm with solvent gradient.

**Table 1.** Solvent Gradient for Reversed-Phase HPLC Analysis of Polyphenolics (Kim and Lee, 2002).

| Time (min) | Solvent A (%) | Solvent B (%) | Solvent C (%) |
|---|---|---|---|
| **0** | 100 | 0 | 0 |
| **4** | 92 | 8 | 0 |
| **10** | 0 | 14 | 86 |
| **22.5** | 0 | 16.5 | 83.5 |
| **27.5** | 0 | 25 | 75 |
| **50** | 0 | 80 | 20 |
| **55** | 100 | 0 | 0 |
| **60** | 100 | 0 | 0 |

### 2.5.8. Statistical Analysis

Analysis of variance (ANOVA) was performed using JMP 12, while box-and-whisker plots were made using both JMP 12 and Minitab 17. A comparison of means was performed using Tukey HSD ($\alpha$=0.05) in JMP 12 program. For unequal variances, a Welch ANOVA was run using JMP 12 software.

# 3. RESULTS AND DISCUSSION

An analysis of variance (ANOVA) test and a means comparison (Tukey HSD) was run to determine differences (α=0.05) across extraction treatments (control, sonication, and reflux), among stave layers (charred layers 1-7 and toasted layers 1-4), between charred and toasted heat treatments, and between 0 and 3 months time. For unequal variances, a Welch ANOVA was run using JMP 12. Interactions between heat treatments and extraction treatments (charred and toasted staves to control, sonication, and reflux), between heat treatments and time (charred and toasted to time 0 and after 3 months), among extraction treatments and time (control, sonication, and reflux to time 0 and after 3 months), between heat treatments and layers (charred and toasted to layers 1 through 7), and among layers and time (layers 1 through 7 and time 0 and after 3 months).

Box-and-whisker plots are used to display the data of each test. Boxes are made up of the upper and lower quartiles with the median between the two while the whiskers are the minimum and maximum data points. A grand mean for the data goes across the box plot chart. Data points were considered outliers if larger or smaller than 1.5 times the interquartile range of the data.

## 3.1. Color

Color was determined by absorbance at 420 nanometers. Significant differences (α=0.05) were found among layers for heat treated charred staves (Fig. 3), across reflux, sonication, and control extraction treatments (Fig. 4), between time 0 and after 3 months (Fig. 5), between heat treated staves among extraction treatments (Fig.6),

among extraction treatments over time (Fig.7), between heat treated staves over time (Fig.8), among layers for charred and toasted staves (Fig.9), and among layers over time (Fig. 10). No significant difference was found between heat treated charred and toasted staves and also among toasted stave layers (Fig. 1 and 2)

.

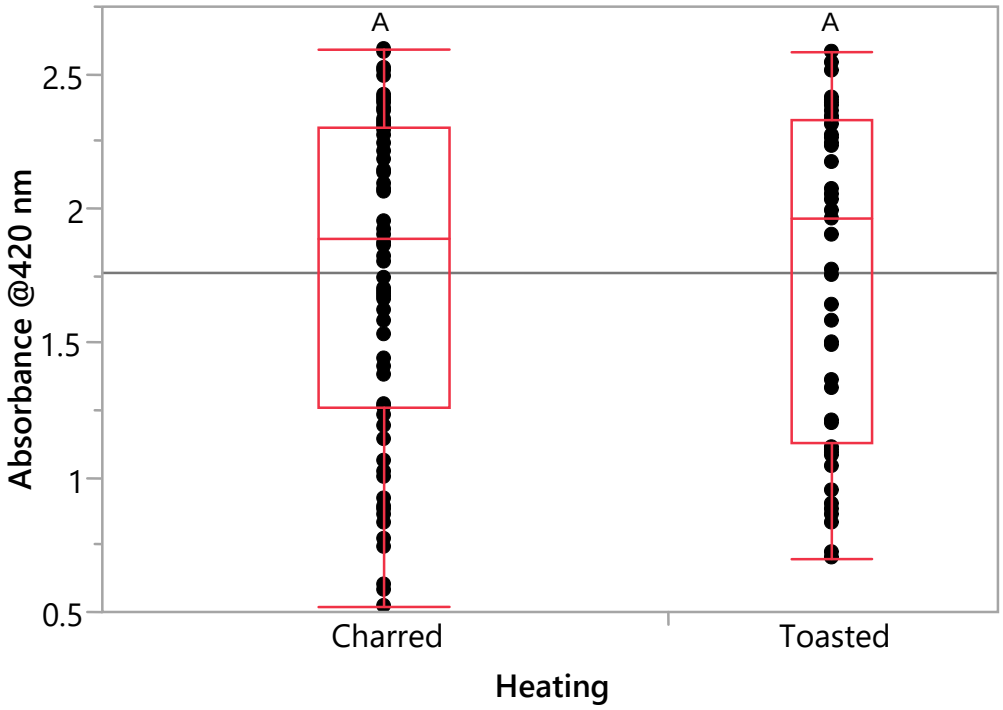


**Figure 1.** Color extracted from charred and toasted staves.

There was no significant difference (α=0.05) in color between charred and toasted staves or among toasted stave layers (Fig. 1 and 2). However, individual charred layers did show a significant difference in color (Fig. 3).

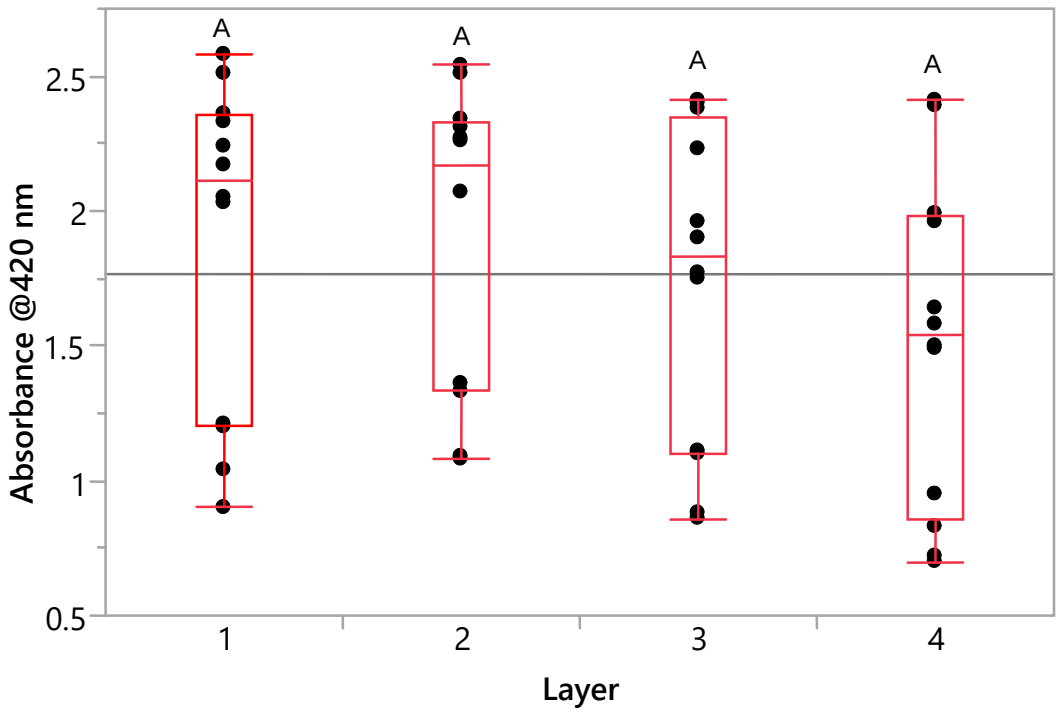


**Figure 2.** Color extracted from each toasted layer.

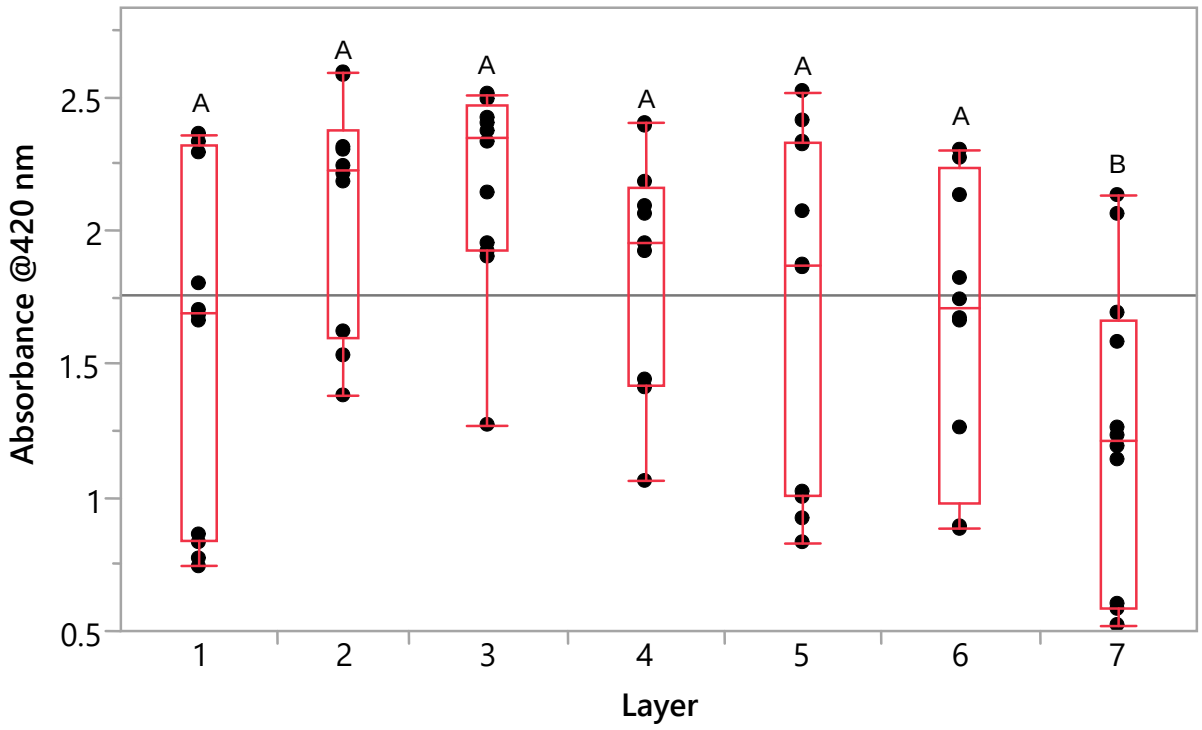


**Figure 3.** Color extracted from each charred layer.

For charred layers, charred layers 1 through 6 had a darker color than charred layer 7(Fig 3). A Welch test was run on individual charred layers and was found to have at least one significant difference ($p=0.0014$).

Heat treatment processes can affect up to a depth of 6 mm (Perry et al., 1990). Each layer in this research was 1 to 2 mm deep, and results show that the inner-most layers did not provide as much color as heat treated layers shown by their decreasing absorbance level at 420 nm. Aging whiskey in toasted or charred barrels provides color, but the two treatments differ in color intensity. Toasted staves undergo less heat treatment than charred barrels, 15-30 seconds versus 45 seconds, which produces less color due to the lack of depth affected and lack of oak cell disruption for oak compound extraction (Mosedale and Puech, 1998; Russell, 2003). Charred layer 1 contributes little color to whiskey as complex ellagitannins are heavily decreased by intense heat and charring, and is instead used to remove undesirable extractive components from whiskey. However, degradation of wood structure in the first layer provides avenues for distillate to reach inner, slightly heat treated layers, such as layers 2 through 6, to extract more oak compounds (Russell, 2003). Oak heartwood in these layers contains the most extractable oak compounds, and thus, produces a darker color (Pandey, 2005). Layers which are not as affected by heat treatment such as charred layer 7 and all toasted layers do not receive as much degradation of hemicelluloses and lignin which decreases the amount of extractable compounds available to provide color (Russell, 2003).

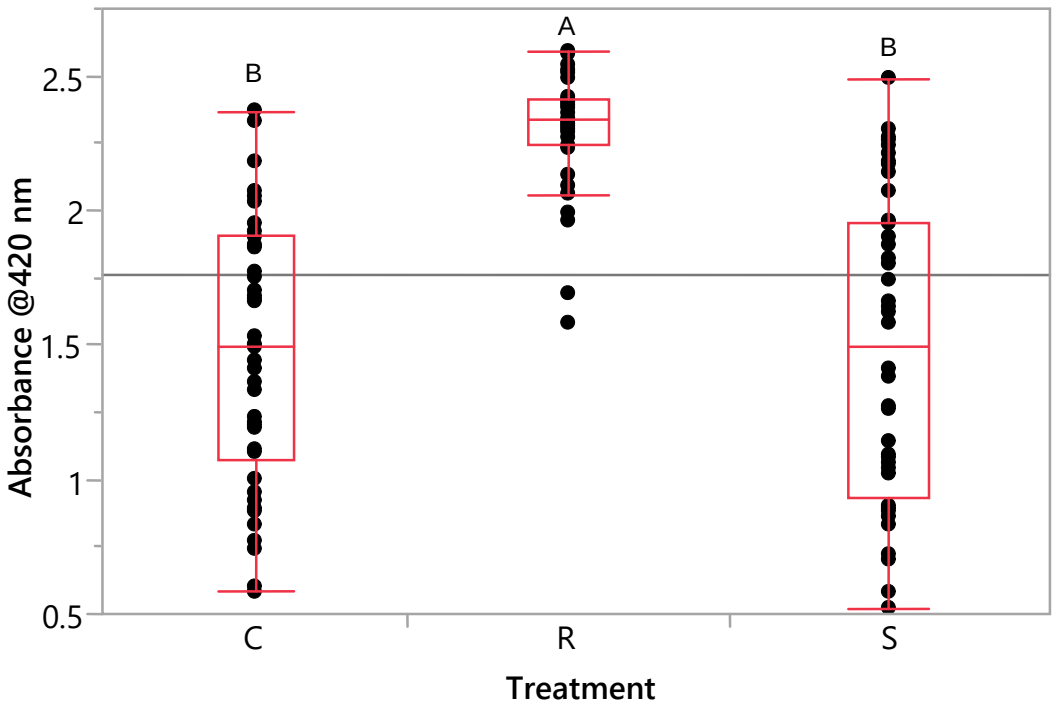


**Figure 4.** Color after each extraction treatment.

Reflux samples were darker in color than sonication and control samples after extraction which suggests reflux extracted more oak compounds (Fig. 4). A Welch test was run for extraction treatments and was found to have at least one significant difference ($p<0.0001$). Extraction of compounds is known to be increased by contact time, surface size, and temperature (Natali et al., 2006; Kulkarni and Rathod, 2014). For all methods, contact time and surface size of shavings remained the same; however, the reflux extraction method requires the boiling of ethanol. In the reflux extraction method, temperature is above the boiling point of ethanol (78°C). A high temperature is known to rapidly evaporate compounds with low boiling points which would open headspace, increase oxidation reactions, and thus, increase the color of the samples (Russell 2003).

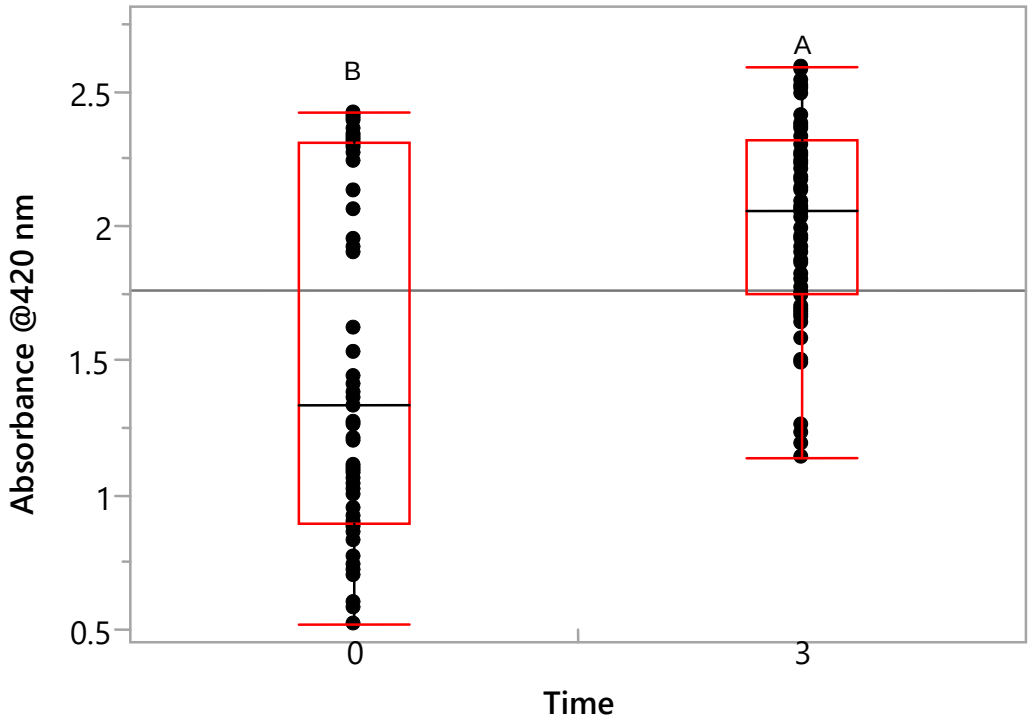


**Figure 5.** Color of extracted samples over time.

Samples were darker after 3 months than at time 0 (Fig. 5). A Welch test was run for difference over time and was found to have at least one significant difference ($p<0.0001$).The darker samples indicate that over time, more oak compounds were extracted. Extraction of oak compounds increases over time due to further degradation and oxidation of hemicelluloses and lignin (Mosedale and Puech, 1998). However, while the color in whiskey will continue to increase over time, the extraction rate of compounds for aroma and flavor will decrease after twelve months. The exact chemical nature of color has yet to be studied, but it is believed that carbonyl and carboxyl groups formed after oxidation produce the color changes seen in whiskey (Russell, 2003; Pandey, 2005).

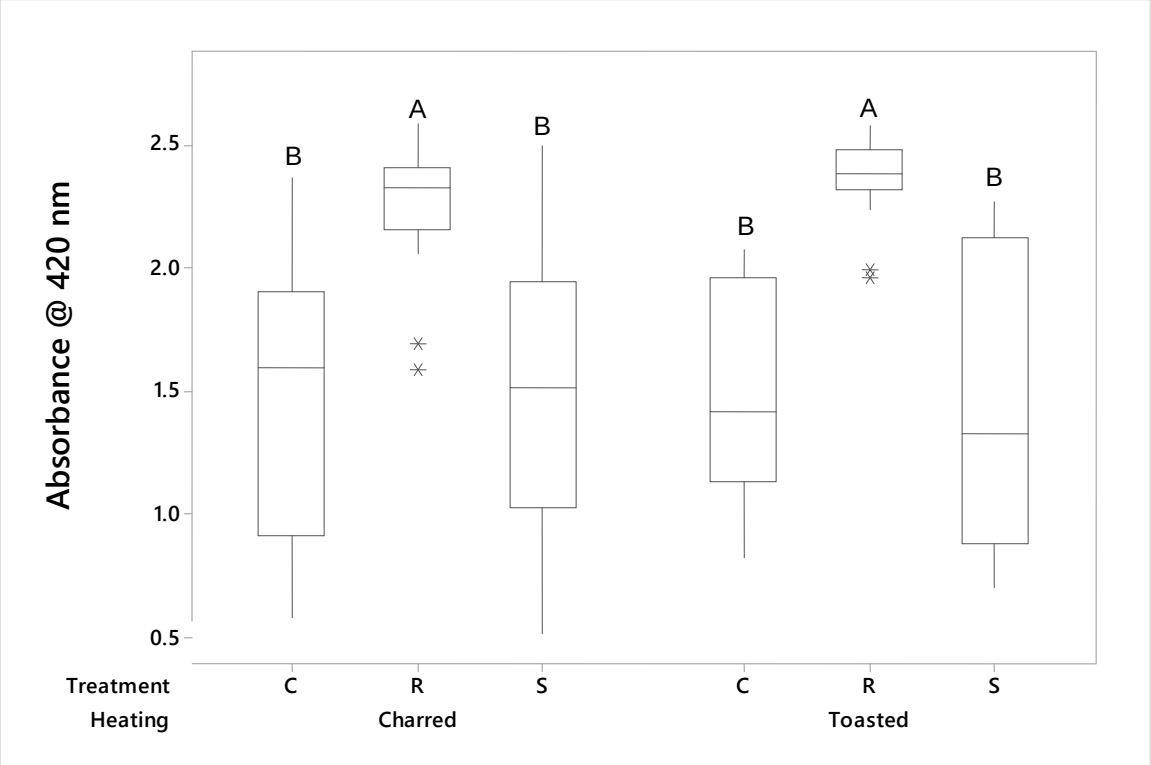


**Figure 6.** Color extracted by each extraction treatment within each heat treatment.

Reflux extraction from toasted and charred staves had a darker color than toasted and charred staves from sonication and control extraction methods (Fig 6). A Welch test was run on extraction treatments within each heat treatment and was found to have at least one significant difference ($p<0.0001$). Because reflux extraction method uses a higher temperature than sonication and control extraction methods, it is able to extract more oak compounds by breaking apart hemicelluloses and lignins which results in production of a darker color (Russell, 2003).

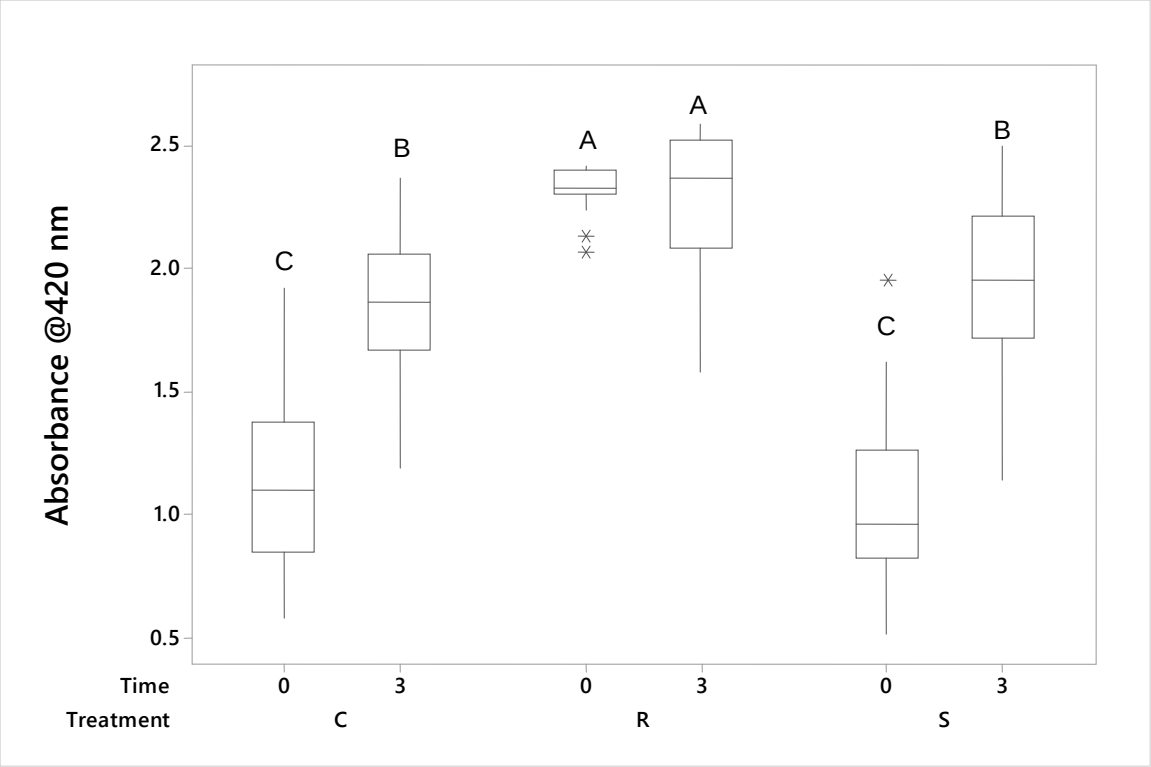


**Figure 7.** Color extracted by each extraction treatment over time.

Reflux at time 0 and after 3 months had a darker color than sonication and control extraction methods at time 0 and after 3 months, while sonication and control after 3 months had a darker color than sonication and control at time 0 (Fig. 7). A Welch test was run on extraction treatments over time and was found to have at least one significant difference ($p<0.0001$). Reflux at both time 0 and after 3 months had the same color level showing that time did not affect reflux extraction treatment also suggesting that reflux initially produced the darkest color and initially extracted the maximum amount of oak compounds capable of producing color. The development of darker color over time for both sonication and control extraction methods after 3 months indicates that time was a factor for these two extraction treatments.

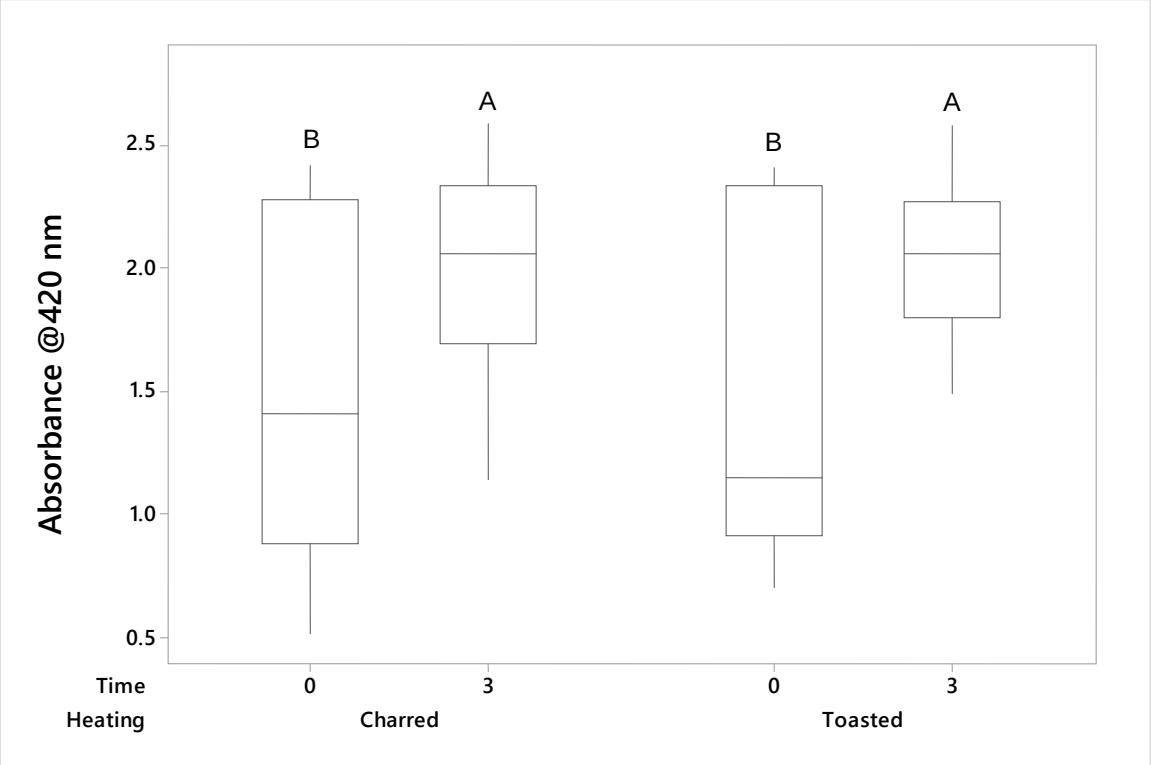


**Figure 8.** Color extracted from each heat treatment over time.

Both charred and toasted oak staves after 3 months had darker color than at time 0 (Fig. 8). A Welch test was run on heat treatments over time and was found to have at least one significant difference ($p<0.0001$). Results indicate that variation decreased and that more oak compounds were extracted from both charred and toasted staves over time which produced a darker color after 3 months than at time 0.

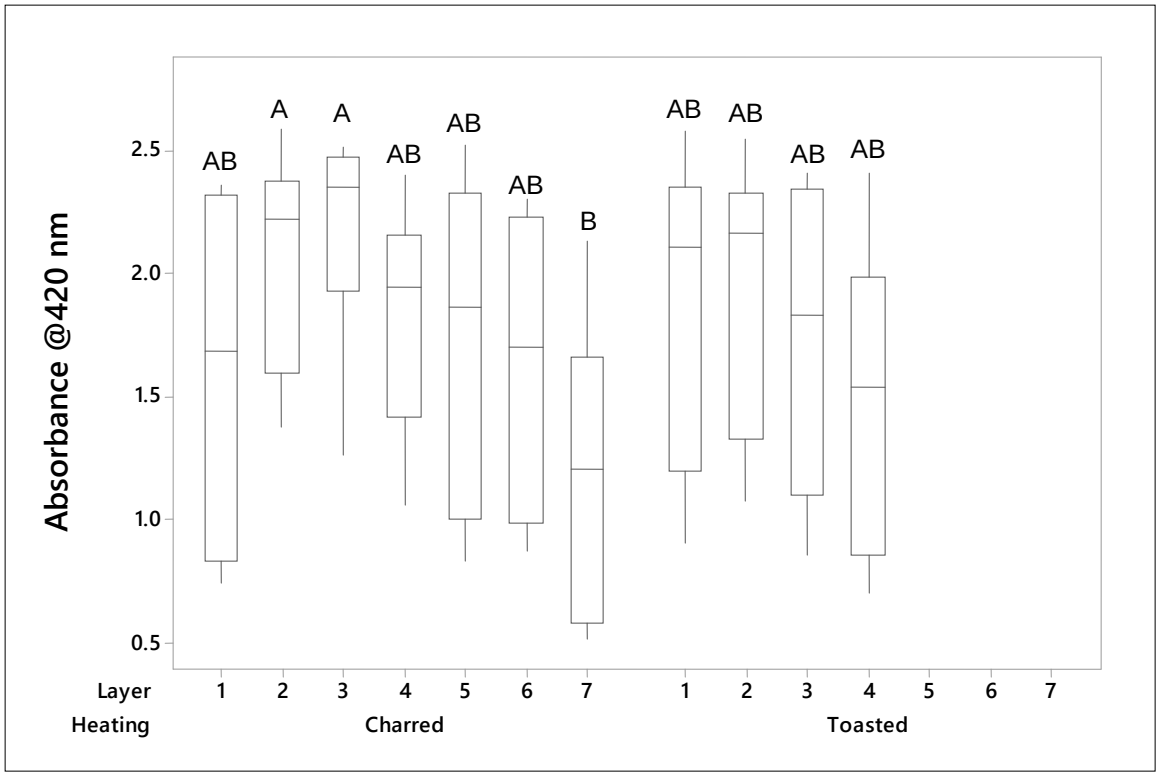


**Figure 9.** Color extracted from each layer within each heat treatment.

Charred layers 2 and 3 extracted a darker color than charred layer 7, but were similar in color to toasted layers 1 through 4 and charred layers 1, 4, 5, and 6. While charred layers 1, 4, 5, and 6 and toasted layers 1 through 4 had a similar color to charred layer 7 (Fig. 9). A Welch test was run on individual layers within heat treatments and was found to have at least one significant difference ($p=0.0035$).

Extraction of oak compounds was increased in charred layers 2 and 3 due to the thermal degradation of cell walls (Russell, 2003; Pandey, 2005). Charred layer 1 and 7 do not impart as much color as other layers due to one being a carbon layer which removes sulfurous compounds, while the other is an inner-most layer which has not been degraded enough during heat treatment to extract oak compounds capable of

producing color. Like charred layer 7, toasted layers 1 through 4 were not degraded enough during heat treatment to produce a dark color.

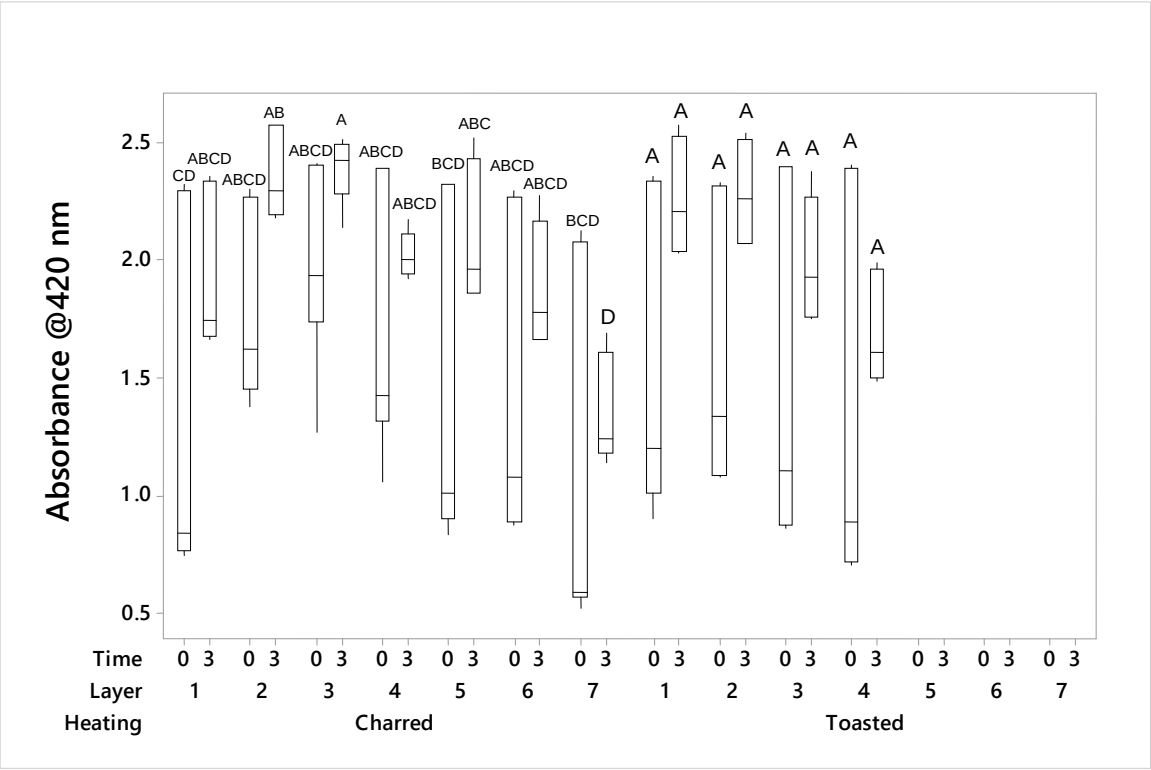


**Figure 10.** Color extracted from each layer over time within each heat treatment.

Charred layer 3 after 3 months had a darker color than charred layers 1, 5, and 7 at time 0 and charred layer 7 after 3 months, but had a similar color to charred layers 2, 3, 4, and 6 at time 0 and charred layers 1, 2, 4, 5, and 6 after 3 months. Charred layer 2 after 3 months had a darker color than charred layers 1 and 7 at time 0, but had a similar color to charred layers 2, 3, 4, 5, and 6 at time 0 and charred layers 1, 4, 5, and 6 after 3 months. Charred layer 5 after 3 months had a darker color than charred layer 7 at time 0, but had a similar color to charred layers 1, 2, 3, 4, 5, and 6 at time 0 and

charred layers 1, 4, 6, and 7 after 3 months. Charred layers 1, 2, 3, 4, 5, 6, and 7 at time 0 and charred layers 1, 4, 6, and 7 after 3 months all had a similar color (Fig. 10). A Welch ANOVA found that at least one value was significantly different ($p<0.0001$). All toasted layers at time 0 and after 3 months had a similar color extracted (Fig. 10). A Welch ANOVA found that at least one value was significantly different ($p=0.003$).

## 3.2. Total Soluble Phenolics

Significant differences ($\alpha=0.05$) were found among layers for heat treated charred staves (Fig. 13), across reflux, sonication, and control extraction treatments (Fig.14), between time 0 and after 3 months (Fig. 15), between heat treated staves among extraction treatments (Fig. 16), among extraction treatments over time (Fig.17), between heat treated staves over time (Fig. 18), among layers for charred and toasted staves (Fig.19), and among layers over time (Fig. 20). However, there was no significant difference found between heat treated charred and toasted staves and also among toasted stave layers (Fig. 11 and 12).

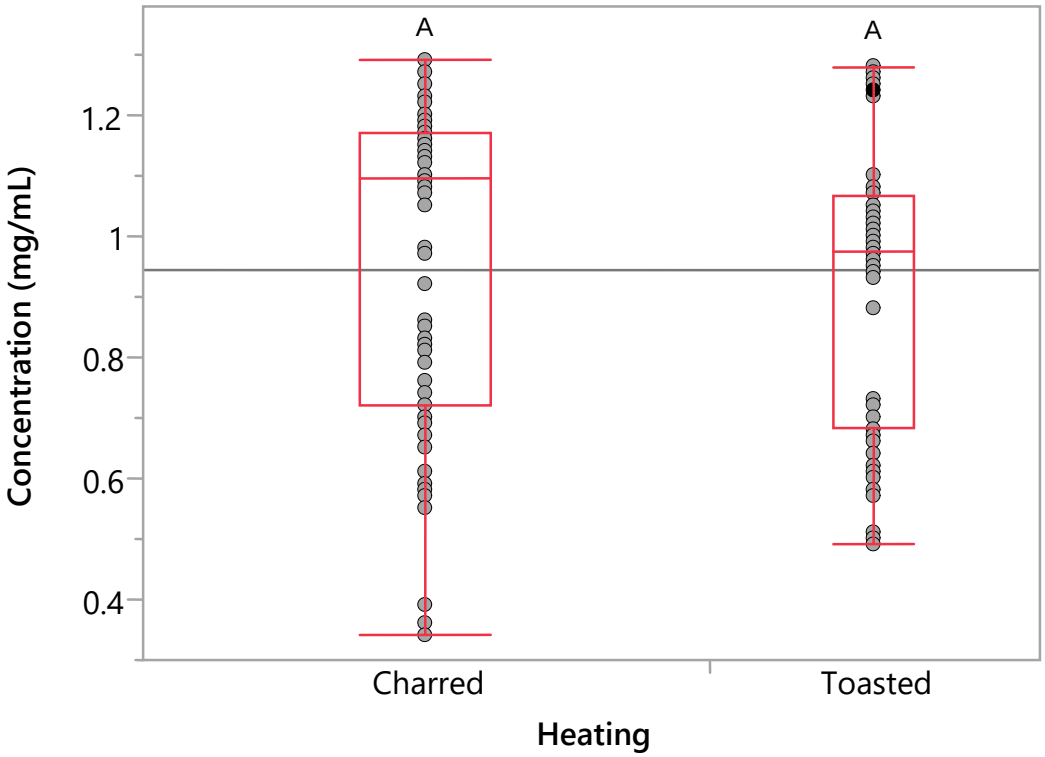


**Figure 11.** Total soluble phenolics extracted from charred and toasted staves.

There was no significant difference (α=0.05) in the amount of total soluble phenolics extracted from charred and toasted staves or from individual toasted layers (Fig. 11 and 12). However, there was a significant difference among charred stave layers (Fig. 13).

There was no significant difference (α=0.05) in the amount of total soluble phenolics extracted from each toasted layer (Figure 12).

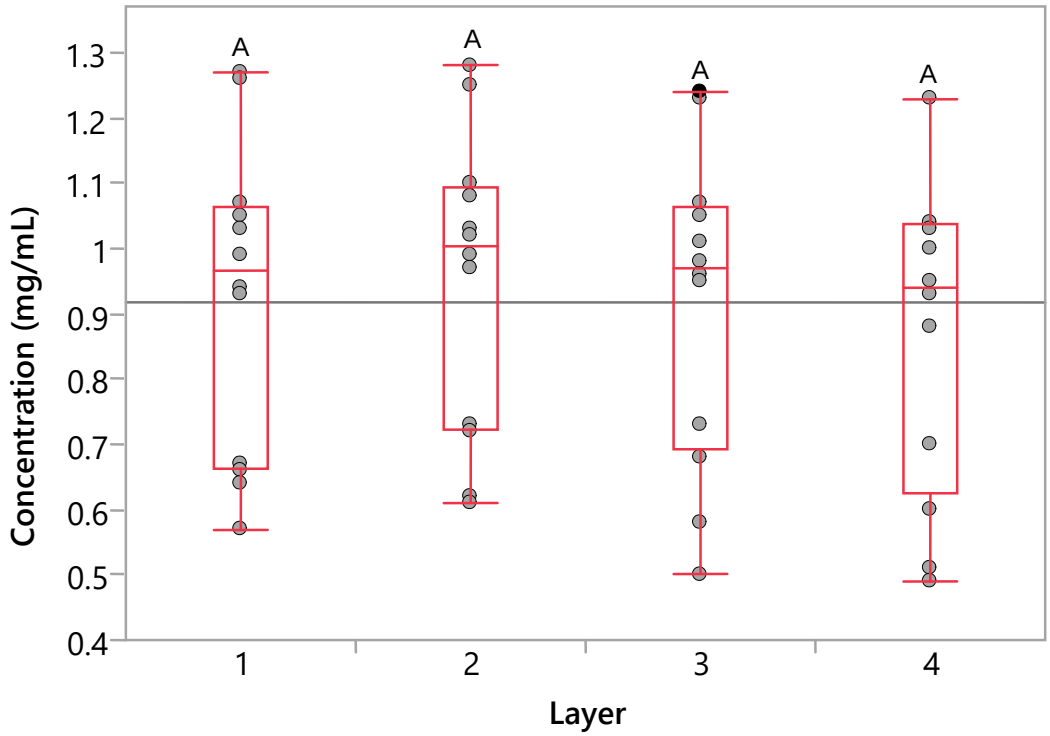


**Figure 12.** Total soluble phenolics extracted from each toasted layer.

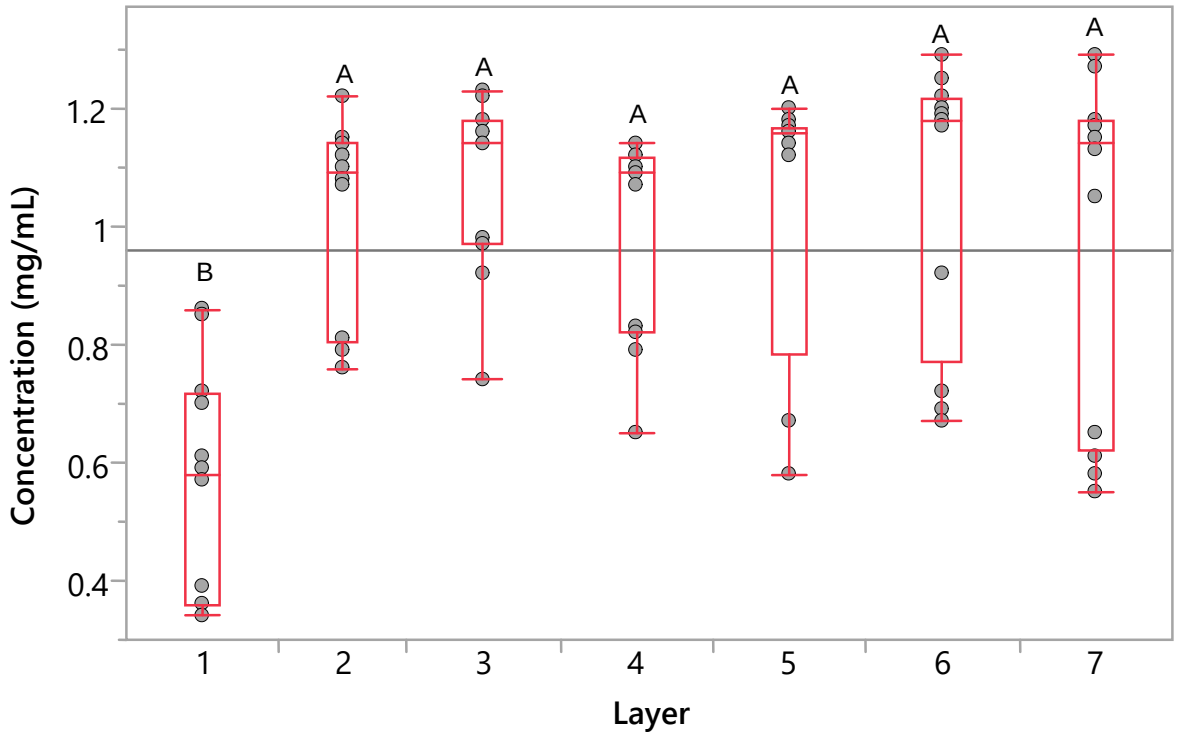


**Figure 13.** Total soluble phenolics extracted from each charred layer

Charred layers 2 through 7 had more total soluble phenolics extracted from charred layer 1 (Figure 13). A Welch test was run on individual charred layers and was found to have at least one significant difference ($p<0.0001$). Most TSP which come from the middle layers are made up of hydrolysable tannins such as gallic acid, ellagic acid, vanillin, syringaldehyde, and sinapaldehyde, to name a few (Russell, 2003). According to Pandey, inner-most layers, such as 6 and 7, should have less available compounds than middle layers, such as 2 through 5 (2005). However, results show that layers 6 and 7 still had a high availability of compounds for extraction similar to earlier layers. Charred layer 1 is not known to have high levels of TSP because of its activated carbon layer which is used to remove sulfurous compounds and not impart compounds (Russell, 2003).

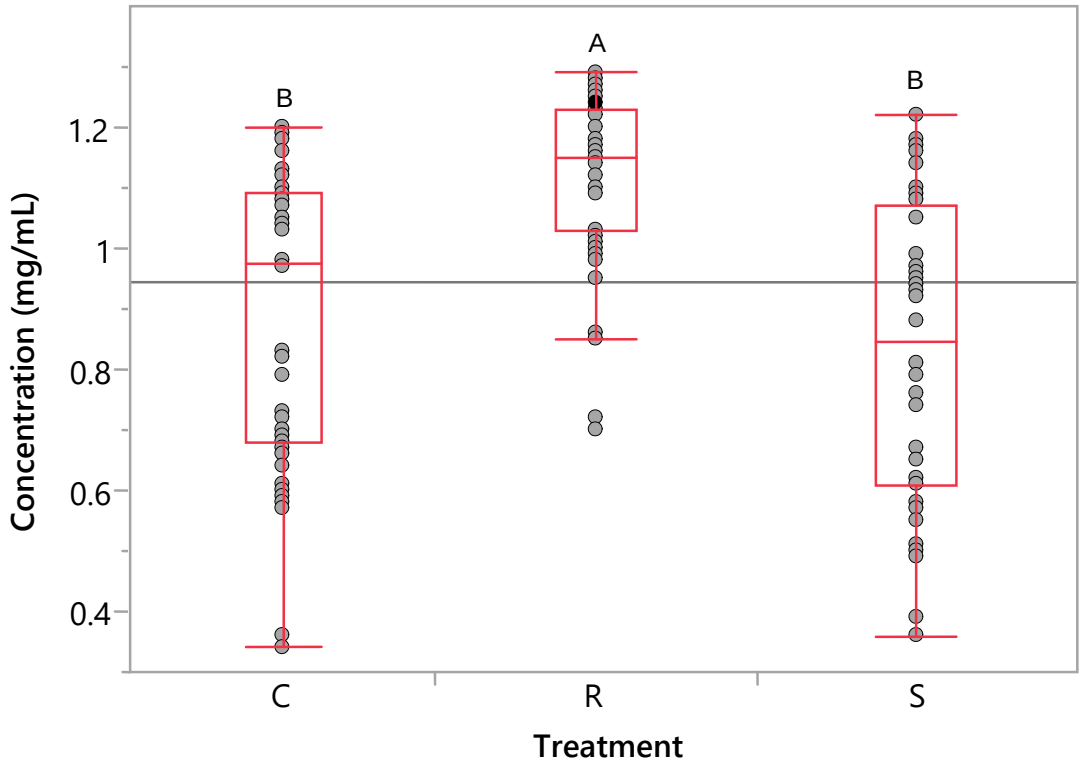


**Figure 14.** Total soluble phenolics extracted from each extraction treatment.

Reflux extracted more total soluble phenolics from sonication and control extraction methods (Fig. 14). A Welch test was run on extraction treatments and was found to have at least one significant difference ($p<0.0001$). The amount of oak compounds extracted and subsequent chemical changes not only depend on the whiskey production process and oak components, but also on the type of technological treatment (Caldeira et al., 2007). Reflux, as a heat intensive extraction method, is able to thermally degrade wood structure and release more oak compounds than either the sonication or control method (Kulkarni and Rathod, 2014). While a solvent with higher temperature releases more phenolic compounds, it can cause heat oxidation and degradation if the high temperature exceeds a short period of time (Rajha et al., 2013). Compounds could produce undesirable and rancid components, such as ketones derived from fatty acids, because of high temperatures (Russell, 2003; Khoddami et al., 2013).

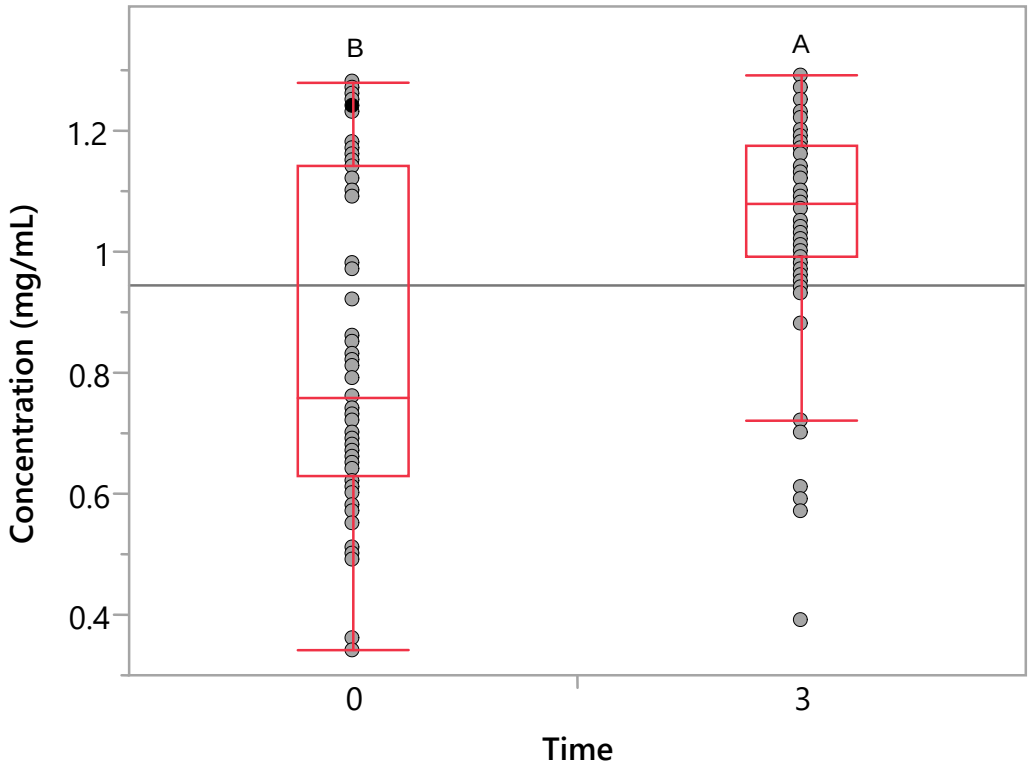


**Figure 15.** Total soluble phenolics extracted over time.

More total soluble phenolics were extracted after 3 months than at time 0 (Fig. 15). A Welch test was run over time and was found to have at least one significant difference ($p<0.0001$). After initial extraction, further extraction of oak compounds displays more cell disruption by the distillate and the oxidation and hydrolysis of oak compounds over time (Russell, 2003). Development of hydrolyzed and oxidized compounds over time is still not well known and would require further time studies (Mosedale and Puech, 1998).

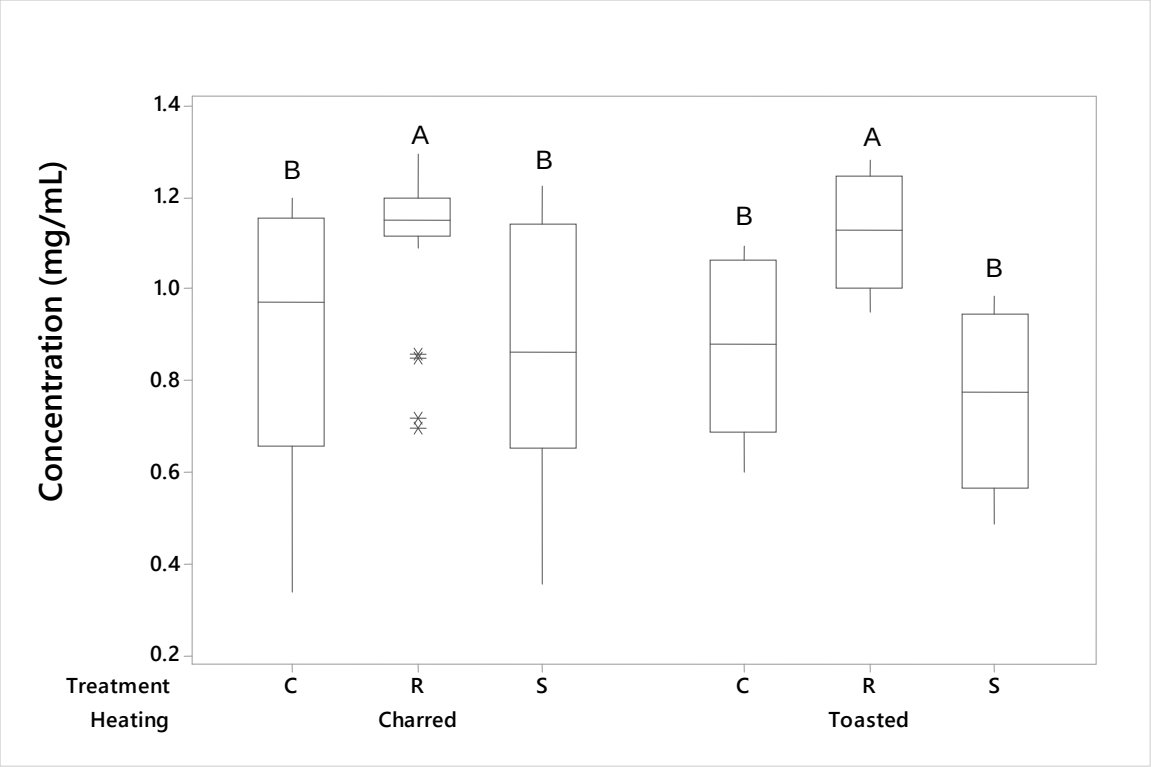


**Figure 16.** Total soluble phenolics extracted by each extraction treatment within each heat treatment.

Reflux extraction treatment extracted more total soluble phenolics from charred and toasted staves than sonication and control extraction methods. Sonication and control extraction methods extracted the same amount of total soluble phenolics from toasted and charred staves (Fig. 16). A Welch test was run on extraction treatments within each heat treatment and was found to have at least one significant difference ($p<0.0001$).

Results indicate reflux extraction treatment was a more effective method of extraction than sonication and control extraction methods.

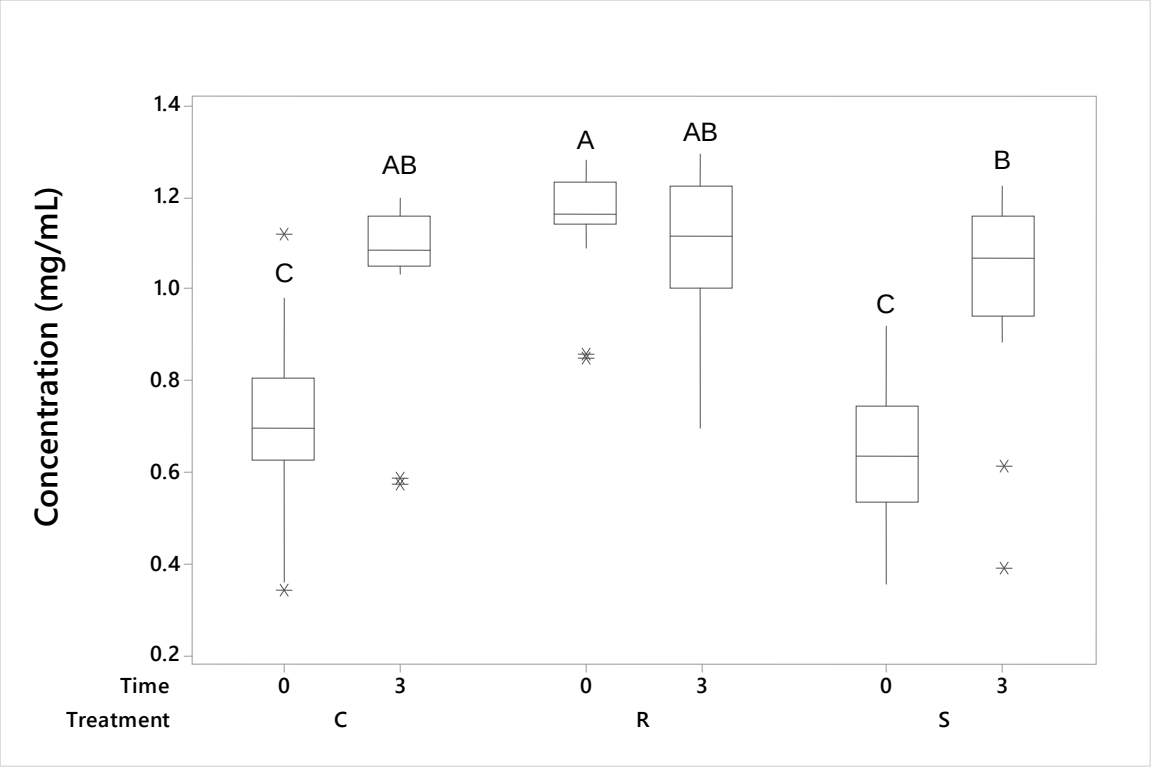


**Figure 17.** Total soluble phenolics extracted by each extraction treatment over time.

Reflux at time 0 extracted more total soluble phenolics than sonication after 3 months and sonication and control extraction treatments at time 0, but was similar to reflux and control extraction treatments after 3 months. Reflux and control treatments after 3 months extracted more total soluble phenolics than sonication and control at time 0, but had a similar amount to sonication after 3 months. Sonication after 3 months extracted more total soluble phenolics than sonication and control methods at time 0 (Fig. 17). A Welch test was run on extraction treatments over time and was found to have at least one significant difference ($p<0.0001$).

More total soluble phenolics were extracted after 3 months than at time 0 and for reflux extraction method than for sonication and control treatments (Fig. 14 and 15).

Because reflux method shows a similar amount of TSP at time 0 and after 3 months, reflux shows ability to extract more compounds initially versus sonication and control extraction treatments which do more extraction over time.

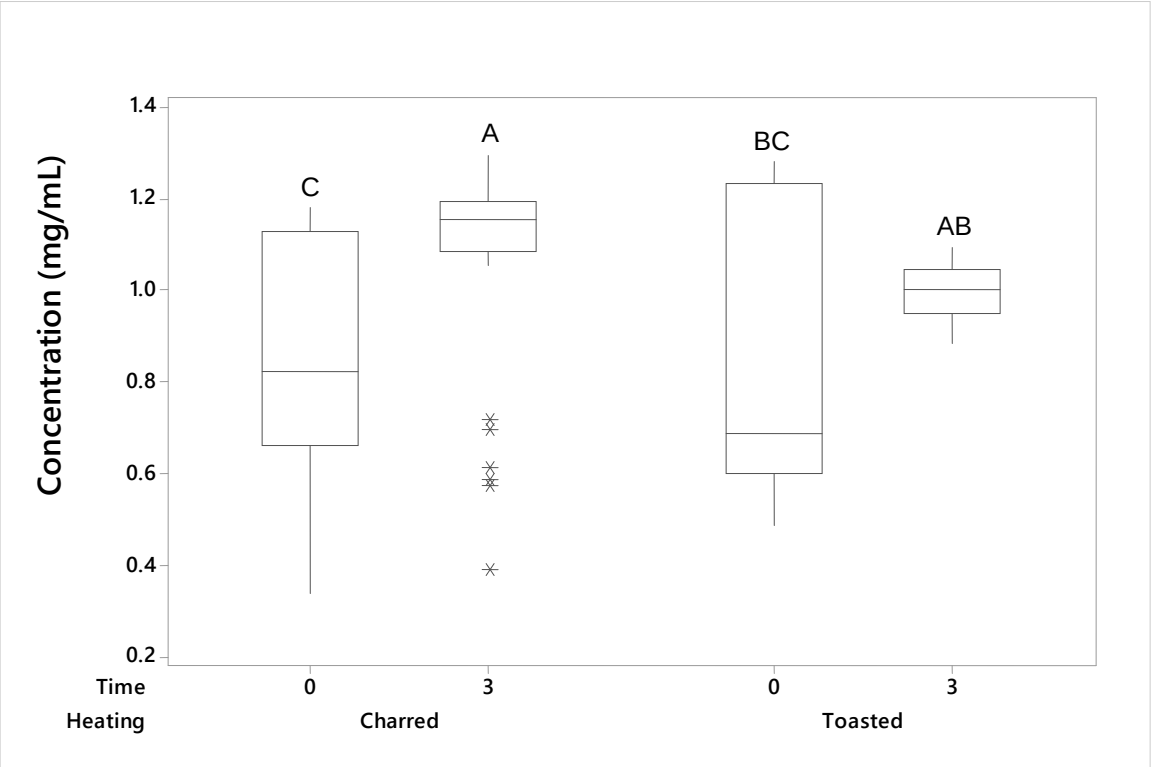


**Figure 18.** Total soluble phenolics extracted from each heat treatment over time.

Charred staves after 3 months extracted more total soluble phenolics than charred and toasted staves at time 0, but extracted a similar amount to toasted staves after 3 months. Toasted staves after 3 months extracted more than charred staves at time 0, but extracted a similar amount to toasted staves at time 0. Toasted staves at time 0 had a similar amount of total soluble phenolics extracted to charred staves at

time 0 (Fig. 18). A Welch test was run on heat treatments over time and was found to have at least one significant difference ($p<0.0001$).

As toasted stave layers were not significantly different from each other, they had a similar amount of TSP extracted at both time 0 and after 3 months; however, charred stave layers were significantly different from each other and extracted a different amount of TSP over time.

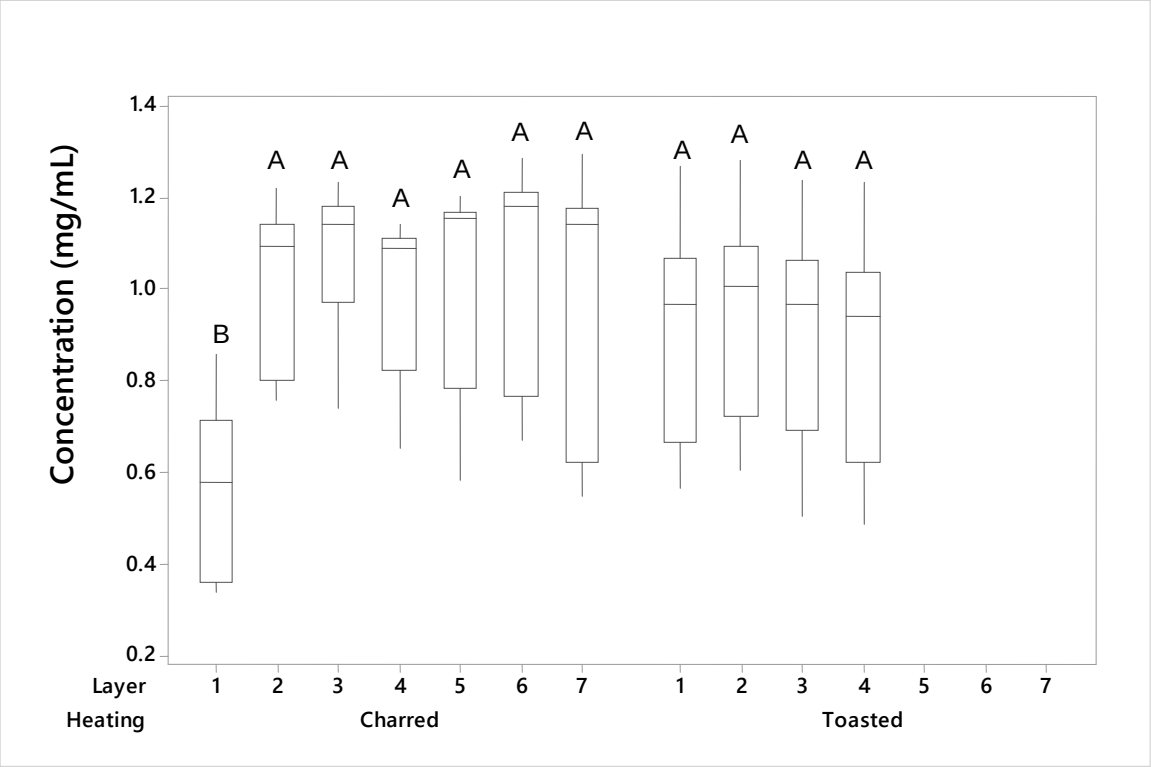


**Figure 19.** Total soluble phenolics extracted from each layer within each heat treatment.

Charred layers 2 through 7 and toasted layers 1 through 4 had more total soluble phenolics extracted than charred layer 1 (Fig. 19). A Welch test was run on individual layers within heat treatments and was found to have at least one significant difference

(p<0.0001). Due to the activated carbon layer, charred layer 1 has less available compounds for extraction (Russell, 2003; Pandey, 2005).

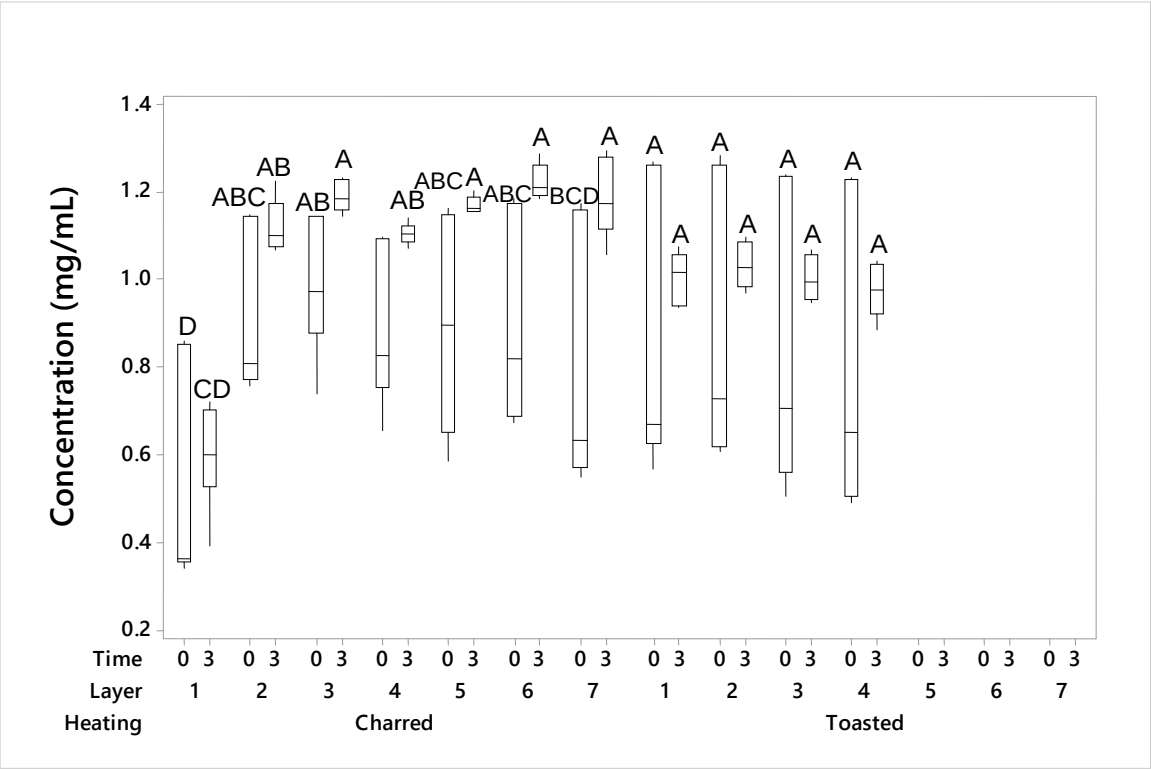


**Figure 20.** Total soluble phenolics extracted from each layer over time.

Charred layers 3, 5, 6, and 7 after 3 months had more total soluble phenolics than charred layers 1 and 7 at time 0 and charred layer 1 after 3 months, but had a similar amount of total soluble phenolics to charred layers 2, 3, 4, 5, and 6 at time 0 and charred layers 2 and 4 after 3 months. Charred layer 3 at time 0 and charred layers 2 and 4 after 3 months had more total soluble phenolics that charred layer 1 at time 0 and after 3 months, but had a similar amount to charred layers 2, 4, 5, 6, and 7 at time 0. Charred layers 2, 3, 5, and 6 had more total soluble phenolics than charred layer 1 at

time 0, but had a similar amount to charred layer 7 at time 0 and charred layer 1 after 3 months. Charred layer 7 at time 0 had a similar amount of total soluble phenolics to charred layer 1 at time 0 and after 3 months (Fig. 20). A Welch ANOVA showed that at least one value was significantly different ($p<0.0001$). Toasted staves layers at time 0 and after 3 months all had a similar amount of total soluble phenolics (Fig. 20).

### 3.3. pH

Significant differences ($\alpha=0.05$) were found among layers for heat treated charred staves (Fig. 23), between time 0 and after 3 months (Fig. 25), among extraction treatments over time (Fig.27), between heat treated staves over time (Fig. 28), among layers for charred and toasted staves (Fig.29), and among layers over time (Fig. 30). However, there was no significant difference found between heat treated charred and toasted staves, among toasted stave layers, among reflux, sonication, and control extraction treatments, and the interaction between heat treated staves among extraction treatments (Fig. 21, 22, 24, and 26).

There was no significant difference ($\alpha=0.05$) in the pH level between charred and toasted staves or for toasted stave layers (Fig. 21 and 22). However, there was a significant difference among charred stave layers (Fig. 23).

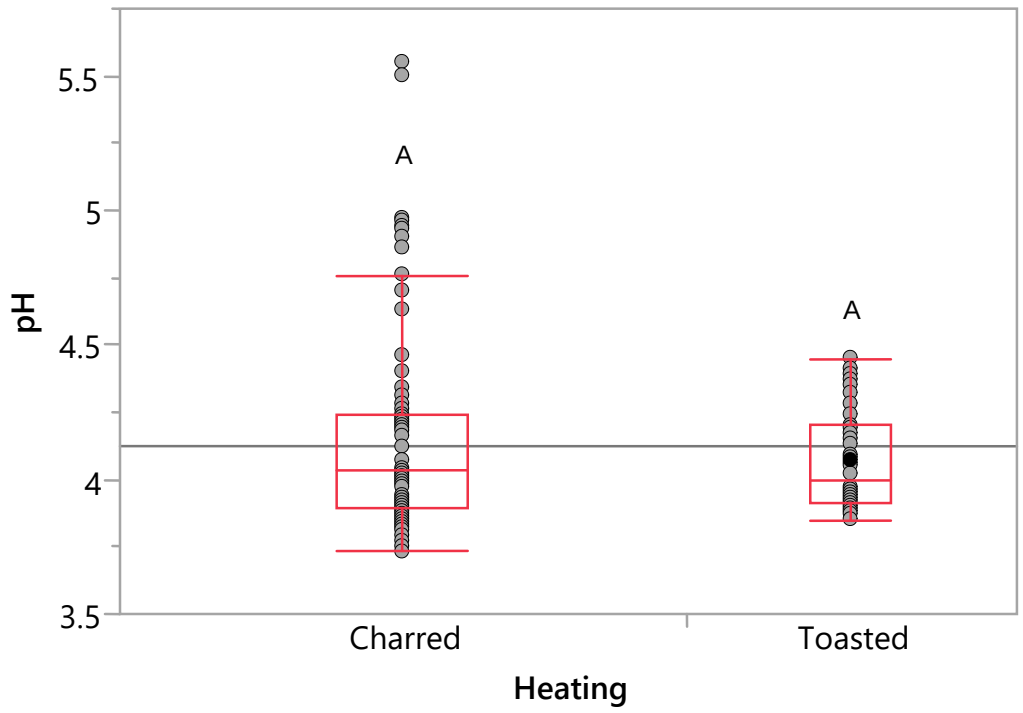


**Figure 21.** Level of pH in charred and toasted staves.

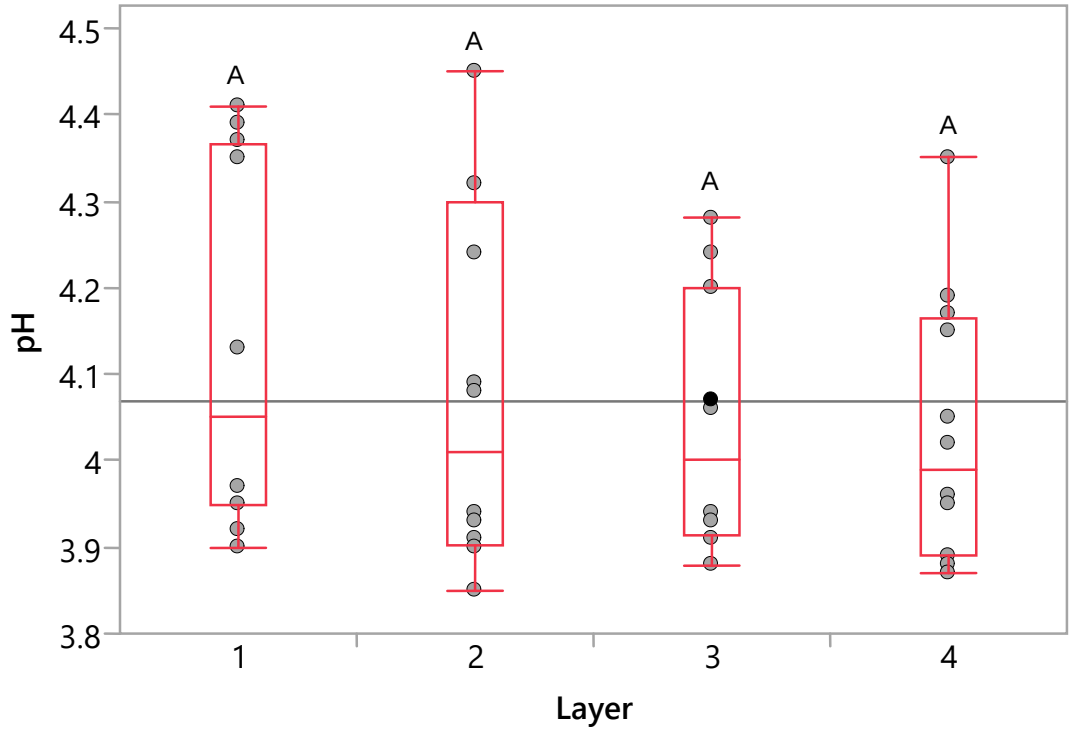


**Figure 22.** Level of pH after extraction from each toasted layer.

There was no significant difference (α=0.05) in the level of pH from toasted layers after extraction treatment (Fig. 22). Results show that pH was the same from each toasted stave layer.

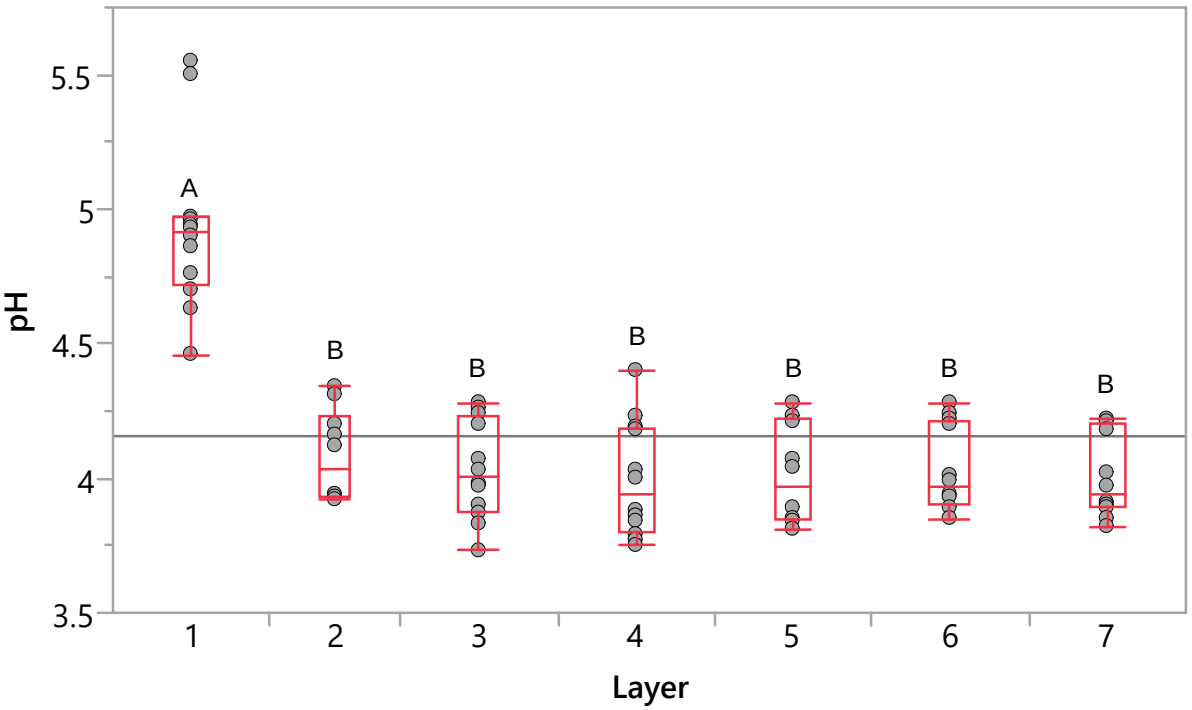


**Figure 23.** Level of pH after extraction from each charred layer.

Charred layer 1 had a higher pH than layers 2 through 7 which all had the same pH (Fig. 23). A Welch test was run on individual charred layers and was found to have at least one significant difference ($p<0.0001$). Due to an active carbon layer, charred layer 1 is more alkaline than other layers (Russell, 2003).

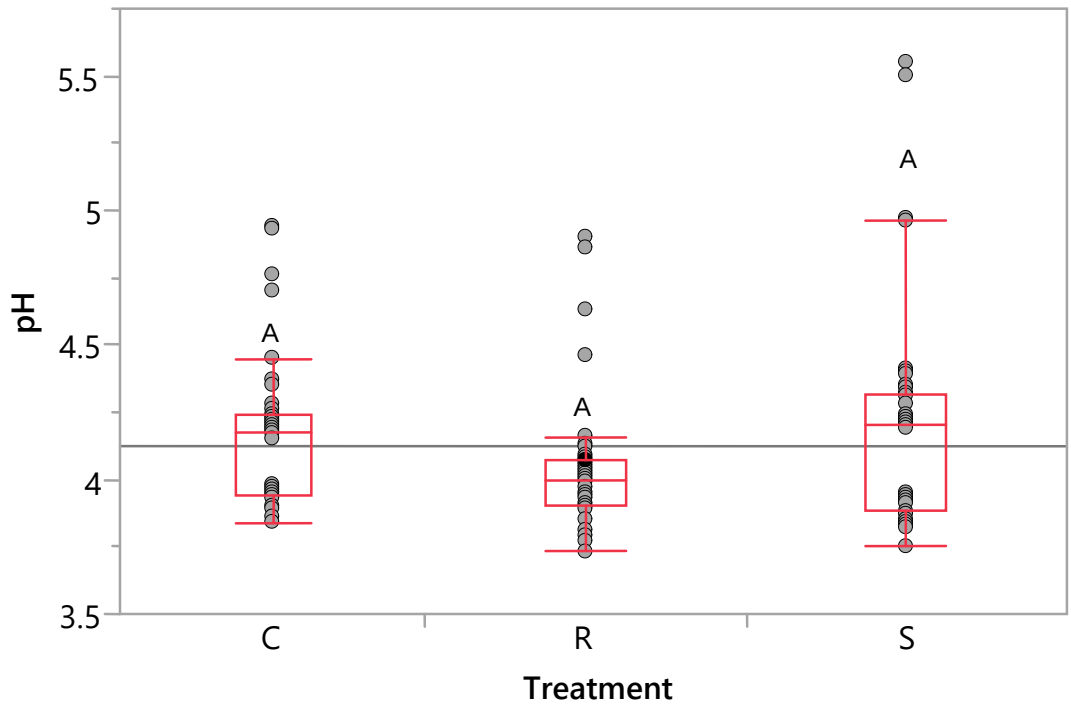


**Figure 24.** Level of pH after each extraction treatment.

There was no significant difference (α=0.05) in the level of pH after each extraction treatment (Fig. 24). A Welch test was run on extraction treatments and was found to have at least one significant difference (p=0.0403). The same pH level between reflux, sonication, or control extraction treatments suggests a similar amount of acids released during extraction (Russell, 2003).

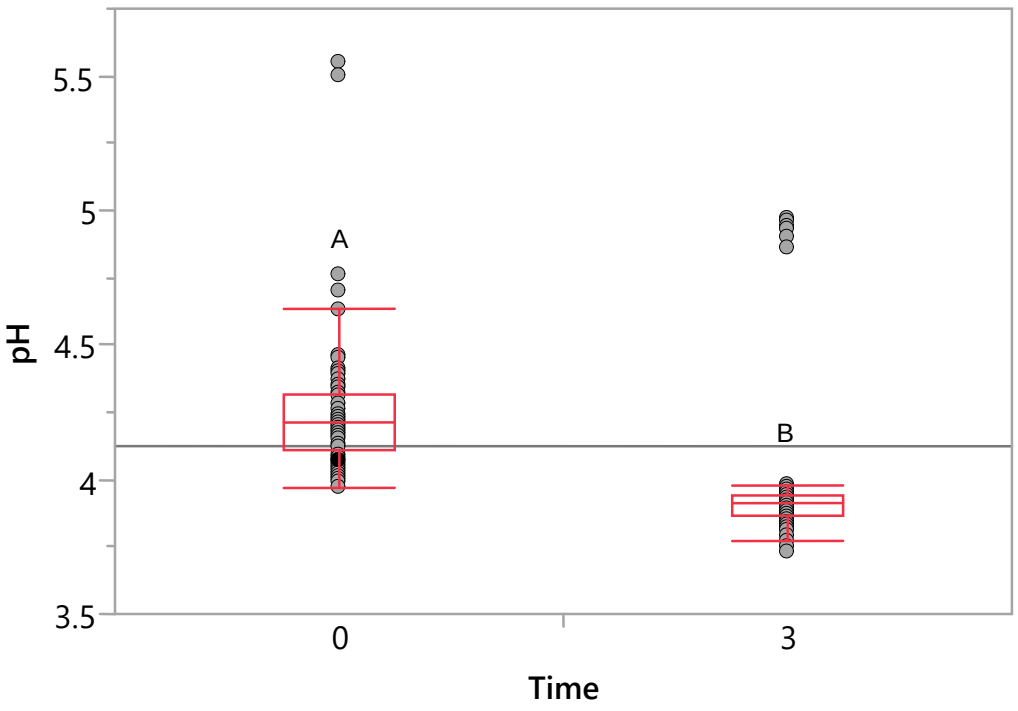


**Figure 25.** Level of pH over time.

Samples at time 0 had a higher pH than after 3 months (Fig. 25). A Welch test was run over time and was found to have at least one significant difference ($p<0.0001$). According to Zelinka and Stone, a drop in pH levels indicates the presence of extractable oak components such as small organic acids, tannins, and phenols (2011). Results show that after 3 months, pH of the samples dropped significantly which is the result of the hydrolysis of hemicelluloses into acetic acid and formic acid and lignin degradation into acidic tannins and phenols (Zelinka and Stone, 2011).

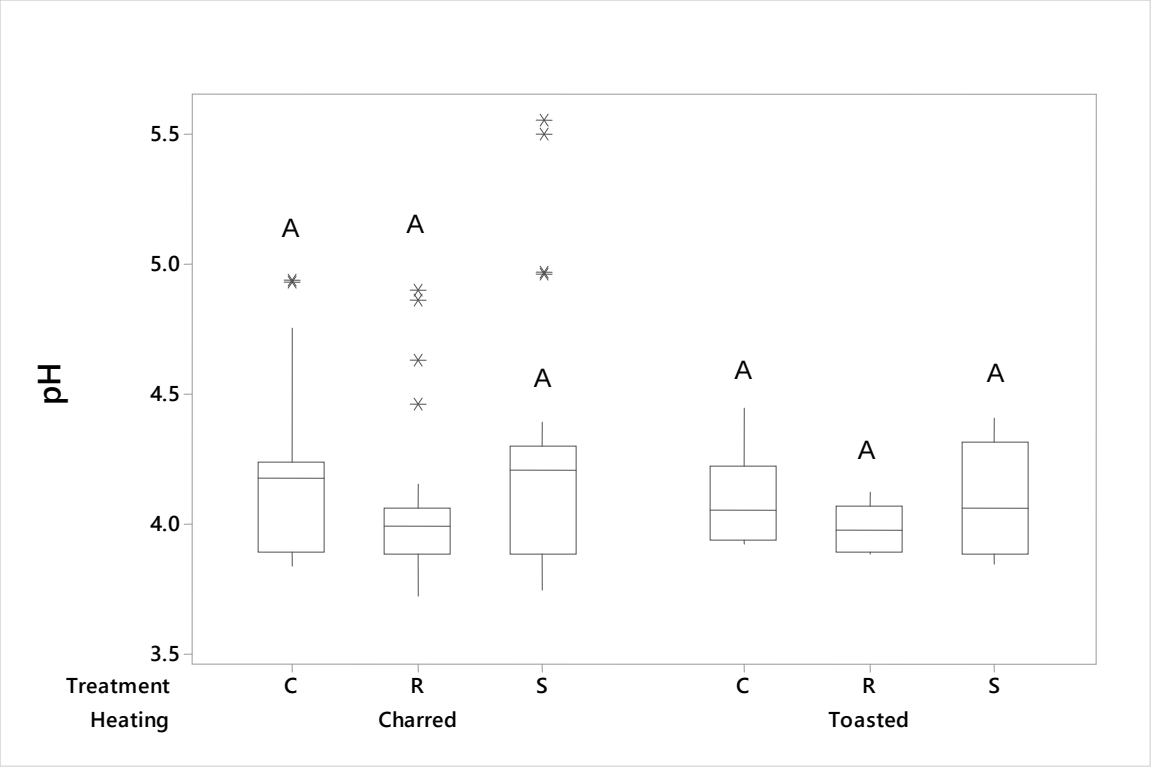


**Figure 26.** Level of pH extracted by each extraction treatment within each heat treatment.

There was no significant difference (α=0.05) in the pH level between toasted and charred oak staves after each extraction treatment (Fig. 26). A Welch test was run on extraction treatments within each heat treatment and was found to have at least one significant difference (p=0.0120).

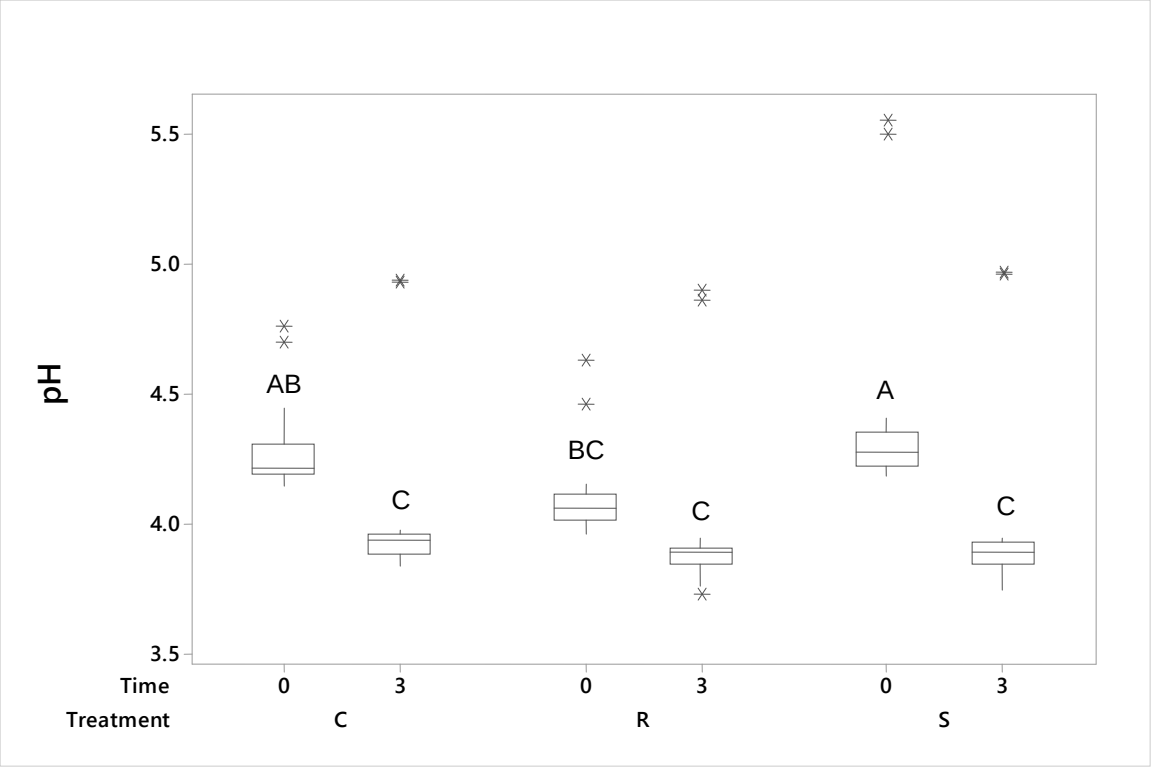


**Figure 27.** Level of pH extracted by each extraction treatment over time.

Sonication at time 0 had a higher pH than reflux at time 0 and sonication, reflux, and control extraction treatments after 3 months, but was similar to the control at time 0. Control at time 0 had a similar level of pH to reflux at time 0, but had a higher pH than sonication, reflux, and control extraction treatments after 3 months. Reflux at time 0 had a similar level of pH to sonication, reflux, and control extraction treatments after 3 months (Fig. 27). A Welch test was run on extraction treatments over time and was found to have at least one significant difference ($p<0.0001$).

The amount of oak compounds released following extraction increases over time as shown by lowered pH levels in sonication, reflux, and control samples after 3 months (Mosedale and Puech, 1998; Russell, 2003). Lower pH is directly correlated to the

further release compounds and the formation of acids during maturation (Pandey, 2005; Zelinka and Stone, 2011).

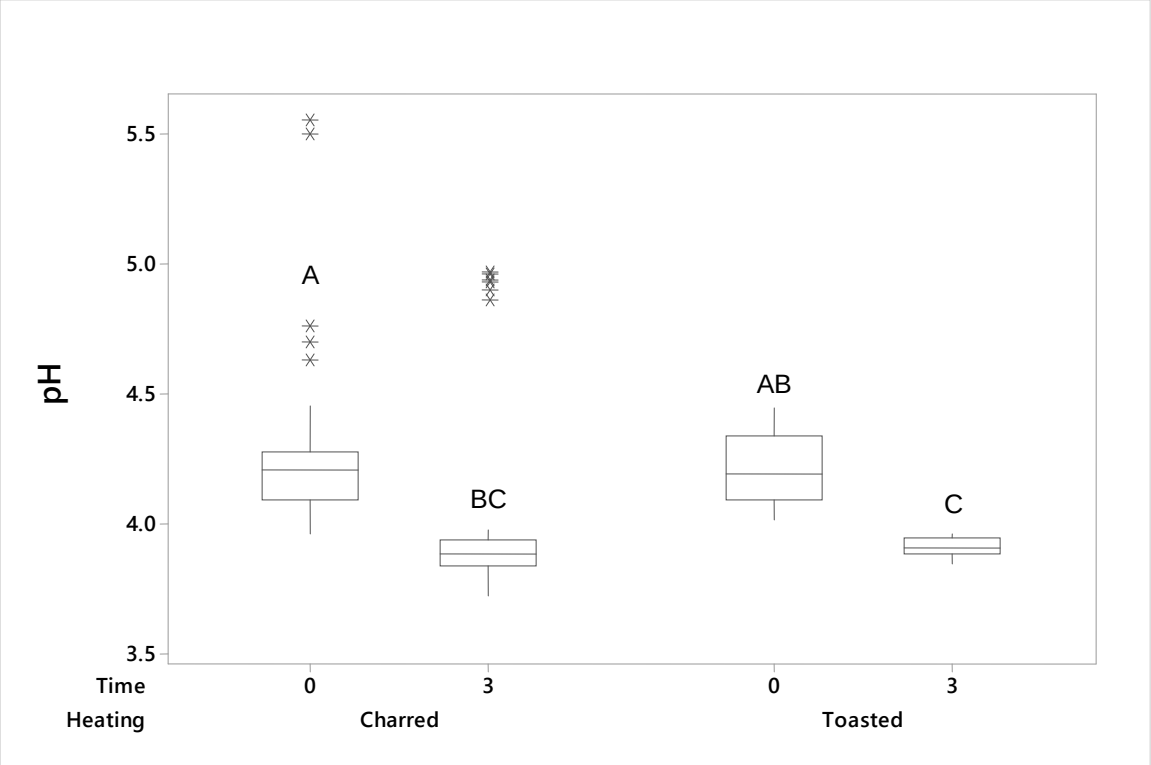


**Figure 28.** Level of pH extracted by each heat treatment over time.

Charred staves at time 0 had a higher pH than charred and toasted staves after 3 months, but had a similar pH level to toasted staves at time 0. Toasted staves at time 0 had a higher pH than toasted staves after 3 months, but had a similar level to charred staves after 3 months (Fig. 28). A Welch test was run on each heat treatment over time and was found to have at least one significant difference ($p<0.0001$). Higher pH levels indicate that ellagitannins were already degraded prior to extraction treatment and were not available for release by oak wood (Russell, 2003; Pandey, 2005).

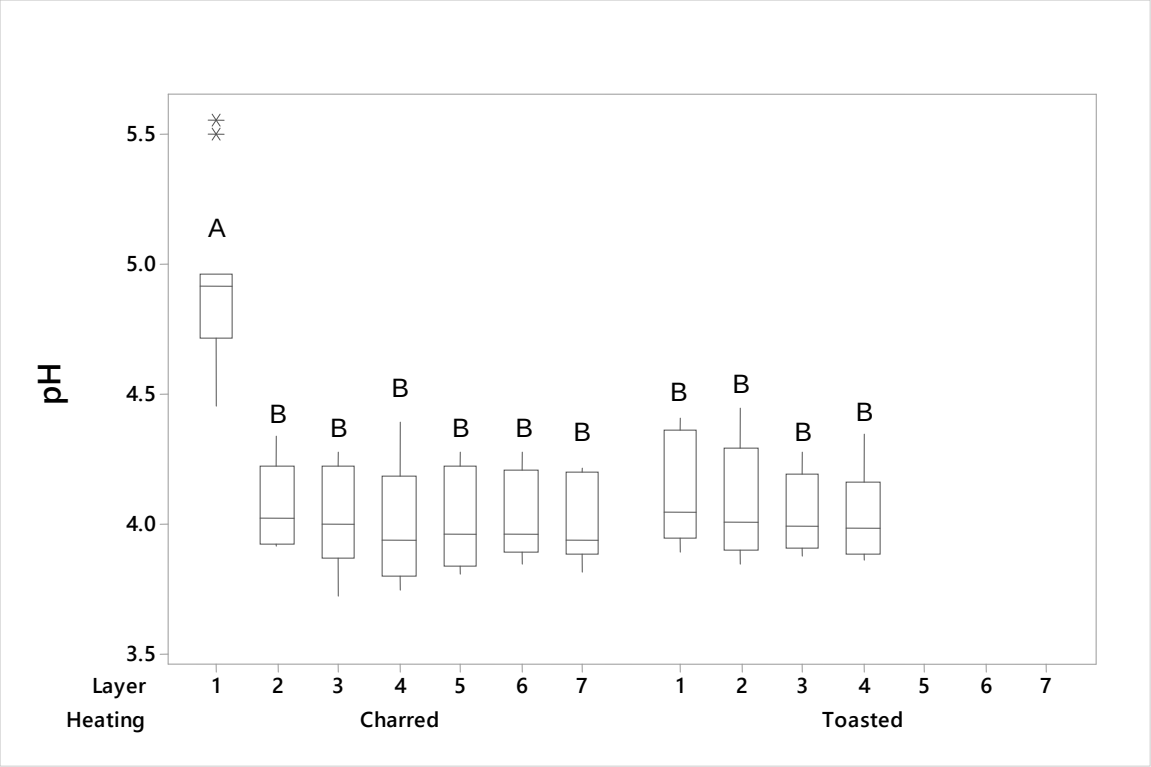


**Figure 29.** Level of pH extracted from each layer within each heat treatment.

Charred layer 1 had a higher pH level than toasted layers 1 through 4 and charred layers 2 through 7. Toasted layers 1 through 4 and charred layers 2 through 7 all had the same pH level (Fig. 29). A Welch test was run on individual layers within each heat treatment and was found to have at least one significant difference ($p<0.0001$).

White oak is one of the most acidic wood types due to its high amount of ellagitannin (Zelinka and Stone, 2011). Apart from charred layer 1, charred layers 2 through 7 and toasted layers 1 through 4 had a high amount of ellagitannin available. The intense charring of charred staves degrades the wood structure more quickly and reduces the amount of available oak compounds (Russell, 2003). Because of

degradation, charred layer 1 produces a solution with a higher pH than charred layers 2 through 7 and toasted layers 1 through 4 which have a lower pH due to the release of more oak compounds (Russell, 2003; Pandey, 2005; Zelinka and Stone, 2011).

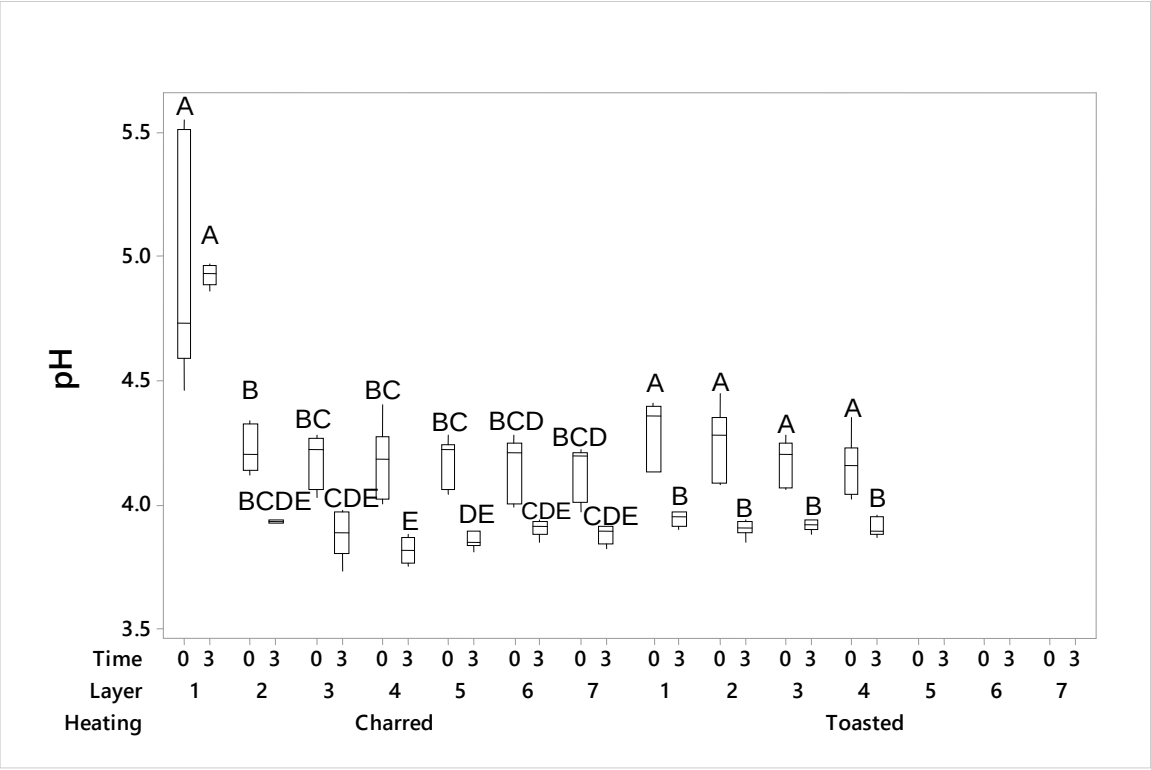


**Figure 30.** Level of pH extracted from each layer over time.

Charred layer 1 at time 0 and after 3 months had a higher pH than all other charred layers. Charred layer 2 at time 0 had a higher pH than charred layers 3, 4, 5, 6, and 7 after 3 months, but had a similar pH to charred layers 3, 4, 5, 6, and 7 at time 0 and charred layer 2 after 3 months. Charred layers 3, 4, and 5 at time 0 had a higher pH than charred layers 4 and 5 after 3 months, but had a similar pH to charred layers 6 and 7 at time 0 and charred layers 2, 3, 6, and 7 after 3 months. Charred layers 6 and 7 at

time 0 had a higher pH than charred layer 4 after 3 months, but had a similar pH to charred layers 2, 3, 5, 6, and 7 after 3 months. Charred layer 2 after 3 months had a similar pH to charred layers 3, 4, 4, 6, and 7 after 3 months (Fig. 30). A Welch ANOVA found that at least one value was significantly different ($p<0.0001$). Toasted stave layers 1 through 4 at time 0 had a higher pH than toasted stave layers 1 through 4 after 3 months (Fig. 30). A Welch ANOVA found that at least one value was significantly different ($p<0.0001$). As time was significant for pH, a lower pH after 3 months demonstrates an increase in chemical reactions of oak compounds over time (Pandey, 2005).

## 3.4. Gas Chromatography

### 3.4.1. Furaldehyde

Significant differences ($\alpha=0.05$) were found among layers between heat treated charred and toasted staves (Fig. 31), among toasted stave layers (Fig. 32), among charred stave layers (Fig. 33), among layers for charred and toasted staves (Fig. 39), and among layers over time (Fig. 40). However, there was no significant difference found among reflux, sonication, and control extraction methods (Fig. 34), between time 0 and after 3 months (Fig. 35), the interaction between heat treated staves among extraction treatments (Fig. 36), among extraction treatments over time (Fig.37), and between heat treated staves over time (Fig. 38).

Toasted staves had more furaldehyde extracted than from charred staves (Fig. 31). Less heat treated layers had a higher availability of furaldehyde for extraction.

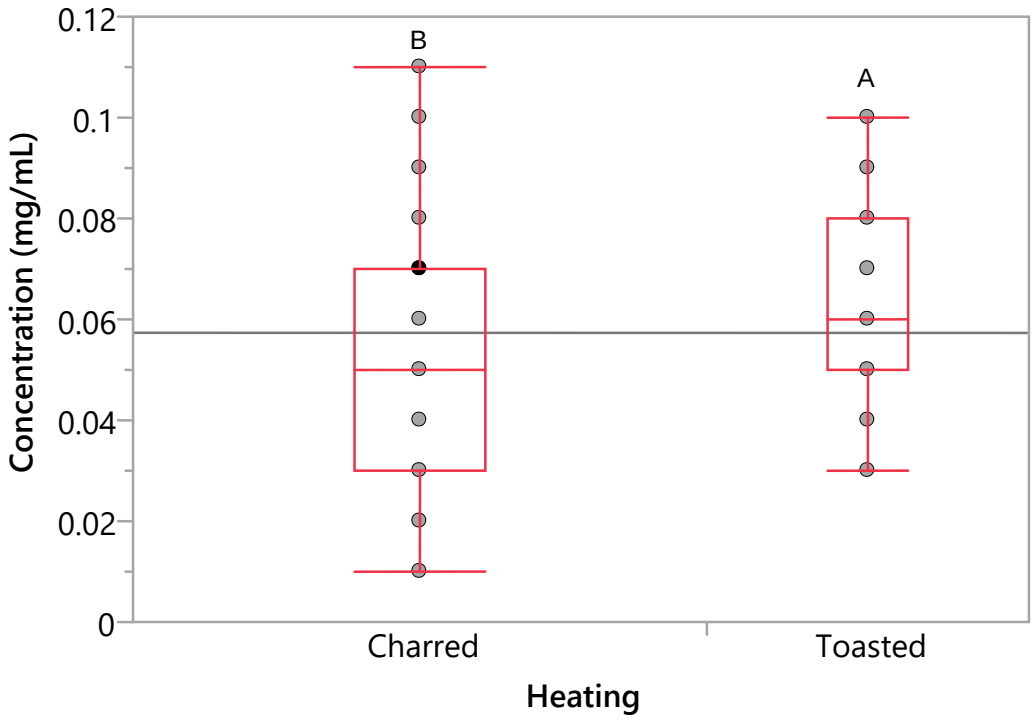


**Figure 31.** Furaldehyde extracted from charred and toasted staves.

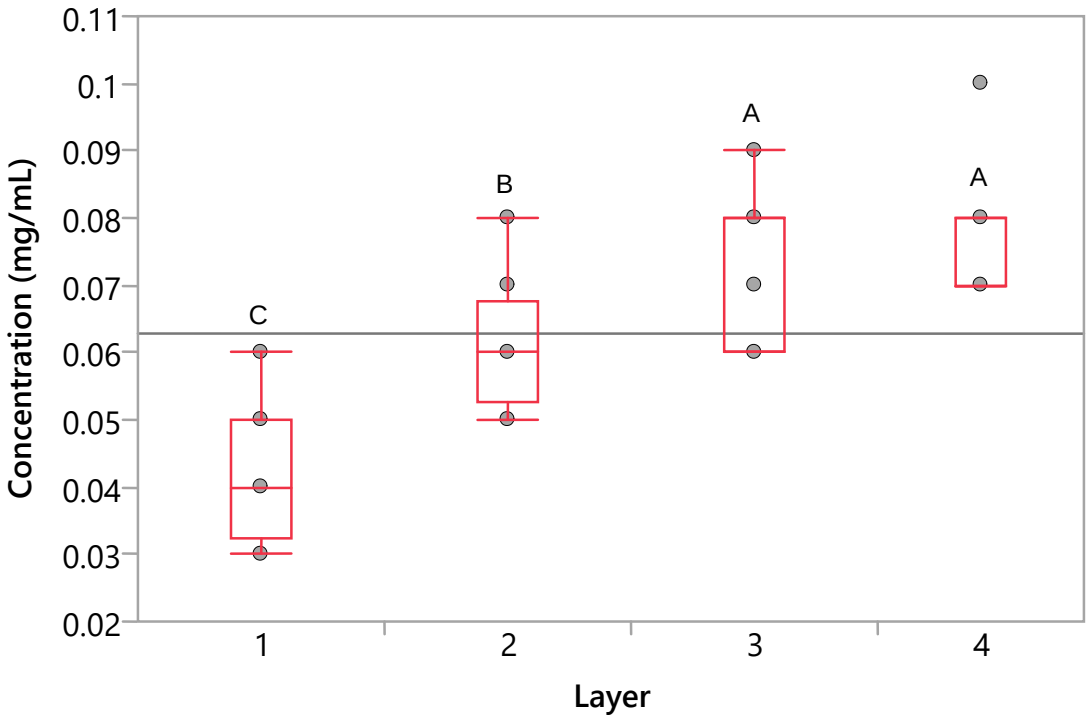


**Figure 32.** Furaldehyde extracted from each toasted layer.

Toasted layers 3 and 4 had a higher amount of furaldehyde extracted than layers 1 and 2. Layer 2 had a higher amount of furaldehyde than toasted layer 1 (Fig. 32). Inner-most layers had a higher amount of furaldehyde than more heat treated layers.

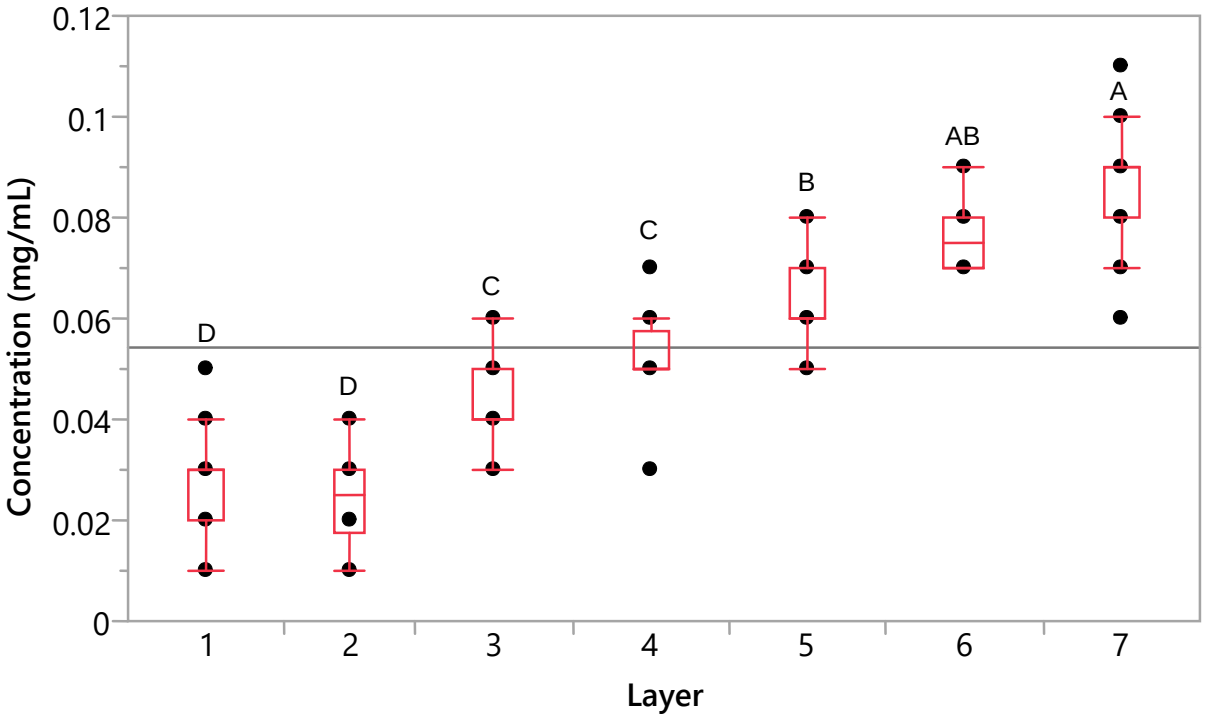


**Figure 33.** Furaldehyde extracted from each charred layer.

Charred layer 7 had a higher amount of furaldehyde than charred layers 1, 2, 3, 4 and 5, but a similar amount to layer 6. Layer 6 had a higher amount of furaldehyde than charred layers 1 through 4, but had a similar amount to layer 5. Layer 5 had a higher amount of furaldehyde than charred layers 1 through 4. Charred layers 3 and 4 had a higher amount of furaldehyde than charred layers 1 and 2 which had the same amount (Fig. 33).

Results show that the least heat treated layers had a higher amount of furaldehyde present (Fig. 31, 32, and 33). Presence of furaldehyde indicates the decomposition of monosaccharides during aging (Alcázar et al., 2005).

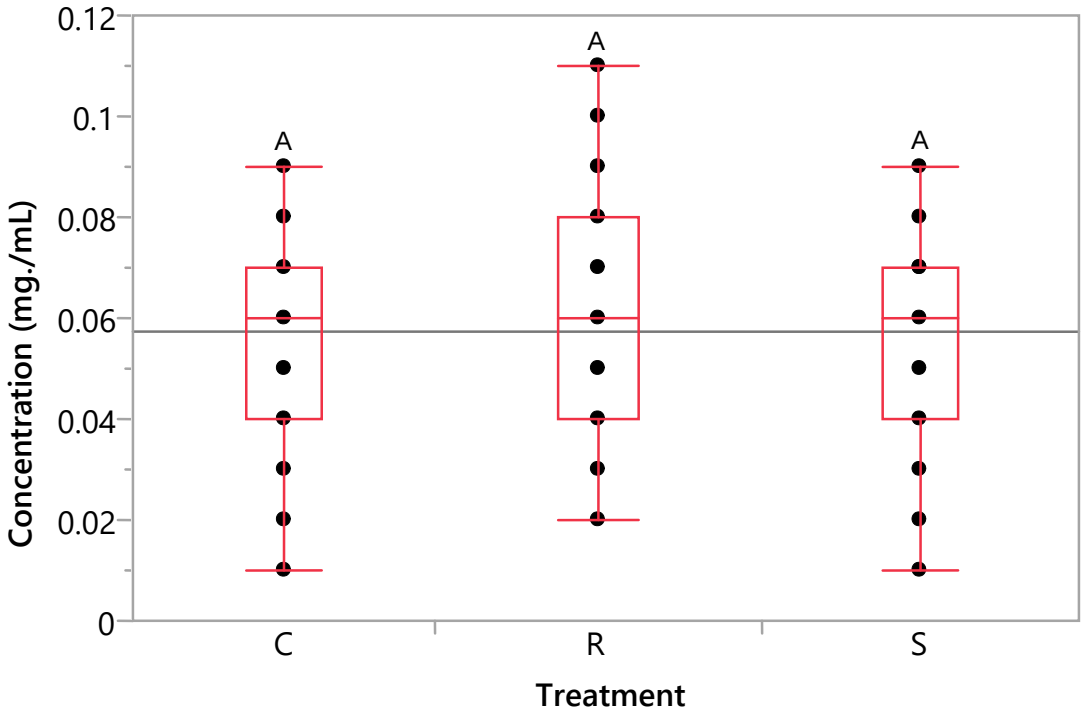


**Figure 34.** Furaldehyde extracted by each extraction treatment.

There was no significant difference (α=0.05) in the amount of furaldehyde extracted during each reflux, sonication, and control extraction treatments (Fig. 34). Extraction treatment had no effect on the extraction of furaldehyde.

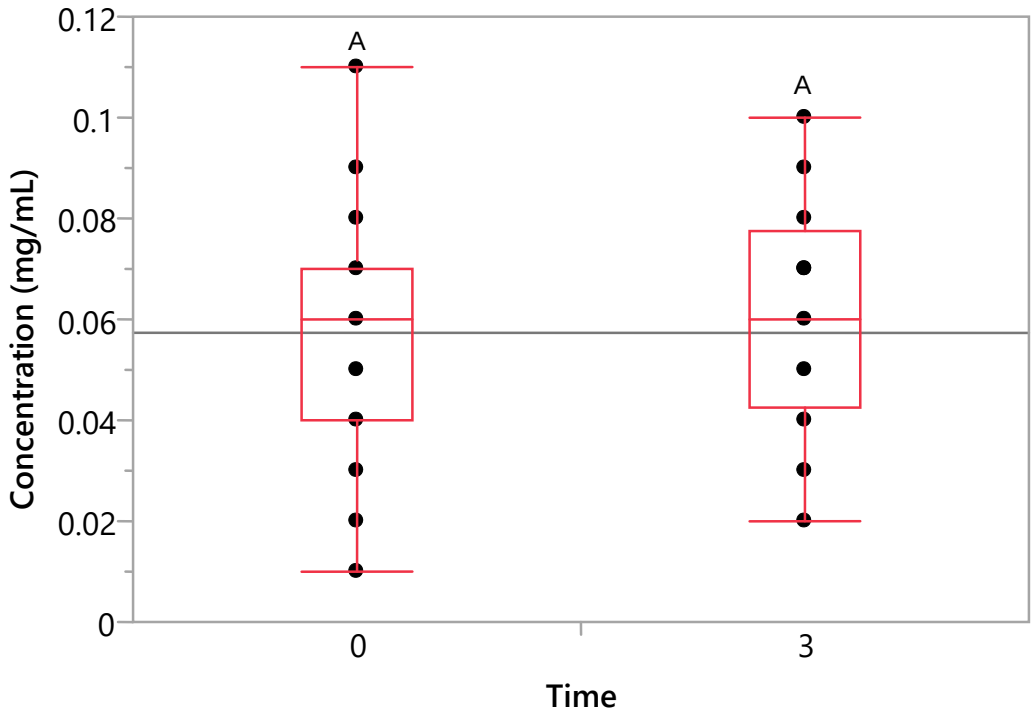


**Figure 35.** Furaldehyde extracted over time.

There was no significant difference (α=0.05) in the amount of furaldehyde extracted over time (Fig. 35). Results show time had no effect on the extraction of furaldehyde.

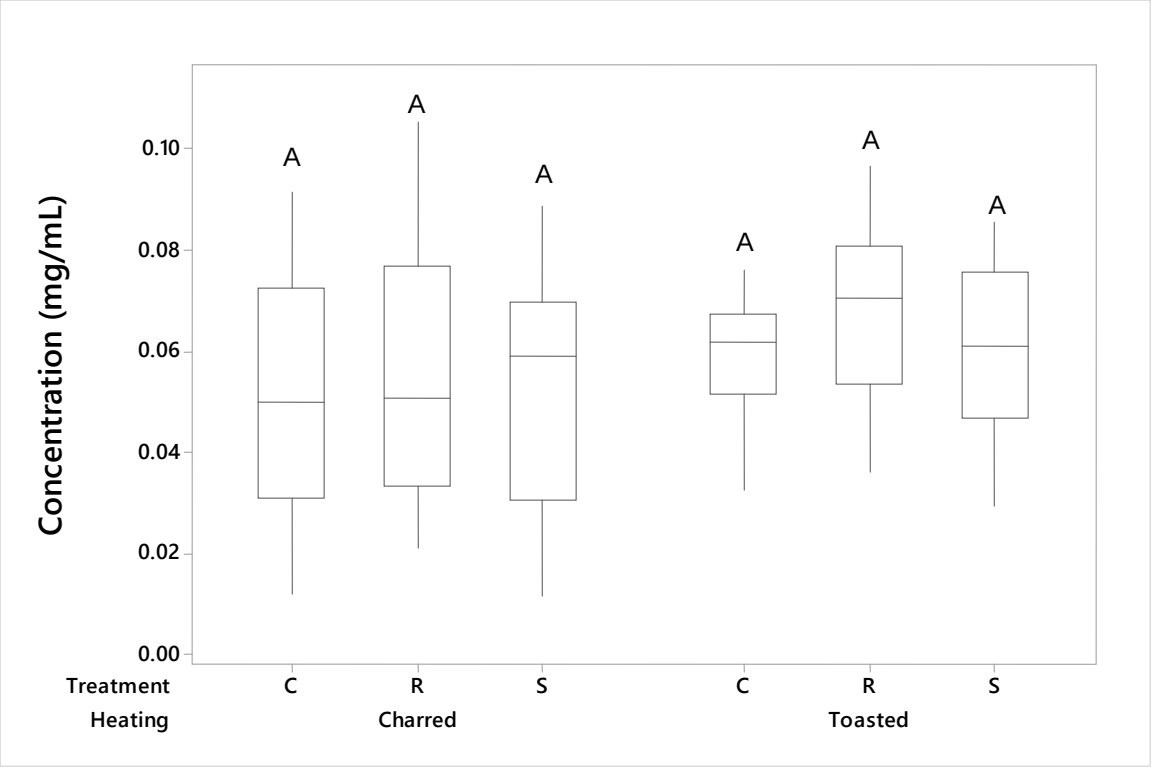


**Figure 36.** Furaldehyde extracted by each extraction treatment within each heat treatment.

There was no significant difference (α=0.05) in the amount of furaldehyde extracted by reflux, sonication, and control extraction treatments from charred and toasted staves (Fig. 36). Results show extraction treatments had no effect on furaldehyde extraction from charred and toasted staves.

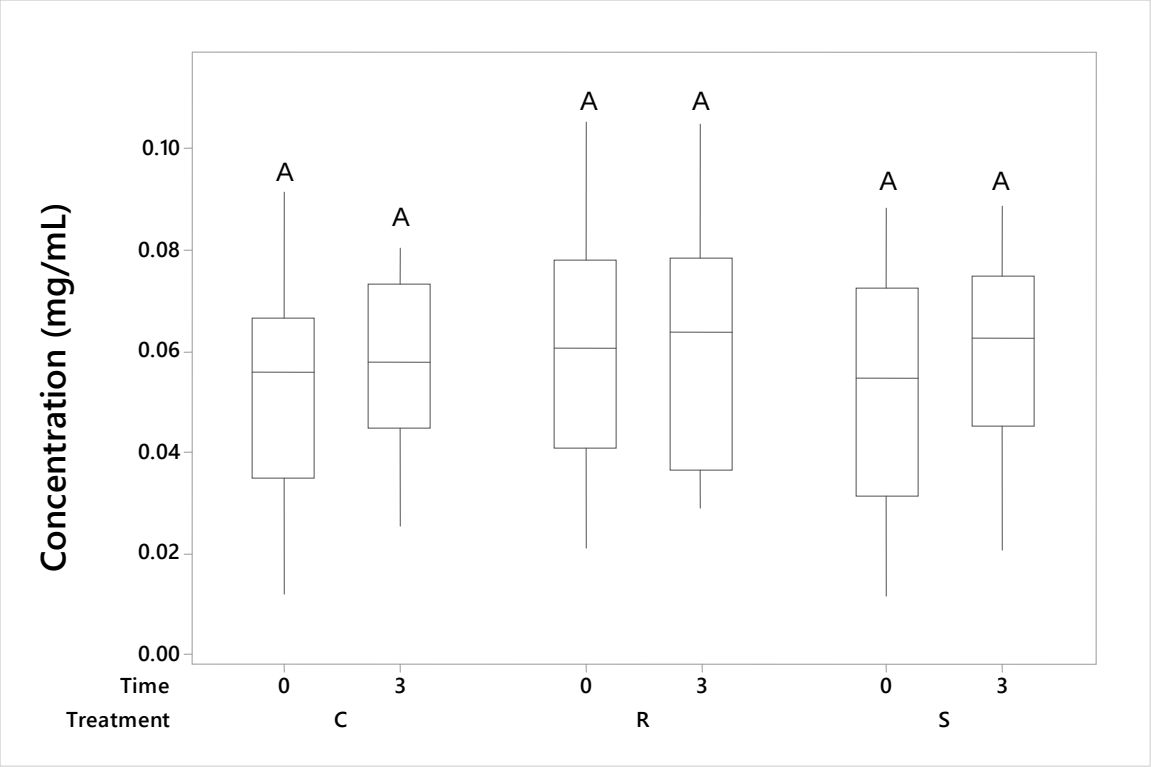


**Figure 37.** Furaldehyde extracted by each extraction treatment over time.

There was no significant difference (α=0.05) in the amount of furaldehyde extracted by reflux, sonication, and control extraction treatments over time (Fig. 37). Time had no effect on the extraction of furaldehyde by reflux, sonication, or control extraction treatments.

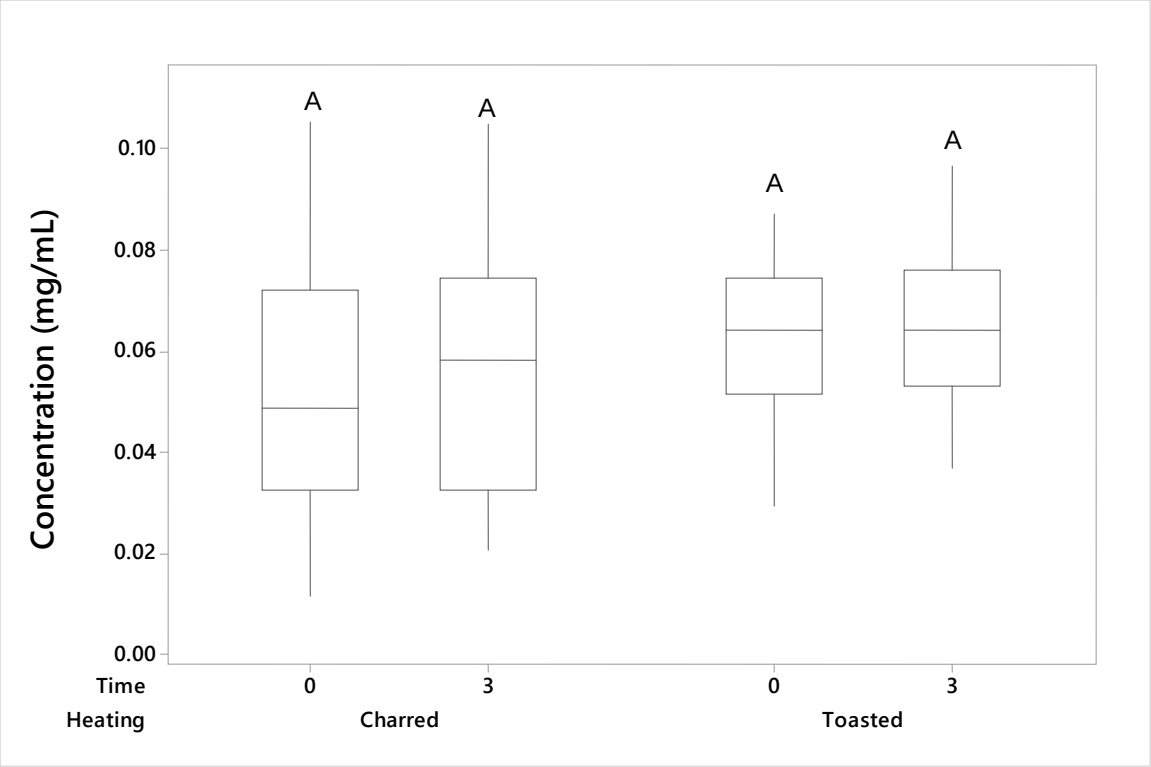


**Figure 38.** Furaldehyde extracted from each heat treatment over time.

There was no significant difference (α=0.05) in the amount of furaldehyde extracted from charred and toasted staves over time (Fig. 38). Results show that time had no effect on the extraction of furaldehyde from charred and toasted oak staves.

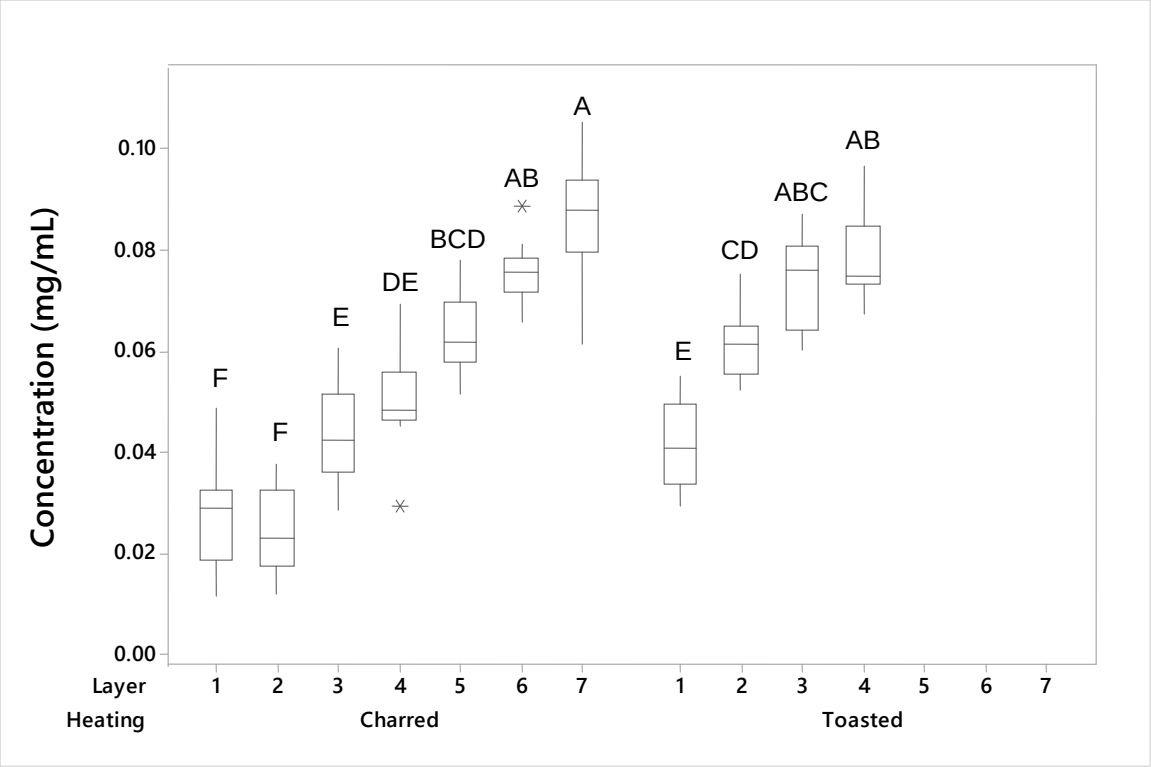


**Figure 39.** Furaldehyde extracted from each layer within each heat treatment.

Charred layer 7 had a higher amount of furaldehyde extracted than charred layers 1 through 5 and toasted layers 1 and 2, but had a similar amount to charred layer 6 and toasted layers 3 and 4. Charred layer 6 and toasted layer 4 had a higher amount of furaldehyde extracted than charred layers 1 through 4 and toasted layers 1 and 2, but had a similar amount to toasted layer 3. Toasted layer 3 had a higher amount of furaldehyde extracted than charred layers 1 through 4 and toasted layer 1, but had a similar amount to toasted layer 2. Charred layer 5 had a higher amount of furaldehyde than charred layers 1 through 3 and toasted layer 1, but had a similar amount to charred layer 4 and toasted layer 2. Toasted layer 2 had a higher amount of furaldehyde than charred layers 1 through 3 and toasted layer 1, but had a similar amount to charred

layer 4. Charred layer 4 had a higher amount of furaldehyde than charred layers 1 and 2, but had a similar amount to charred layer 3 and toasted layer 1. Charred layer 3 and toasted layer 1 had a similar amount of furaldehyde to charred layers 1 and 2 which had the same amount (Fig. 39).

Inner-most layers such as charred layers 6 and 7 and toasted layers 3 and 4 had more furaldehyde extracted than direct heat treated layers such as charred layers 1 and 2 and toasted layer 1. Results show that heat treatment affects the availability of furaldehyde for extraction for both charred and toasted stave layers.

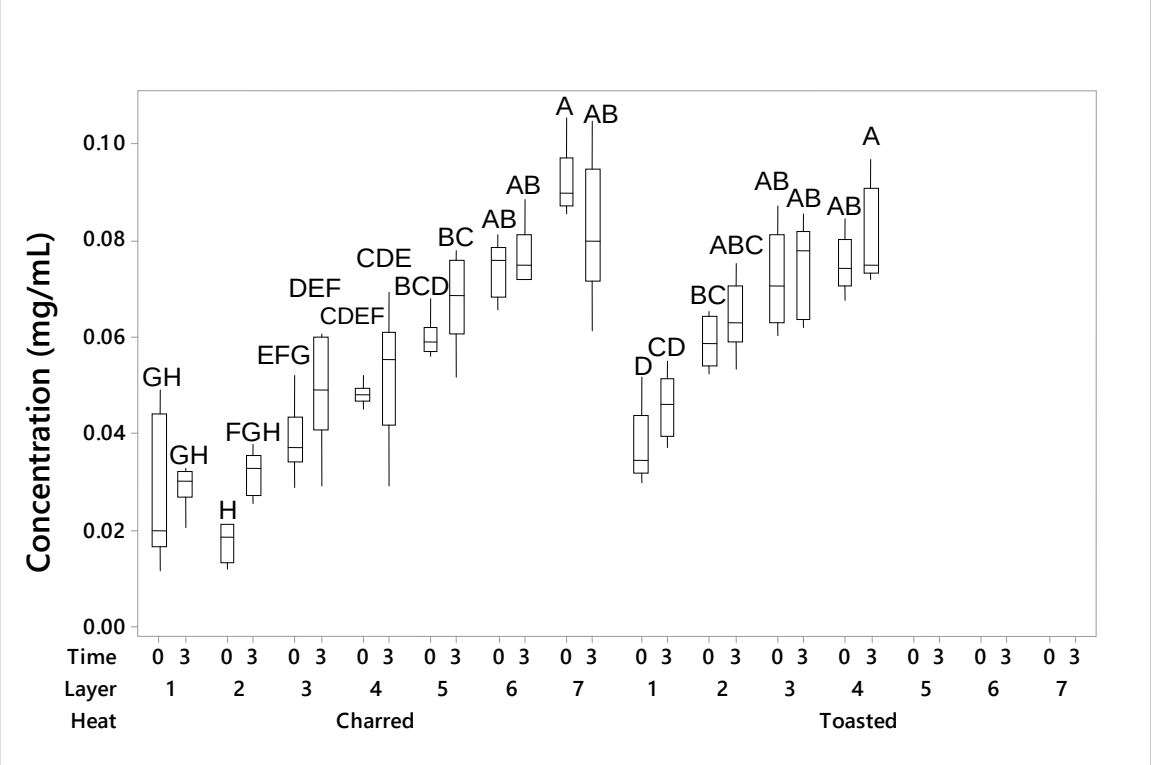


**Figure 40.** Furaldehyde extracted from each layer over time.

Charred layer 7 at time 0 had more furaldehyde than charred layers 1, 2, 3, 4, and 5 at time 0 and charred layers 1, 2, 3, 4, and 5 after 3 months, but had a similar amount to charred layer 6 at time 0 and charred layers 6 and 7 after 3 months. Charred layer 6 at time 0 and charred layers 6 and 7 after 3 months had more furaldehyde than charred layers 1, 2, 3, and 4 at time 0 and charred layers 1, 2, 3, and 4 after 3 months, but had a similar amount to charred layer 5 at time 0 and after 3 months. Charred layer 5 after 3 months had more furaldehyde than charred layers 1, 2, and 3 at time 0 and charred layers 1, 2, and 3 after 3 months, but had a similar amount to charred layers 4 and 5 at time 0 and charred layer 4 after 3 months. Charred layer 5 at time 0 had more furaldehyde than charred layers 1, 2, and 3 at time 0 and charred layers 1 and 2 after 3 months, but had a similar amount to charred layer 4 at time 0 and charred layers 3 and 4 after 3 months. Charred layer 4 after 3 months had more furaldehyde than charred layers 1 and 2 at time 0 and after 3 months, but had a similar amount to charred layers 3 and 4 at time 0 and charred layer 3 after 3 months. Charred layer 4 at time 0 had more furaldehyde than charred layer 1 and 2 at time 0 and charred layer 1 after 3 months, but had a similar amount to charred layer 3 at time 0 and charred layers 2 and 3 after 3 months. Charred layer 3 after 3 months had more furaldehyde than charred layers 1 and 2 at time 0 and charred layer 1 after 3 months, but had a similar amount to charred layer 3 at time 0 and charred layer 2 after 3 months. Charred layer 3 at time 0 had more furaldehyde than charred layer 2 at time 0, but had a similar amount to charred layer 1 at time 0 and charred layers 1 and 2 after 3 months. Charred layer 2 after 3 months had a similar amount of furaldehyde to charred layers 1 and 2 at time 0

and charred layer 1 after 3 months (Fig. 40). A Welch ANOVA found that at least one value was significantly different ($p<0.0001$). Toasted layer 4 after 3 months was found to have more furaldehyde than toasted layers 1 and 2 at time 0 and toasted layer 1 after 3 months, but had a similar amount to toasted layers 3 and 4 at time 0 and toasted layers 2 and 3 after 3 months. Toasted layer 3 and 4 at time 0 and toasted layer 3 after 3 months had more furaldehyde than toasted layer 1 at time 0 and after 3 months, but had a similar amount to toasted layer 2 at time 0 and after 3 months. Toasted layer 2 after 3 months had more furaldehyde than toasted layer 1 at time 0, but had a similar amount of furaldehyde to toasted layer 2 at time 0 and toasted layer 1 after 3 months. Toasted layer 2 at time 0 had more furaldehyde than toasted layer 1 at time 0, but had a similar amount to toasted layer 1 after 3 months. Toasted layer 1 after 3 months had a similar amount of furaldehyde to toasted layer 1 at time 0 (Fig. 40). A Welch ANOVA found that at least one value was significantly different ($p<0.0001$).

Results show extraction of furaldehyde was similar between individual layers over time. Because time 0 was not significantly different ($\alpha=0.05$) than after 3 months, individual layers had a similar amount of furaldehyde extracted over time.

### 3.4.2 Vanillin

Significant differences ($\alpha=0.05$) were found among layers between heat treated charred and toasted staves (Fig. 41), among toasted stave layers (Fig. 42), among charred stave layers (Fig. 43), between time 0 and after 3 months (Fig. 45), the interaction between heat treated staves among extraction treatments (Fig. 46), among extraction treatments over time (Fig. 47), and between heat treated staves over time

(Fig. 48).among layers for charred and toasted staves (Fig. 49), and among layers over time (Fig. 50). However, there was no significant difference found among reflux, sonication, and control extraction methods (Fig. 44).

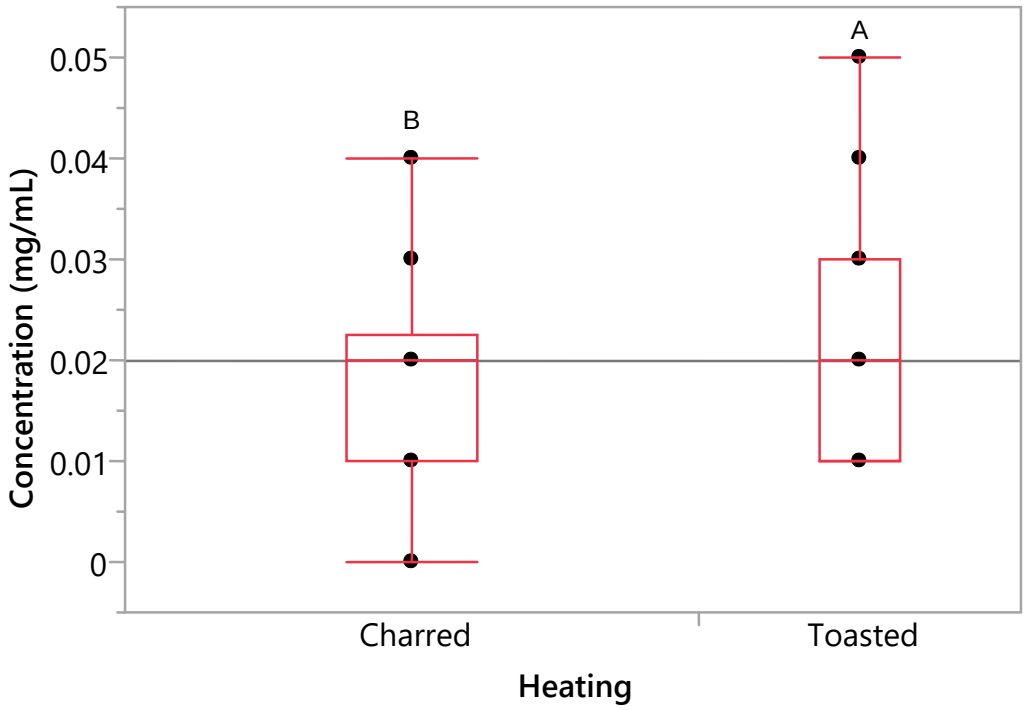


**Figure 41.** Vanillin extracted from charred and toasted staves.

Toasted staves had more vanillin than charred staves (Fig. 41). Results show that toasted staves had more vanillin available for extraction than charred stave.

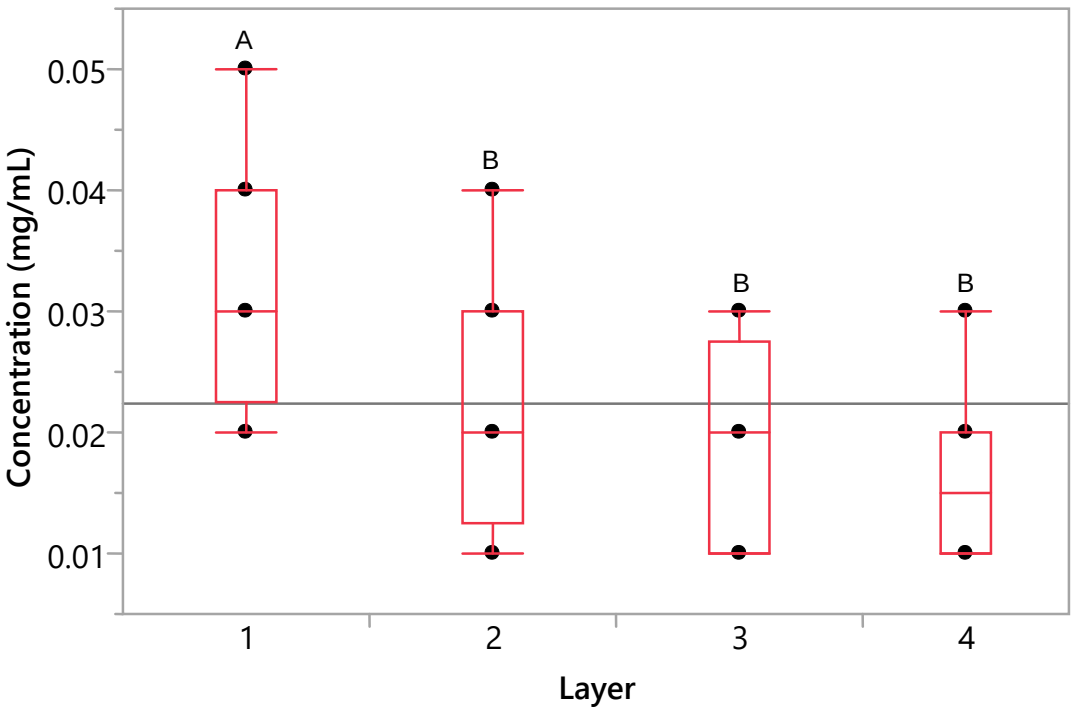


**Figure 42.** Vanillin extracted from each toasted layer.

Toasted layer 1 had more vanillin extracted than toasted layers 2, 3, and 4 which all extracted the same amount of vanillin (Fig. 42). Results show that toasted layer 1 had a higher availability of vanillin for extraction than other layers.

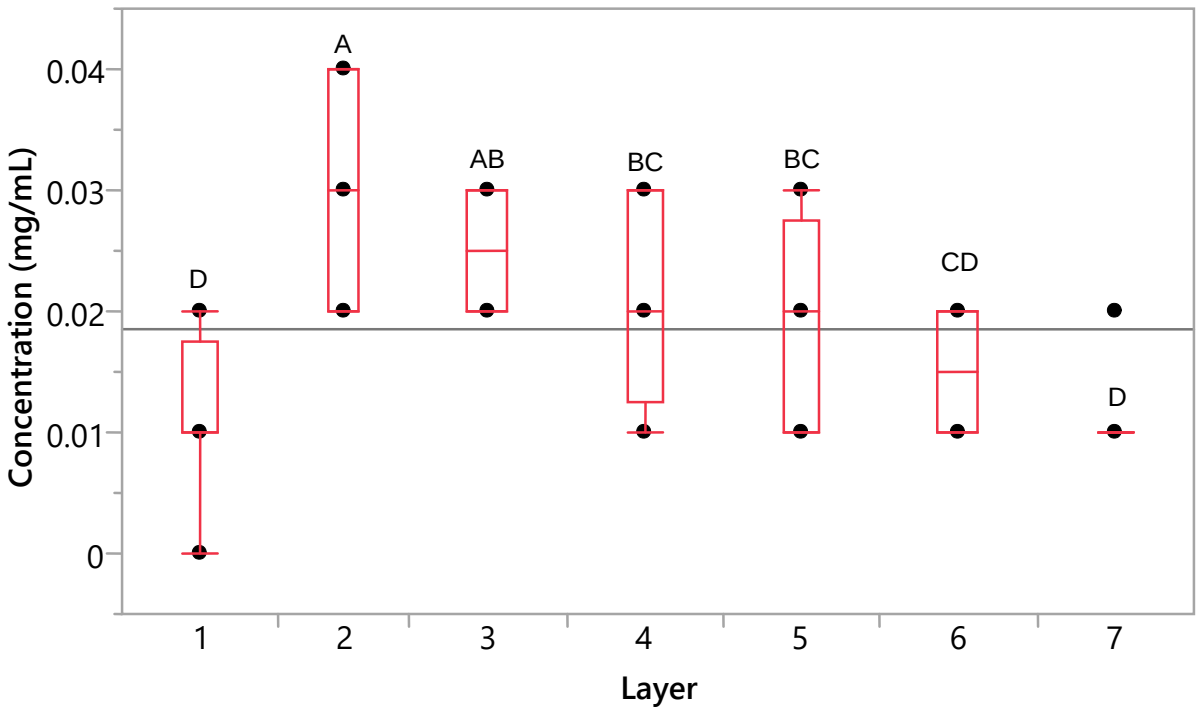


**Figure 43.** Vanillin extracted from each charred ayer.

More vanillin was extracted from charred layer 2 than from charred layers 4 through 7, but a similar amount was extracted from layer 3. Charred layer 3 had more vanillin extracted than from layers 1, 6, and 7, but a similar amount was extracted from layers 4 and 5. Charred layer 4 had more vanillin extracted than from layers 1 and 7, but a similar amount was extracted from layers 5 and 6. Charred layer 5 extracted a similar amount of vanillin to charred layers 1, 6, and 7 (Fig. 43).

Results show that inner-layers, like charred layer 2 and 3, had a higher availability of vanillin for extraction than other layers. The directly heat treated layer, charred layer 1, had less vanillin due to strong degradation of oak wood during heat treatment, while layers further removed from heat treatment, charred layers 6 and 7,

had less vanillin availability because they did not have enough degradation of oak wood during heat treatment for extraction of oak compounds.

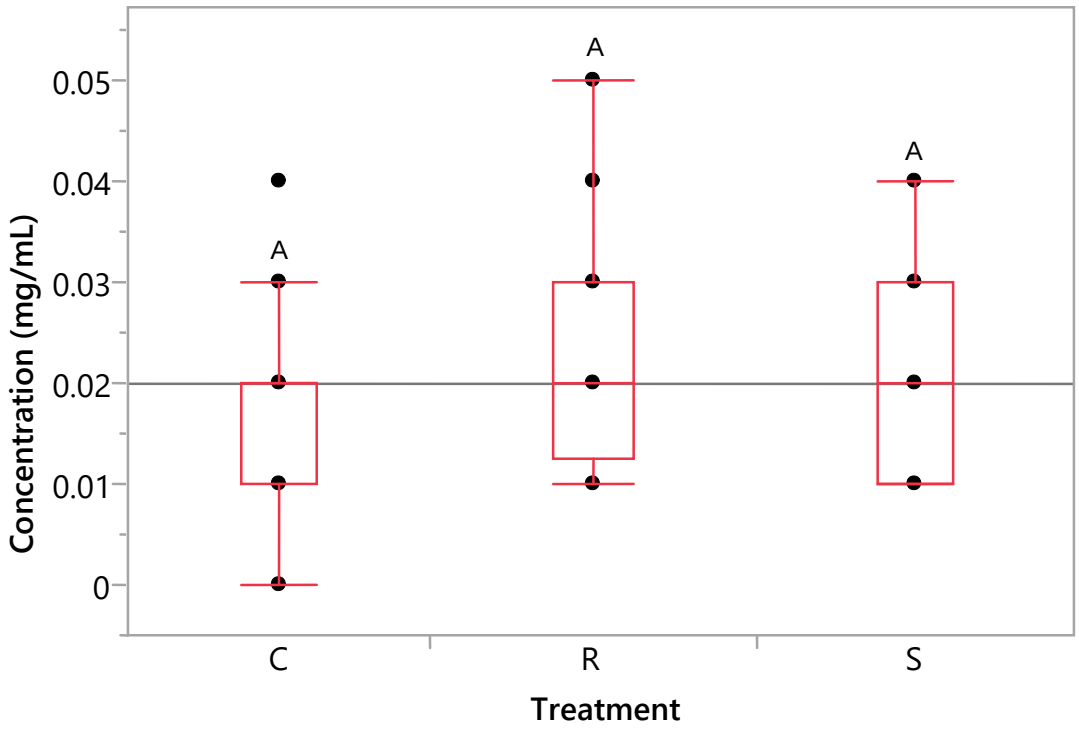


**Figure 44.** Vanillin from each extraction treatment.

There was no significant difference in the amount of vanillin extracted from each extraction treatment (Fig. 44). Results show that extraction treatment had no effect on the extraction of vanillin from oak staves.

More vanillin was extracted after 3 months than initially (Fig. 45). Results show that time is a significant factor in the extraction of vanillin.

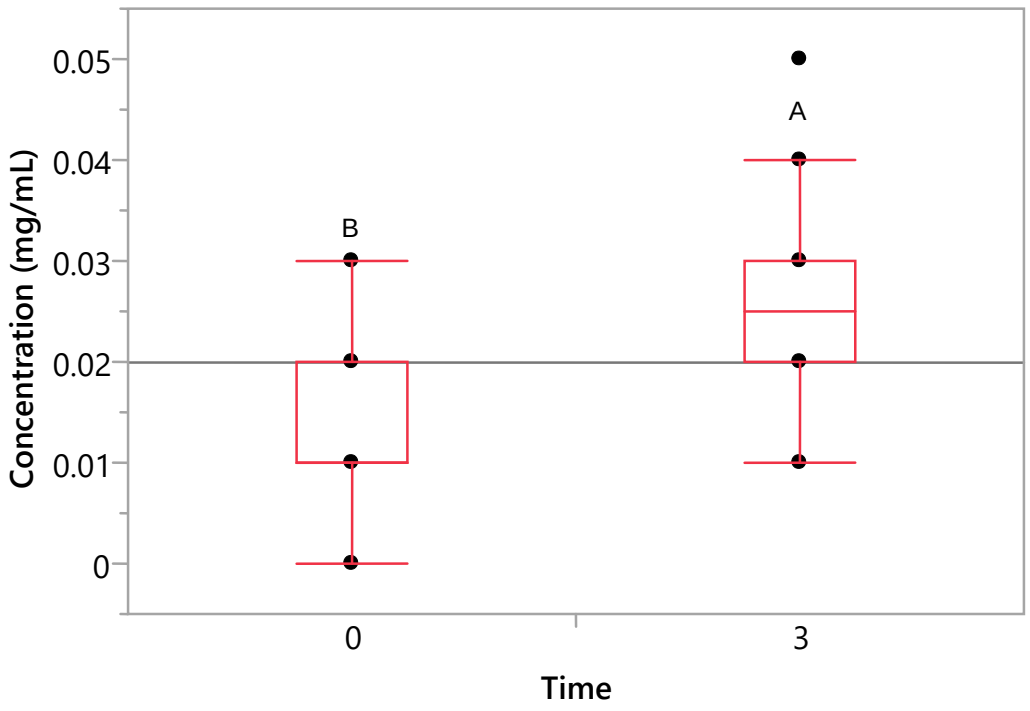


**Figure 45.** Vanillin extracted over time.

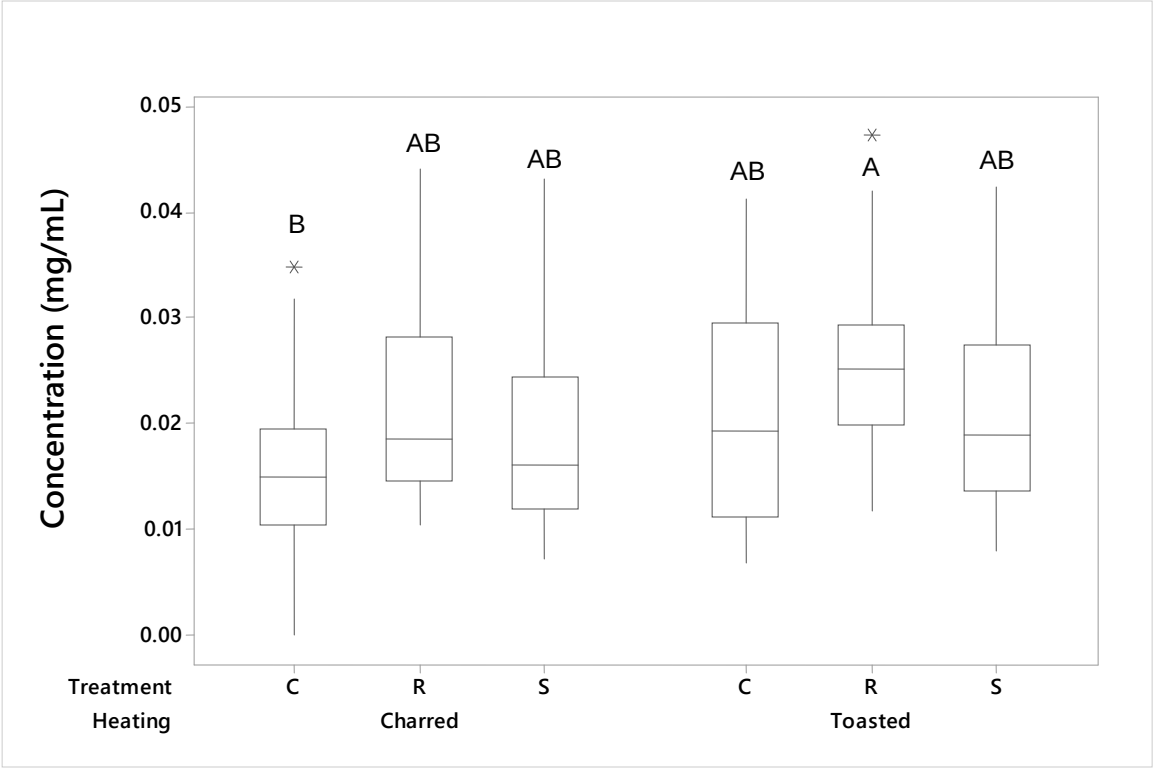


**Figure 46.** Vanillin extracted by each extraction treatment within each heat treatment.

Toasted staves had more vanillin extracted by reflux than vanillin in charred staves extracted by control extraction treatment, but had a similar amount to vanillin from toasted staves extracted by sonication and control methods and to vanillin from charred staves extracted by reflux and control extraction treatments. Extraction of toasted staves using sonication and control extraction methods and extraction of charred staves using sonication, reflux, and control extraction treatments all extracted a similar amount of vanillin (Fig. 46).

Results show that while toasted staves had more vanillin extracted than from charred staves, extraction treatment did not have a strong effect on the amount of vanillin extracted from toasted and charred staves.

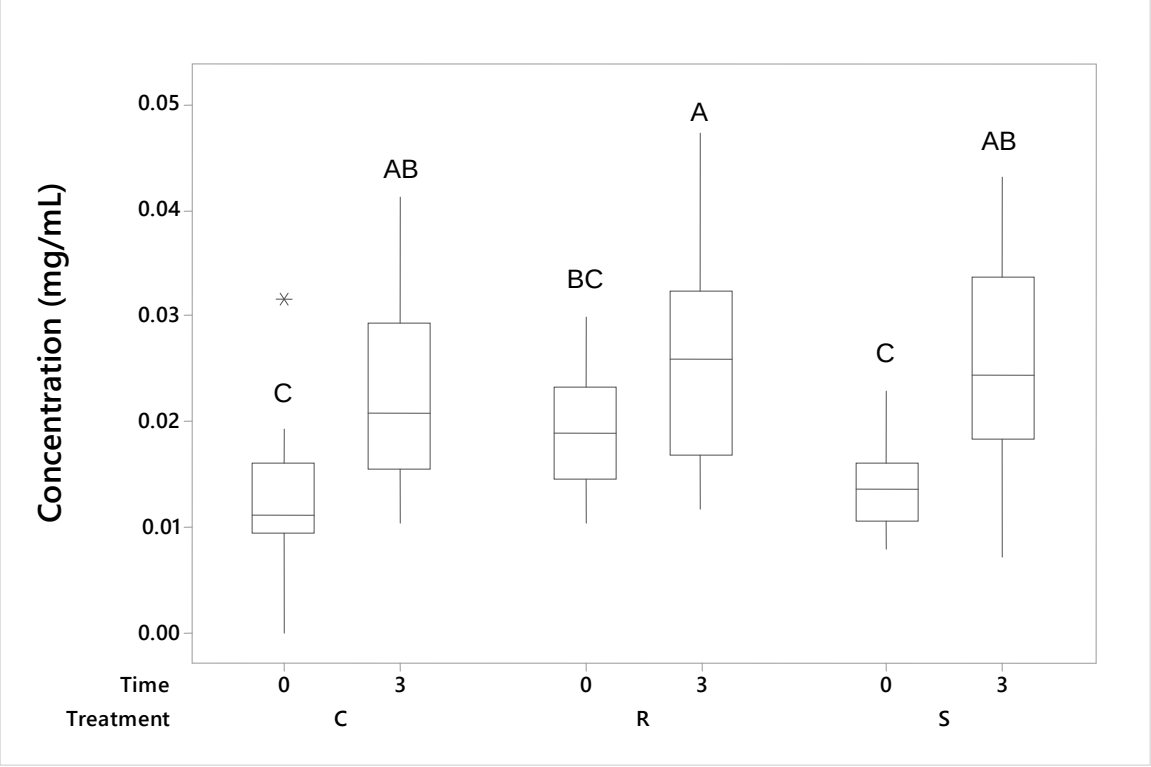


**Figure 47.** Vanillin extracted by each extraction treatment over time.

Reflux after 3 months extracted more vanillin than reflux, sonication, and control methods at time 0, but is similar to sonication and control extraction treatments after 3 months. Sonication and control after 3 months extracted more vanillin than sonication and control at time 0, but extracted a similar amount to reflux extraction treatment at time 0. Reflux at time 0 extracted a similar amount of vanillin to sonication and control extraction treatments (Fig. 47).

Results show that extraction of vanillin increased after 3 months from all extraction treatments compared to extraction of vanillin at time 0.

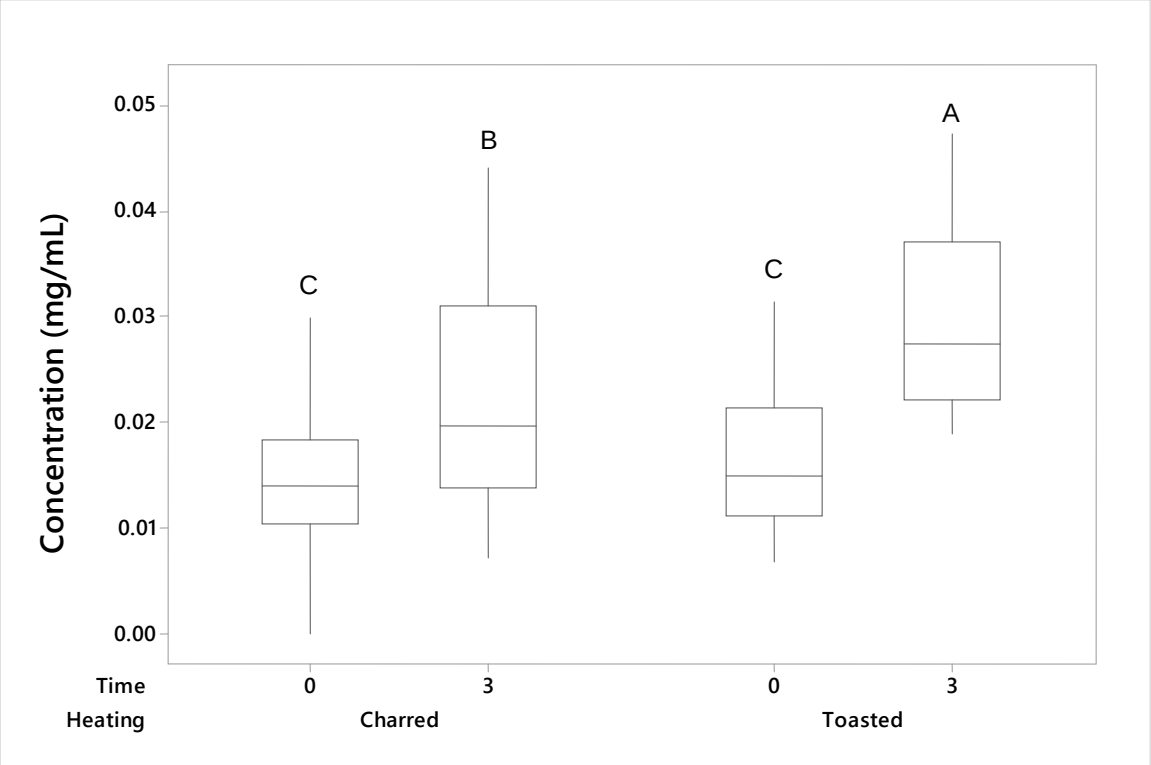


**Figure 48.** Vanillin extracted from each heat treatment over time.

Toasted staves after 3 months extracted more vanillin than charred staves at time 0 and after 3 months and toasted staves at time 0. Charred staves after 3 months

extracted more vanillin than charred and toasted staves at time 0 which extracted the same amount of vanillin (Fig. 48).

Results show that toasted staves extracted more vanillin after 3 months than charred staves; however, toasted and charred staves extracted the same amount of vanillin at time 0. The differentiation of vanillin extraction after three months between charred and toasted staves indicates a higher availability of vanillin for extraction in toasted staves, but not in charred.

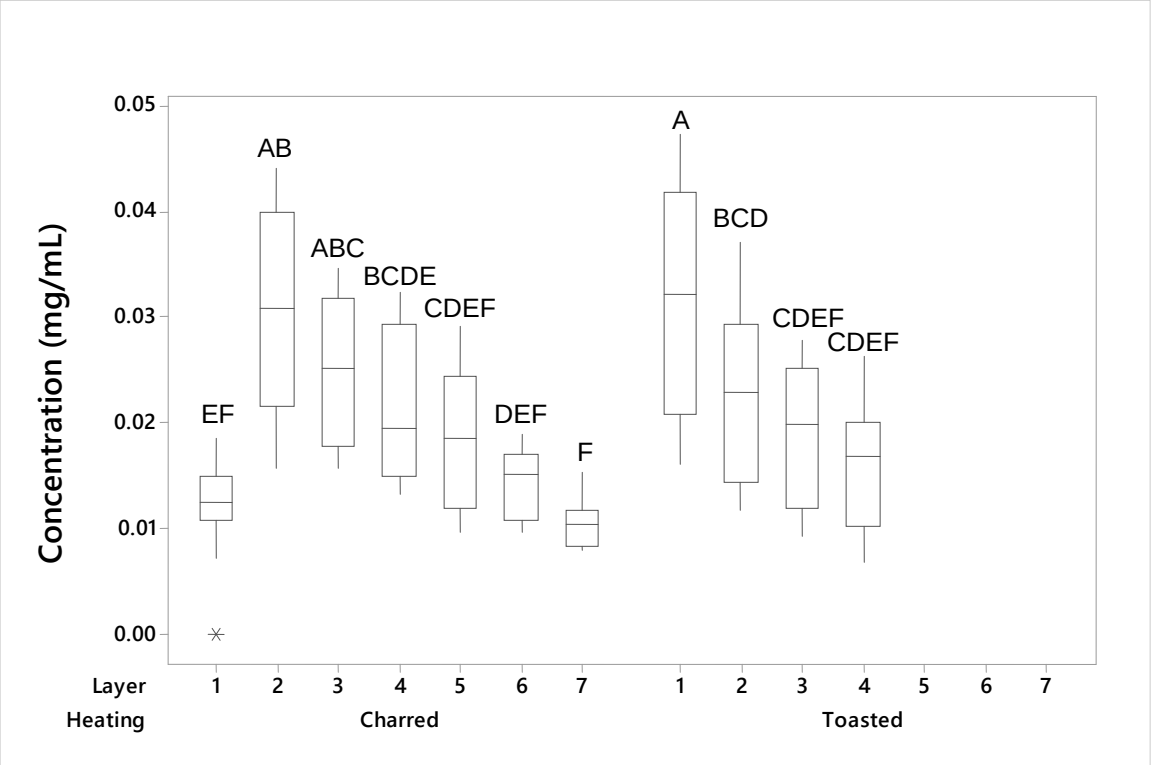


**Figure 49.** Vanillin extracted by each layer within each heat treatment.

Toasted layer 1 had more vanillin extracted than charred layers 1, 4, 5, 6, and 7 and toasted layers 2, 3, and 4, but had a similar amount extracted to charred layers 2 and 3. Charred layer 2 extracted more vanillin than charred layers 1, 4, 5, 6, and 7 and

toasted layers 2, 3, and 4, but had a similar amount to charred layer 3. Charred layer 3 had more vanillin extracted than charred layers 1, 6, and 7, but had a similar amount to charred layers 4 and 5 and toasted layers 2, 3, and 4. Toasted layer 2 had more vanillin extracted than charred layers 1 and 7, but had a similar amount extracted to charred layers 4, 5, and 6 and toasted layers 3 and 4. Charred layer 4 had more vanillin extracted than charred layer 7, but had a similar amount extracted to charred layers 1, 5, and 6 and toasted layers 3 and 4. Charred layer 5 and toasted layers 3 and 4 had a similar amount of vanillin extracted to charred layers 1, 6, and 7 (Fig. 49).

Results show that toasted layer 1 and charred layers 2 and 3 extracted more vanillin available for extraction than other layers. Toasted layer 1 and charred layers 2 and 3 had slight degradation of oak wood cells during heat treatment which allows more oak compounds, such as vanillin, to be extracted more readily from wood than less heat treated layers such as charred layers 6 and 7 and toasted layers 3 and 4 and completely degraded layers such as charred layer 1.

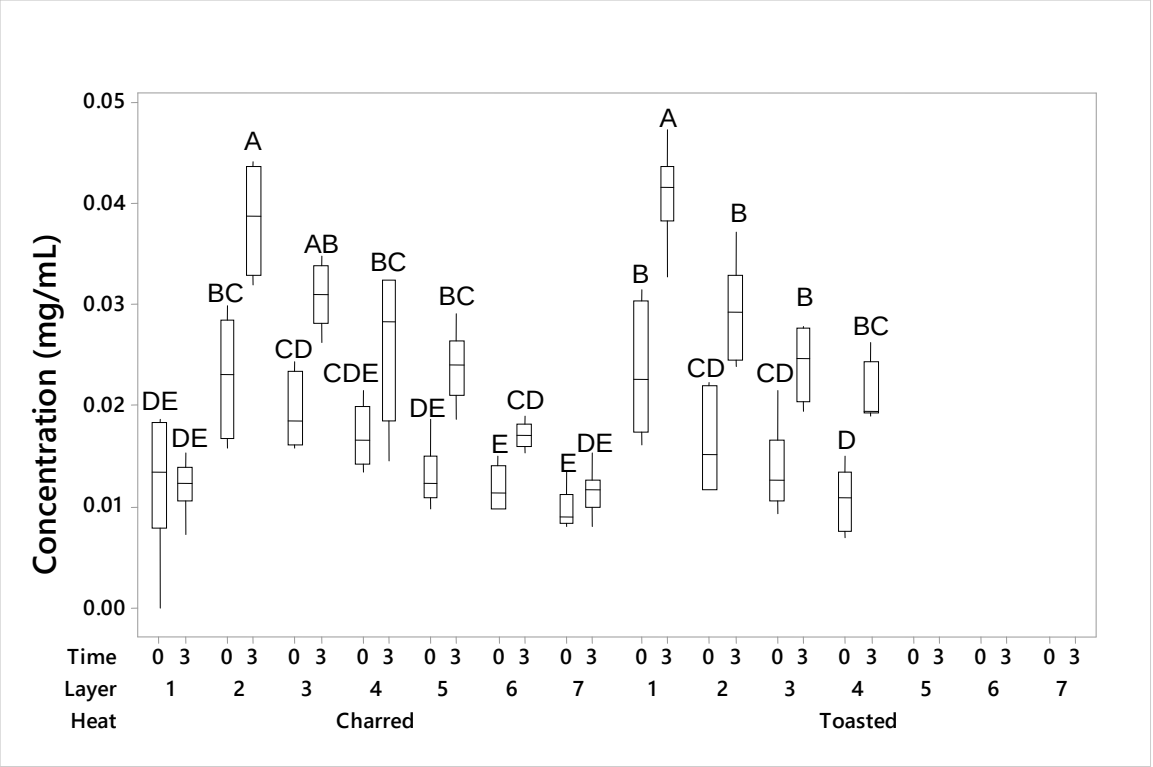


**Figure 50.** Vanillin extracted from each layer over time.

Charred layer 2 after 3 months had more vanillin extracted than from charred layers 1, 2, 3, 4, 5, 6, and 7 at time 0 and charred layers 1, 4, 5, 6, and 7 after 3 months, but had a similar amount to charred layer 3 after 3 months. Charred layer 3 after 3 months had more vanillin than charred layers 1, 3, 4, 5, 6, and 7 at time 0 and charred layers 1, 6, and 7 after 3 months, but had a similar amount to charred layer 2 at time 0 and charred layers 4 and 5 after 3 months. Charred layer 2 at time 0 and charred layers 4 and 5 after 3 months had more vanillin than charred layers 1, 5, 6, and 7 at time 0 and charred layers 1 and 7 after 3 months, but had a similar amount to charred layers 3 and 4 at time 0 and charred layer 6 after 3 months. Charred layer 3 at time 0 and charred layer 6 after 3 months had more vanillin than charred layers 6 and 7 at time 0, but had a

similar amount to charred layers 1, 4, and 5 at time 0 and charred layers 1 and 7 after 3 months. Charred layer 4 at time 0 had a similar amount of vanillin to charred layers 1, 5, 6, and 7 at time 0 and charred layers 1 and 7 after 3 months (Fig. 50).

Toasted layer 1 after 3 months had more vanillin than toasted layers 1 through 4 at time 0 and toasted layers 2 through 4 after 3 months. Toasted layer 1 at time 0 and toasted layer 2 after 3 months had more vanillin than toasted layers 2 through 4 at time 0, but had a similar amount to toasted layer 4 after 3 months. Toasted layer 4 after 3 months had more vanillin than toasted layer 4 at time 0, but had a similar amount to toasted layers 2 and 3 at time 0. Toasted layers 2 through 4 at time 0 had a similar amount of vanillin ( Fig. 50). Results show that layers extracted more vanillin after 3 months than at time 0, but were similar in the amount of vanillin within each extraction time.

## 3.5. Liquid Chromatography

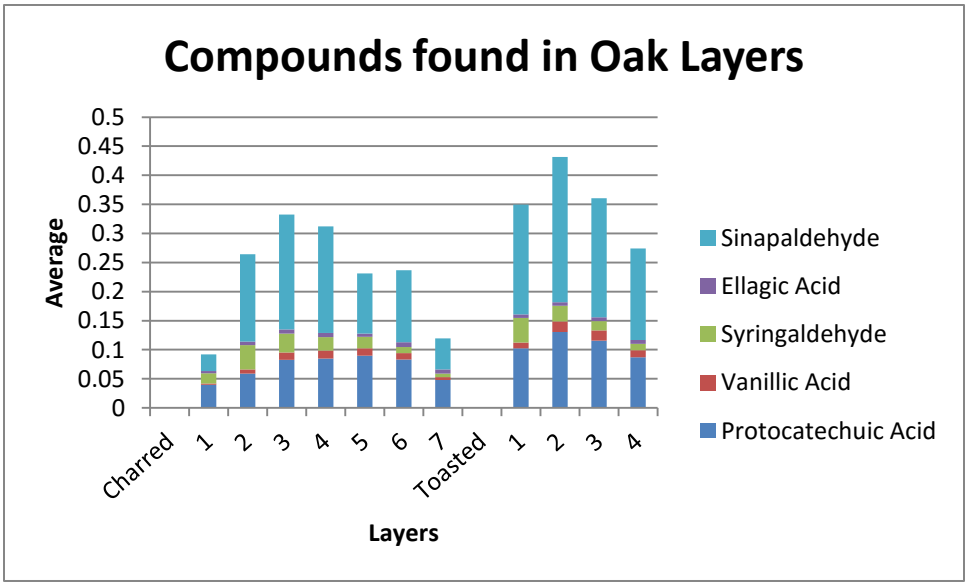


**Figure 51.** Average amount of oak compounds found in each layer.

More compounds were extracted from charred layer 3 and toasted layer 2 (Fig.51). Of the identified compounds, sinapaldehyde was the most extracted compound found in each oak stave layer followed by ellagic acid, syringealdehyde, vanillic acid, and protocatechuic acid. Results indicate that of the identified compounds, there were more aldehydes extracted than acids which is supported by findings from Simón et al.,(1999).

Identification and comparison of non-volatile compounds between charred and toasted staves and their individual stave layers were determined using HPLC with a photodiode array detector. Of the non-volatile compounds identified, protocatechuic acid (PA), vanillic acid (VA), syringaldehyde, ellagic acid (EA), and sinapaldehyde were found in charred and toasted staves and were analyzed.

A low concentration of gallic acid (GA) was found in charred stave layers 3, 4, 5, 6, and 7 for all treatments at time 0 months, but disappeared after 3 months. GA was also found in toasted stave layers 2, 3, and 4 for all treatments, except for missing layer 2 control, at time 0, but also disappeared after 3 months. Methyl gallate was only found in sonication extracted, charred layers 1 and 2 at time 0. Protocatechuic aldehyde and scoleptin were not found in these staves using these extraction methods. These compounds were not analyzed as they were not found in abundant amounts. Because these compounds are considered low molecular weight, they only make up a minor portion (about 14%) of the total phenols extracted from oak staves (Viriot et al., 1993).

Degradation of ellagitannins occurs first followed by the degradation of lignins during heat treatment and extraction forms and produces many compounds due to

hydrolyzation and oxidation (Viriot et al., 1993). Coniferyl and sinapyl alcohols oxidize into volatile phenols, conifer- and sinapaldehydes, which then further oxidize into vanillin and syringaldehyde (Russell, 2003; Niciforovic and Abramovic, 2013). Other phenols are produced following extraction by ethanol (Russell, 2003).

### 3.5.1. Sinapaldehyde

Significant differences ($\alpha=0.05$) were found among layers between heat treated charred and toasted staves (Fig. 52), among toasted stave layers (Fig. 53), for heat treated charred staves (Fig. 54), across reflux, sonication, and control extraction treatments (Fig. 55), between heat treated staves among extraction treatments (Fig.57), among extraction treatments over time (Fig.58), between heat treated staves over time (Fig.59), among layers for charred and toasted staves (Fig.60), and among layers over time (Fig. 61). However, there was no significant difference found between time 0 and after 3 months (Fig. 56).

Toasted staves had more sinapaldehyde than charred staves (Fig. 52). A Welch test was run on heat treated staves and was found to have at least one significant difference ($p<0.0001$). Sinapaldehyde existed in higher amounts from toasted staves than from charred staves due to higher availability in toasted staves than in charred staves.

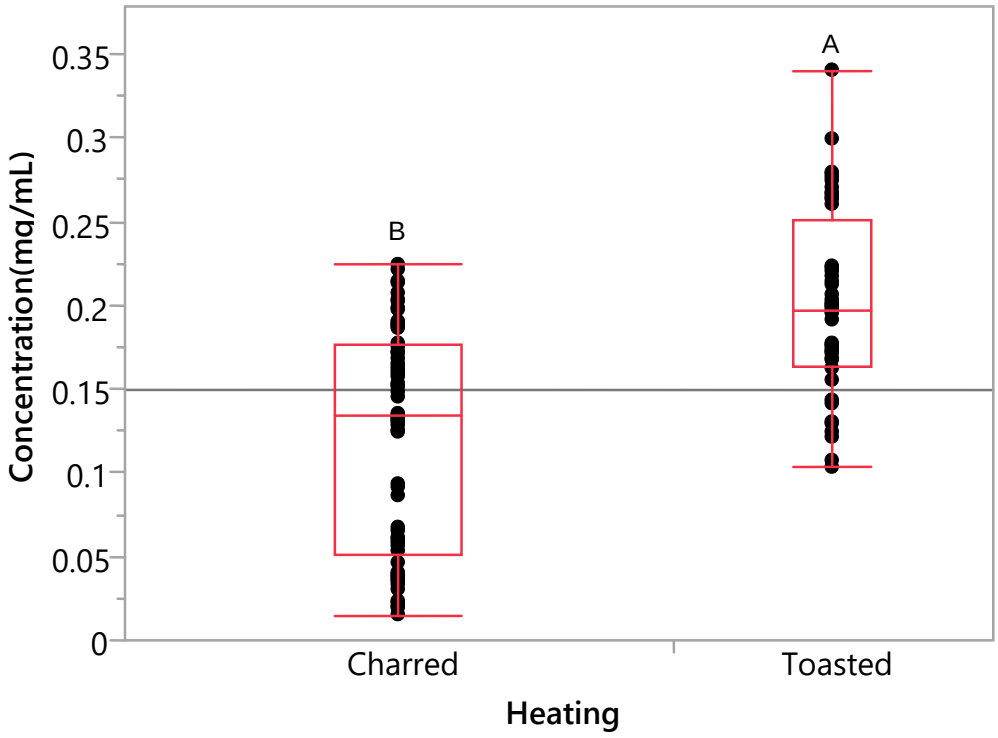


**Figure 52.** Sinapaldehyde extracted from charred or toasted staves.

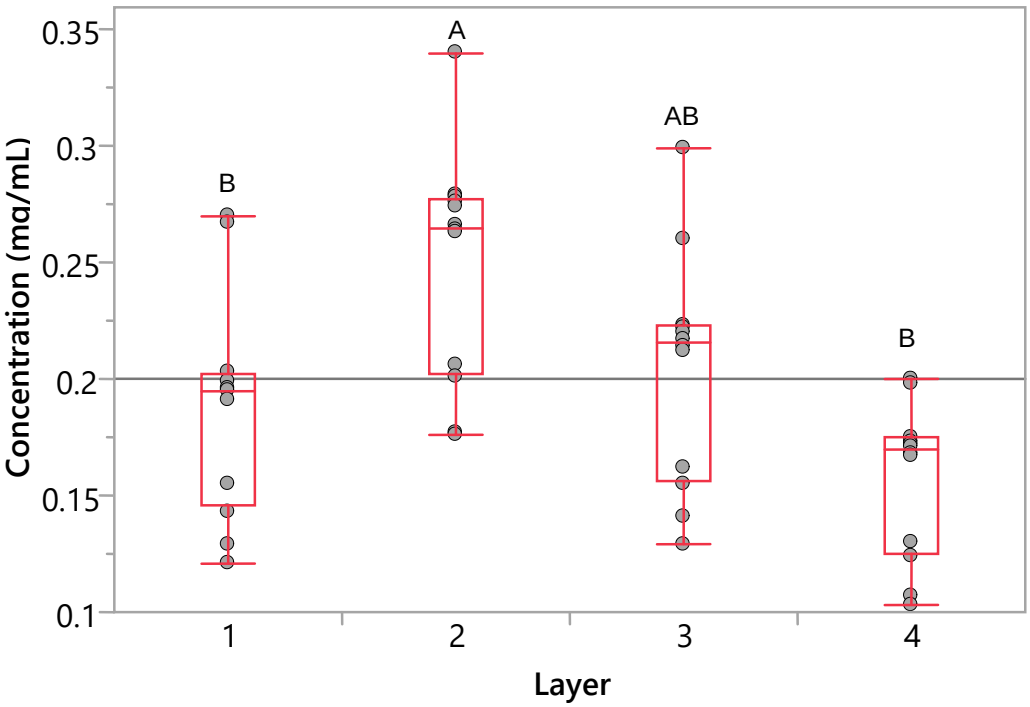


**Figure 53.** Sinapaldehyde extracted from each toasted layer.

Toasted layer 2 had a similar amount of sinapaldehyde extracted to toasted layer 3 but had more toasted layers 1 and 4. Toasted layer 3 had a similar amount of sinapaldehyde extracted to layer 1 and 4 (Fig. 53). A Welch test was run on individual toasted layers and was found to have at least one significant difference (p=0.0002). Results show that toasted inner layers had a higher availability of sinapaldehyde than other layers.

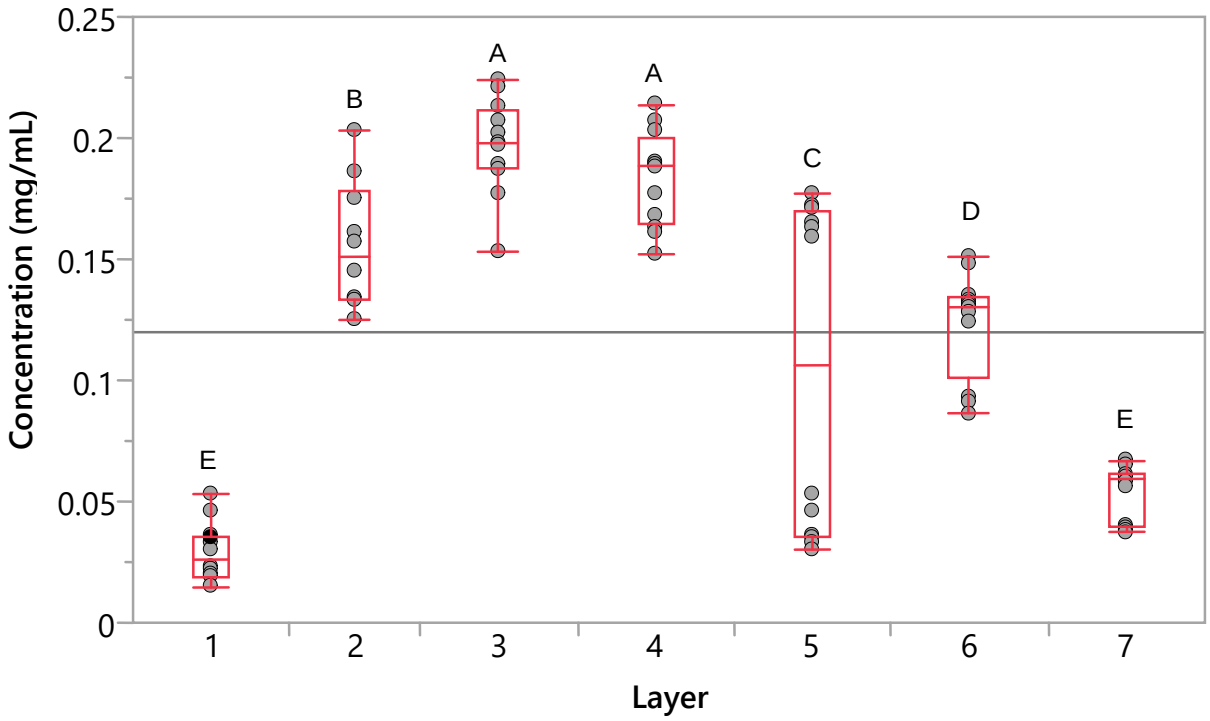


**Figure 54.** Sinapaldehyde extracted from each charred layer.

Charred layer 3 had a similar amount of sinapaldehyde extracted to charred layer 4, but had more than charred layers 1, 2, 5, 6, and 7. Charred layer 4 had more sinapaldehyde than layers 1, 5, 6, and 7, but had a similar amount to layer 2. Charred layer 2 had more sinapaldehyde than charred layers 1, 5, and 7 but was similar in

amount to layer 6. Charred layer 6 was similar to layer 5 but had more than layers 1 and 7. Charred layers 1 and 7 had the same amount of sinapaldehyde extracted (Fig. 54). A Welch test was run on individual charred layers and was found to have at least one significant difference ($p<0.0001$). Results show that inner layers had a higher availability of sinapaldehyde than charred layer 1 and charred layer 7. Charred layer 1 had a higher level of degradation reducing the amount of extractable oak compounds while charred layer 7 was not degraded enough to extract more oak compounds such as sinapaldehyde.

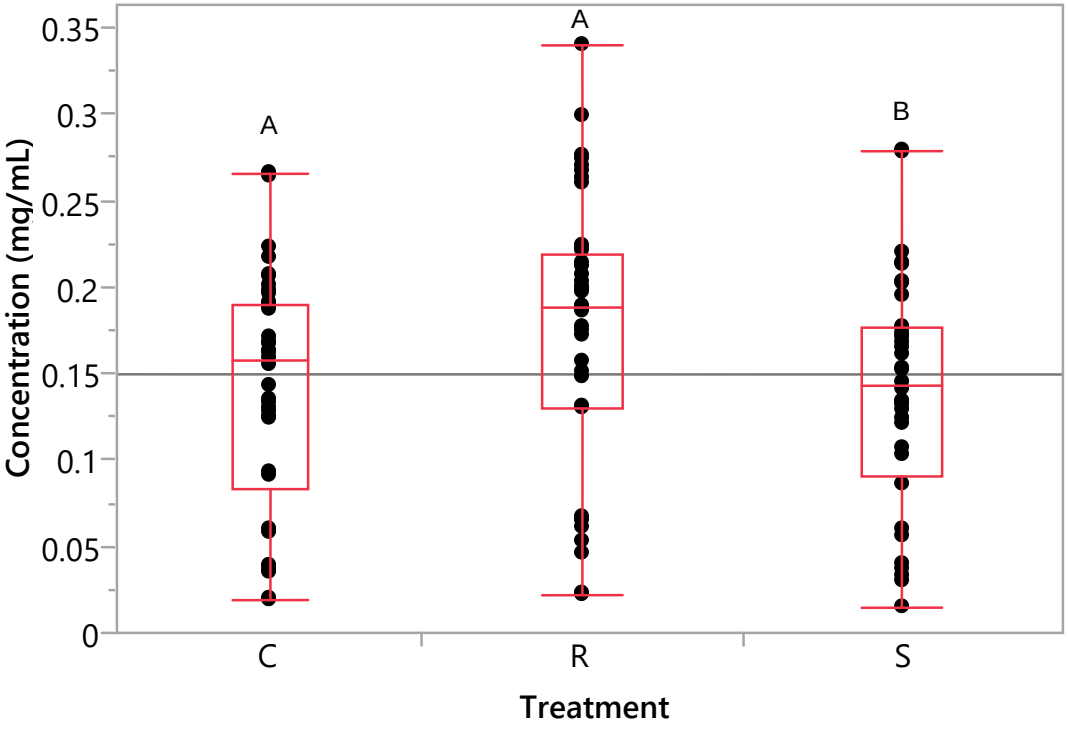


**Figure 55.** Sinapaldehyde extracted from each extraction treatment.

Reflux extracted more sinapaldehyde than sonication, but was similar in the amount of extraction to the control (Fig 55). Results show that reflux extracted more sinapaldehyde than sonication extraction treatment; however, both reflux and sonication extracted a similar amount of sinapaldehyde.

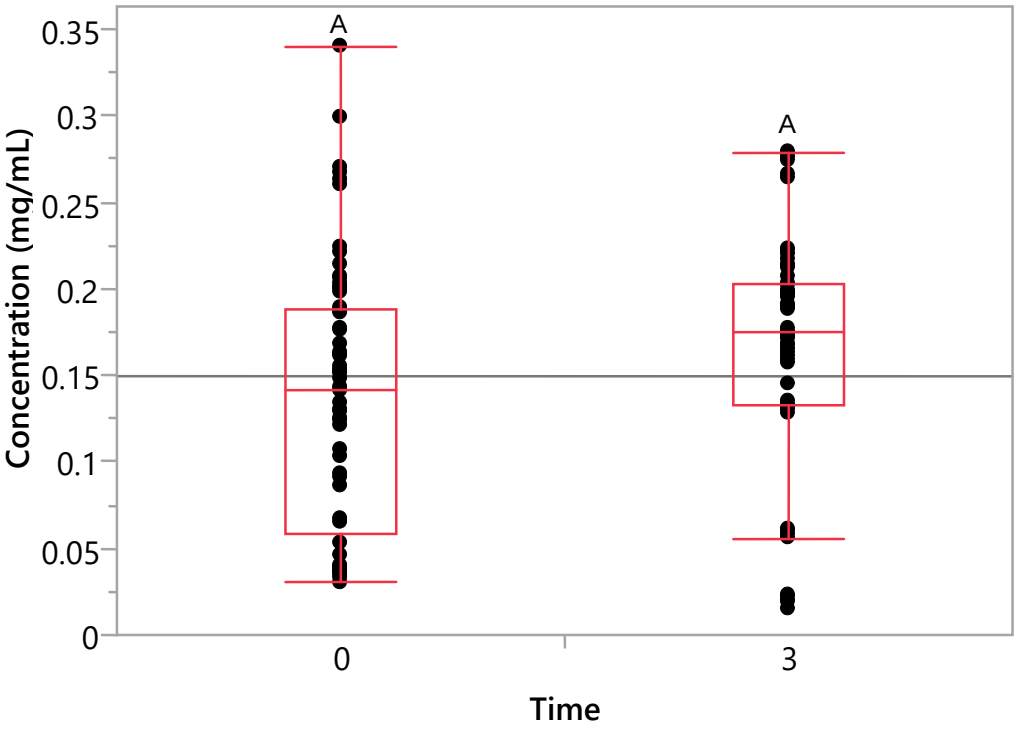


**Figure 56.** Sinapaldehyde extracted over time.

There was no significant difference in the amount of sinapaldehyde extracted at time 0 and after 3 months (Fig. 56). Results show that time had no effect on the extraction of sinapaldehyde from oak staves.

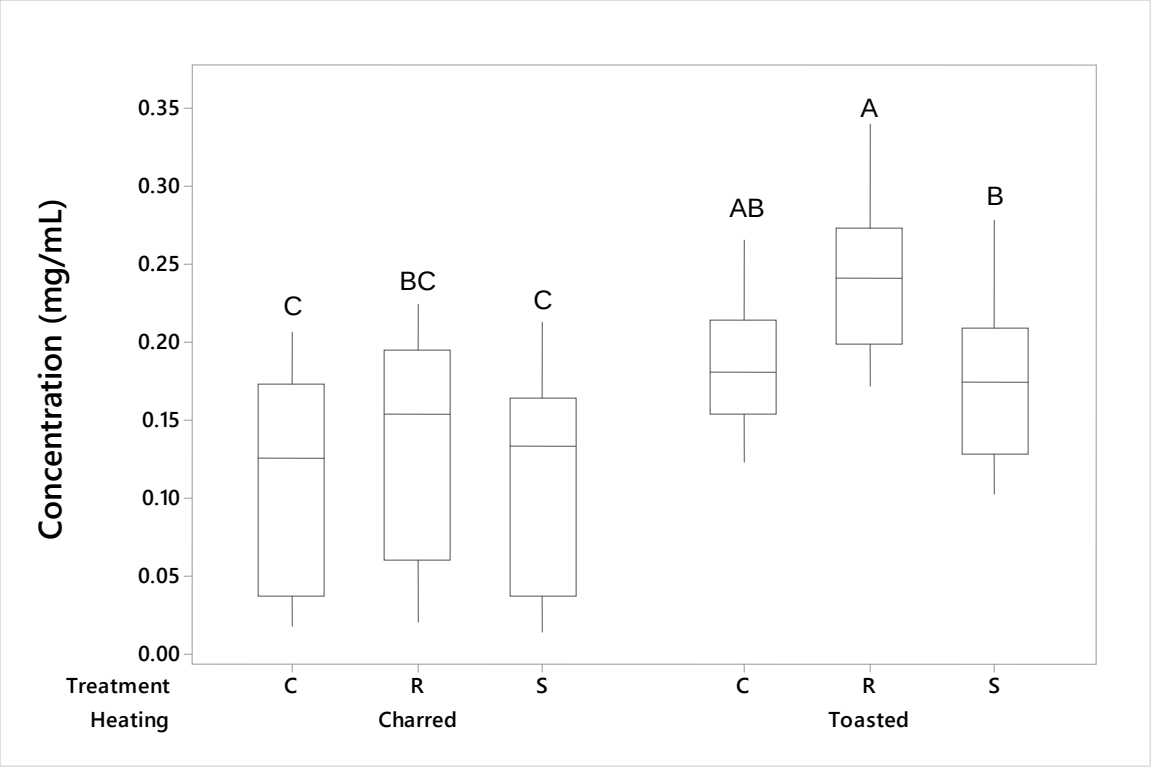


**Figure 57.** Sinapaldehyde extracted by each extraction treatment within each heat treatment.

Toasted staves had more sinapaldehyde extracted by reflux than sonicated, but were similar to the control, while charred staves had a similar amount of sinapaldehyde extracted by reflux, sonication, and control extraction treatments (Fig. 57). A Welch test was run on extraction treatments within each heat treatment and was found to have at least one significant difference ($p<0.0001$). Results show that more sinapaldehyde was extracted more from toasted staves than charred staves for all extraction treatments; however, reflux extraction treatment extracted more sinapaldehyde from toasted staves than sonication, but both reflux and sonication extraction treatments extracted a similar amount of sinapaldehyde from charred staves.

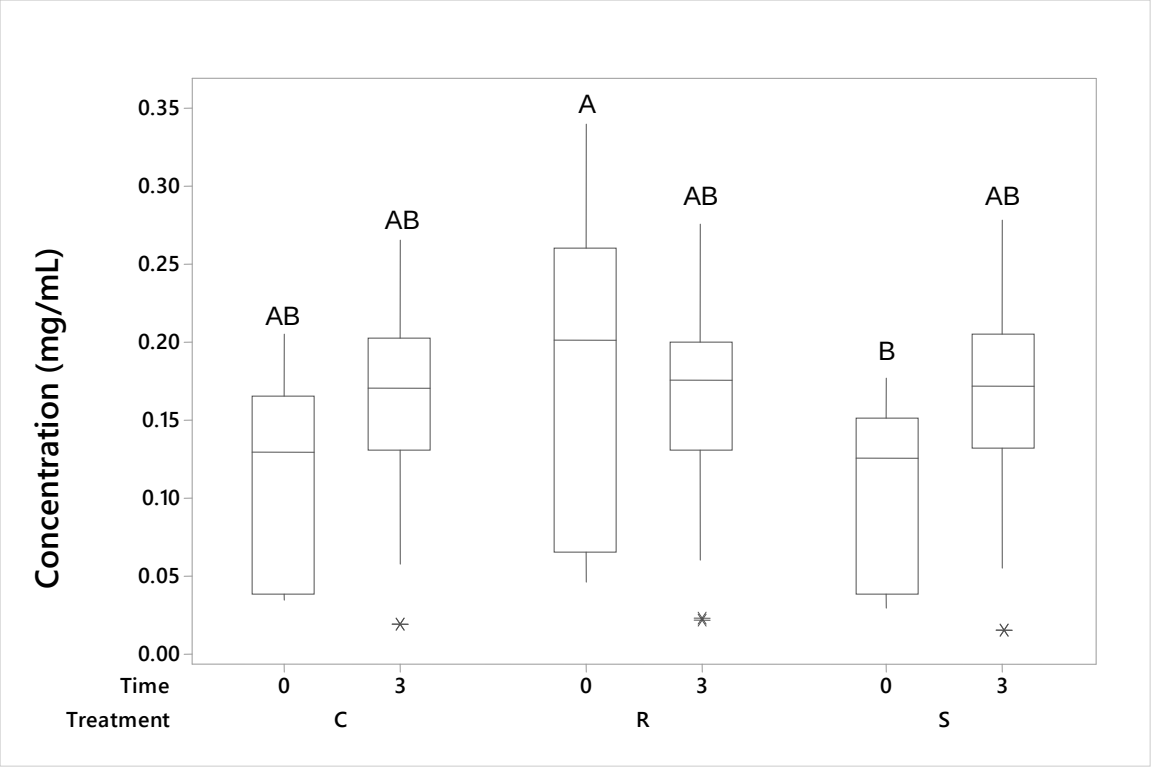


**Figure 58.** Sinapaldehyde extraction by each extraction treatment over time.

Reflux at time 0 had more sinapaldehyde extracted than sonicated at time 0 but was similar to the amount extracted after 3 months for all treatments. Reflux, sonication, and control extraction treatments after 3 months extracted more than sonication at time 0, but were similar to the control at time 0 (Fig.58). A Welch test was run on extraction treatments over time and was found to have at least one significant difference ($p=0.0038$).

The interaction between extraction treatment and time shows that sinapaldehyde amount is equal for all three treatments after three months. However, it also shows that sinapaldehyde amount in sonication and the control increased after 3 months, thus these two extraction methods are still capable of extracting sinapaldehyde.

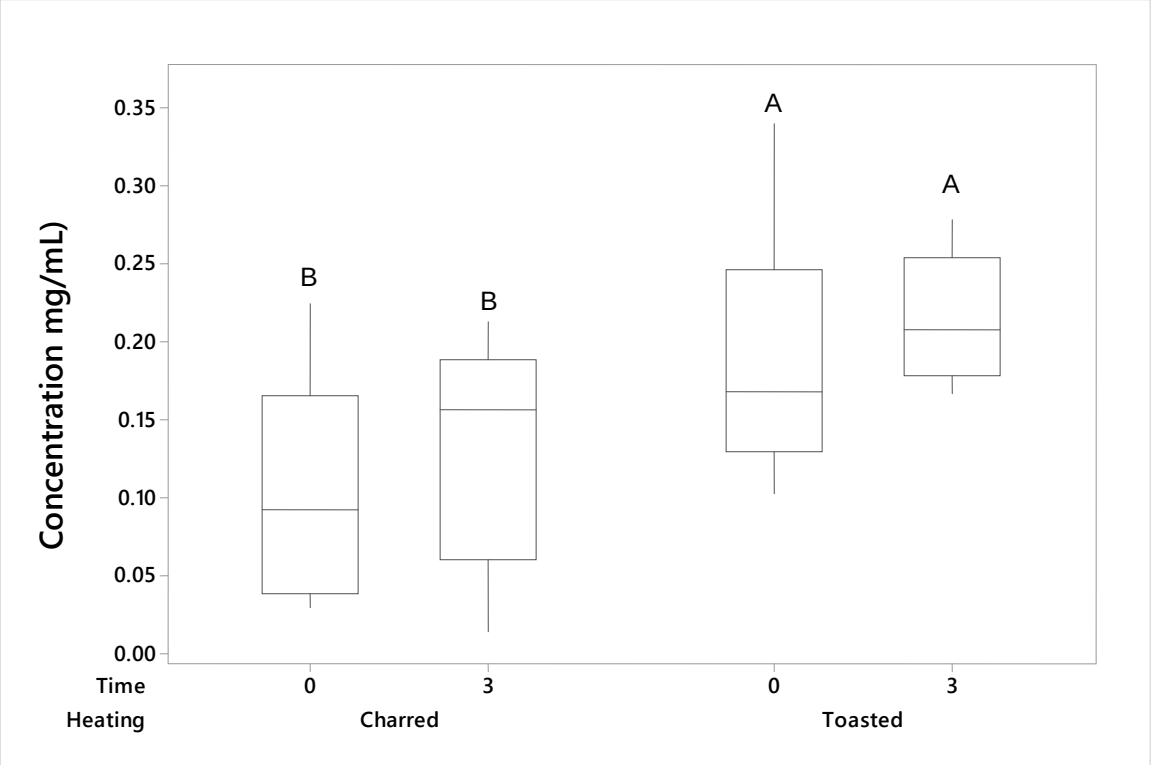


**Figure 59.** Sinapaldehyde extracted from each heat treatment over time.

As time was not significant, there was no difference between sinapaldehyde extracted at time 0 and after 3 months for toasted or for charred staves (Fig. 59). A Welch test was run on heat treatments over time and was found to have at least one significant difference ($p<0.0001$). However, there was a significant difference in the amount of sinapaldehyde extracted between toasted and charred staves. Toasted staves at time 0 and after 3 months had more sinapaldehyde extracted than charred staves at both time 0 and after 3 months.

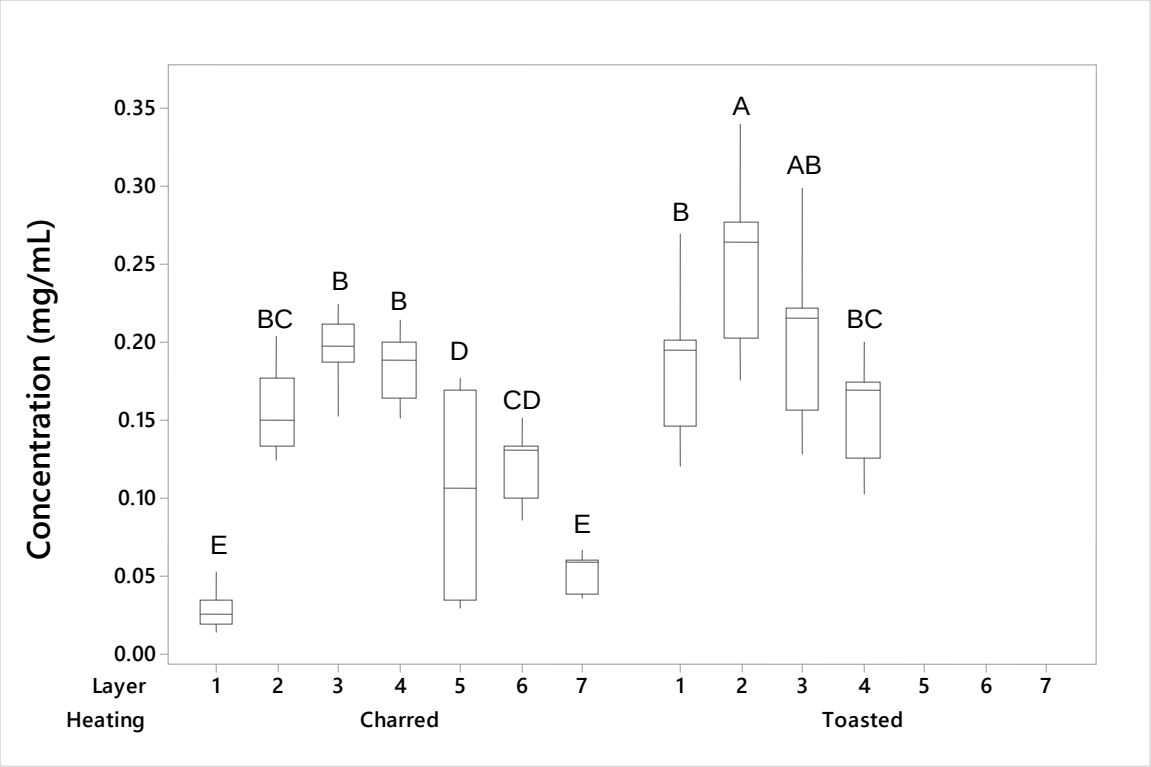


**Figure 60.** Sinapaldehyde extracted from each layer within each heat treatment.

Toasted layer 2 had more sinapaldehyde extracted than charred layers 1, 2, 3, 4, 5, 6, and 7 and toasted layers 1 and 4, but had a similar amount to toasted layer 3. Toasted layer 3 had more sinapaldehyde than charred layers 1, 5, 6, and 7, but was similar to charred layers 2, 3, and 4 and toasted layers 1 and 4. Charred layers 3 and 4 and toasted layer 1 were similar to toasted layer 4 and charred layer 2, but had more than charred layers 1, 5, 6, and 7. Toasted layer 4 and charred layer 2 had a similar amount of sinapaldehyde to charred layer 6, but had more than charred layers 1, 5, and 7. Charred layer 6 had a similar amount to charred layer 5, but had more sinapaldehyde than charred layers 1 and 7. Charred layer 5 had more sinapaldehyde extracted than charred layers 1 and 7. Charred layers 1 and 7 had the same amount of sinapaldehyde

extracted (Fig. 60). A Welch test was run on individual layers within each heat treatment and was found to have at least one significant difference ($p<0.0001$).

Results show that inner-most layers, such as toasted layers 2 and 3 and charred layers 3 and 4, had a higher availability of sinapaldehyde. Charred layers 1 and 7 had the least amount of sinapaldehyde due to over-degradation and under-degradation during heat treatment.

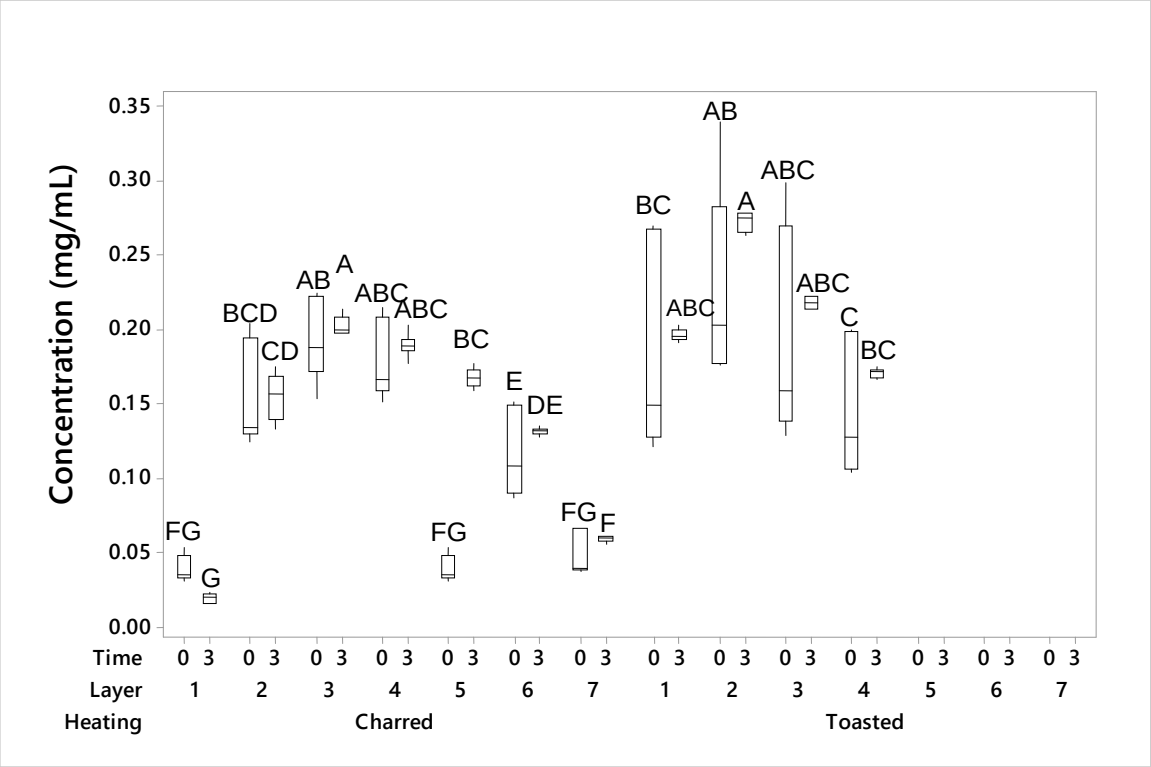


**Figure 61.** Sinapaldehyde from each layer over time.

Charred layer 3 after 3 months had more sinapaldehyde than charred layers 1, 2, 5, and 6 at time 0 and charred layers 1, 2, 5, 6, and 7 after 3 months, but had a similar amount to charred layers 3 and 4 at time 0 and charred layer 4 after 3 months. Charred

layer 3 at time 0 had more sinapaldehyde than charred  layer 1, 5, 6, and 7 at time 0 and charred layers 1, 2, 6, and 7 after 3 months, but had a similar amount to charred layers 2 and 4 at time 0 and charred layers 4 and 5 after 3 months. Charred layer 4 at time 0 and after 3 months had more sinapaldehyde than charred layers 1, 5, 6, and 7 at time 0 and charred layers 1, 6, and 7 after 3 months, but had a similar amount to charred layer 2 at time 0 and charred layers 2 and 7 after 3 months. Charred layer 5 after 3 months had more sinapaldehyde than charred layers 1, 5, 6, and 7 at time 0 and charred layers 1, 6, and 7 after 3 months, but had a similar amount to charred layer 2 at time 0 and after 3 months. Charred layer 2 at time 0 had more sinapaldehyde extracted than charred layers 1, 5, 6, and 7 at time 0 and charred layers 1 and 7 after 3 months, but had a similar amount to charred layers 2 and 6 after 3 months. Charred layer 2 after 3 months had more sinapaldehyde extracted than charred layers 1, 5, 6, and 7 at time 0 and charred layers 1 and 7 after 3 months, but had a similar amount to charred layer 6 after 3 months. Charred layer 6 after 3 months had more sinapaldehyde than charred layers 1, 5, and 7 at time 0 and charred layers 1 and 7 after 3 months, but had a similar amount to charred layer 6 at time 0. Charred layer 6 at time 0 had more sinapaldehyde than charred layers 1, 5, and 7 at time 0 and charred layers 1 and 7 after 3 months. Charred layer 7 after 3 months had more sinapaldehyde than charred layer 1 after 3 months, but had a similar amount to charred layers 1, 5, and 7 at time 0. Charred layers 1, 5, and 7 at time 0 had a similar amount of sinapaldehyde to charred layer 1 after 3 months (Fig. 61). A Welch ANOVA determined that at least one value was significantly different ($p<0.0001$). Toasted layer 2 after 3 months had more sinapaldehyde than

toasted layers 1 and 4 at time 0 and toasted layer 4 after 3 months, but had a similar amount to 2 and 3 at time 0 and toasted layers 1 and 3 after 3 months. Toasted layer 2 at time 0 had more sinapaldehyde than toasted layer 4 at time 0, but had a similar amount to toasted layers 1 and 3 at time 0 and toasted layers 1, 3, and 4 after 3 months. Toasted layer 3 at time 0 and toasted layers 1 and 3 after 3 months had more sinapaldehyde than toasted layers 1 and 4 at time 0 and toasted layer 4 after 3 months. Toasted layer 1 at time 0 and toasted layer 4 after 3 months had a similar amount to toasted layer 4 at time 0 (Fig. 61). A Welch ANOVA determined that at least one value was significantly different ($p<0.0001$).

Results show that layers had a similar amount of sinapaldehyde extracted at time 0 and after 3 months indicating that time was not a significant factor ($\alpha=0.05$) among layers.

### 3.5.2. Protocatechuic Acid

Significant differences ($\alpha=0.05$) were found among layers between heat treated charred and toasted staves (Fig. 62), among toasted stave layers (Fig. 63), for heat treated charred staves (Fig. 64), between heat treated staves among extraction treatments (Fig.67), between heat treated staves over time (Fig.69), among layers for charred and toasted staves (Fig.70), and among layers over time (Fig. 71). However, there was no significant difference found across reflux, sonication, and control extraction treatments (Fig. 65), between time 0 and after 3 months (Fig. 66), and among extraction treatments over time (Fig.68).

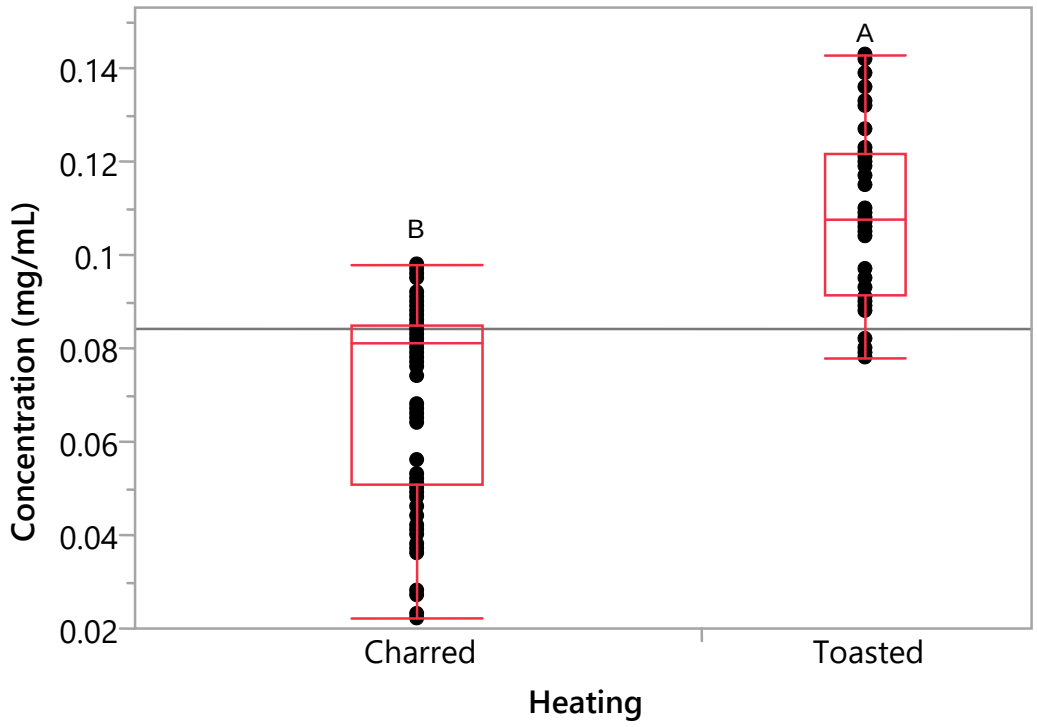


**Figure 62.** Protocatechuic acid extracted from charred and toasted staves.

More PA was extracted from toasted staves than from charred staves (Fig 62). A Welch test was run on heat treated staves and was found to have at least one significant difference ($p<0.0001$). Results suggest that a lighter heat treatment was more beneficial for the extraction of PA.

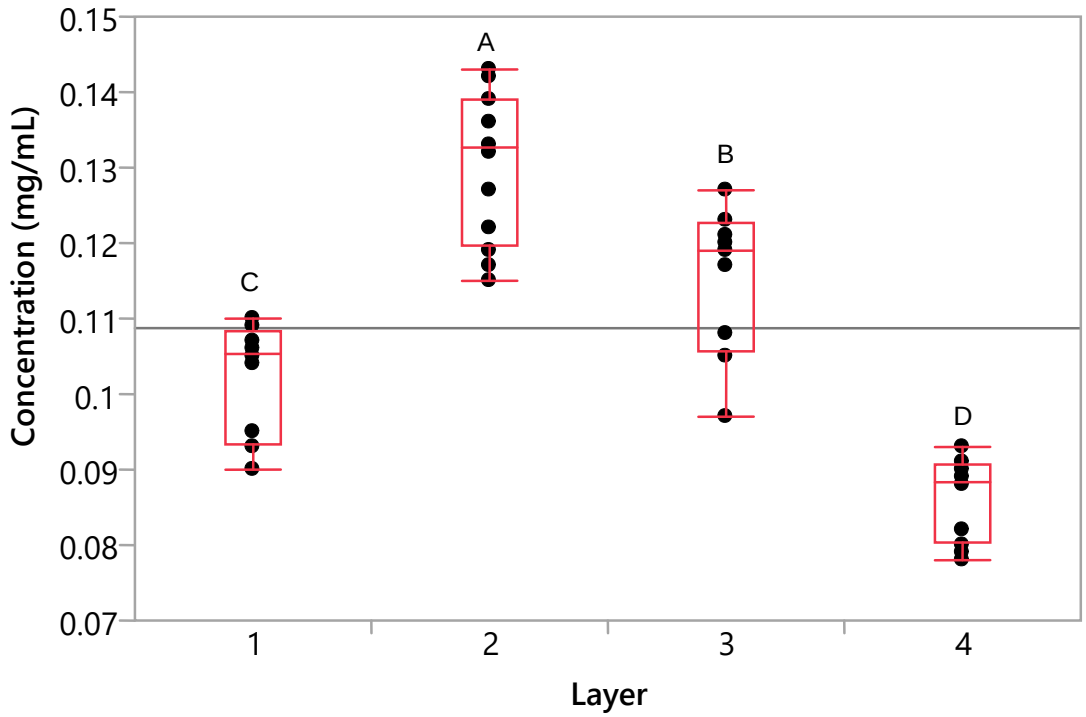


**Figure 63.** Protocatechuic acid extracted from each toasted layer.

Toasted layer 2 had the most PA extracted compared to all toasted layers. Toasted layer 3 had more PA than toasted layers 1 and 4 while toasted layer 1 had more PA than toasted layer 4 (Fig. 63). A Welch test was run on individual toasted stave layers and was found to have at least one significant difference ($p<0.0001$). Results show that toasted inner layer 2 had the highest availability of PA for extraction than other layers. The extraction of PA from layer 2 indicates a higher degradation of layer 1 during heat treatment than layer 2 for more PA extraction availability.

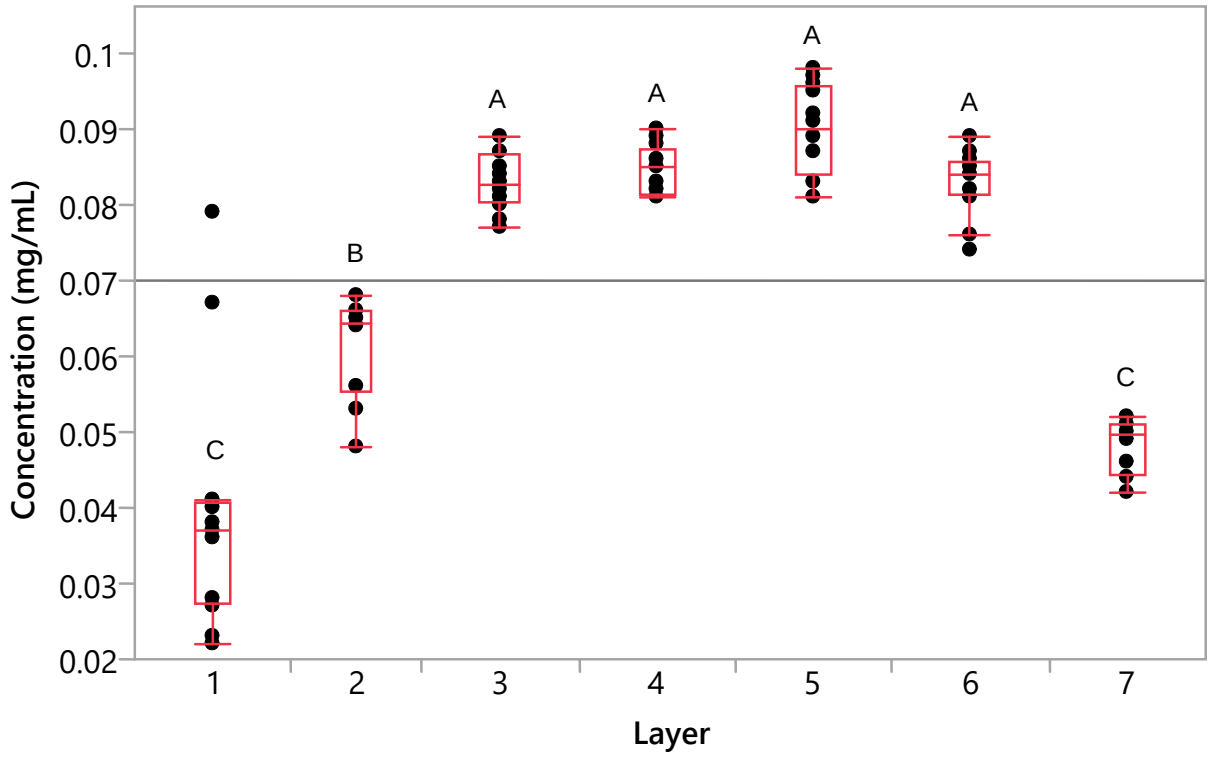


**Figure 64.** Protocatechuic acid extracted from each charred layer.

Charred layer 5 had the most PA extracted than layers 1, 2, 3, 6, and 7, but is similar in amount of PA extracted to charred layer 4. Charred layer 4 had similar amounts of PA extracted to layers 3 and 6, but more PA than charred layers 1, 2, and 7. Charred layer 2 had more PA than charred layer 1 and 7 while charred layer 7 had more PA than charred layer 1 (Fig 64). A Welch test was run on individual charred stave layers and was found to have at least one significant difference ($p<0.0001$).

Results show that inner charred layers had a greater availability than charred layer 1 and 7. Charred layer 1 goes through a burning process during heat treatment which degrades most oak wood cells and which decreases the amount of available oak compounds such as PA. Charred layer 7 is furthest from the heat treatment and has oak wood cells not degraded enough for full extraction of PA.

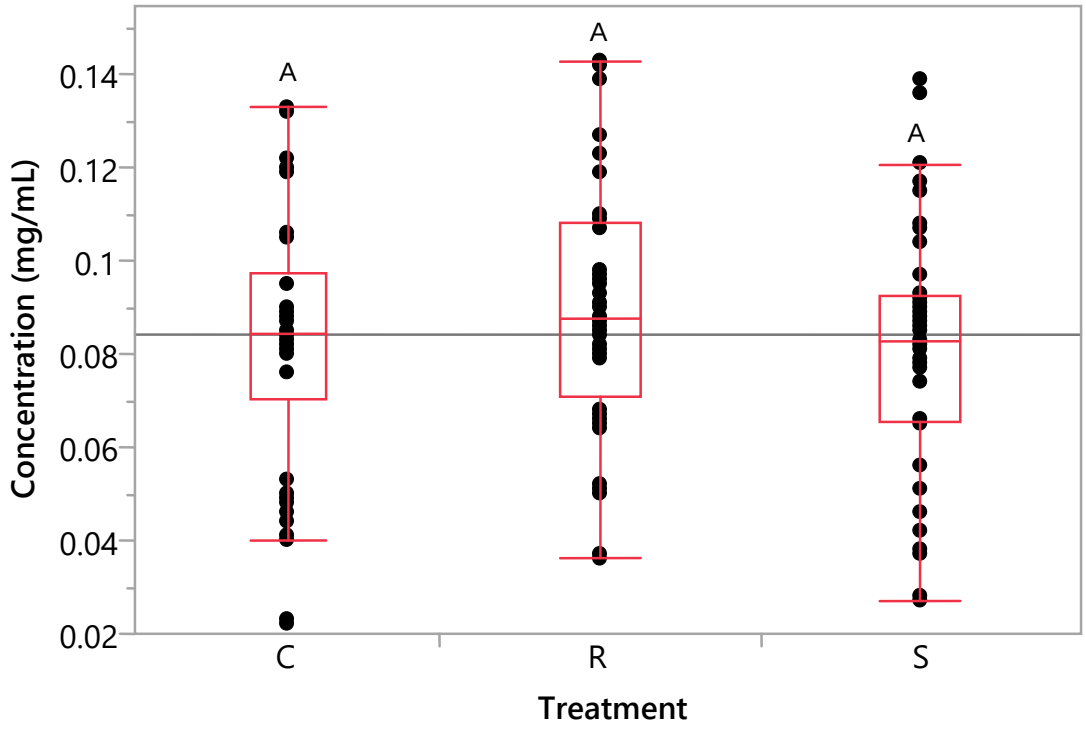


**Figure 65.** Protocatechuic acid extracted by each treatment.

There was no significant difference found in the extraction of PA across reflux, sonication, and control extraction methods (Fig 65). Results show that the control was similar to extraction of PA from both reflux and sonication extraction methods which suggests that neither the reflux or sonication method accelerated the extraction of PA from each layer. However, both methods may have an accelerated extraction elsewhere.

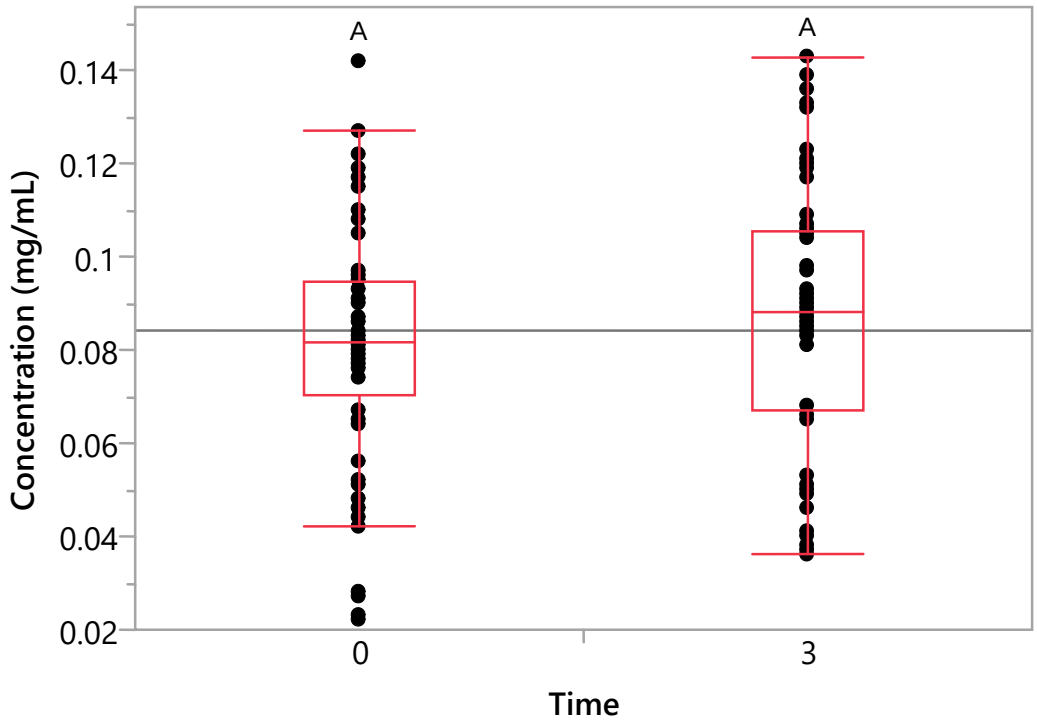


**Figure 66.** Protocatechuic acid extracted over time.

There was no significant difference in the amount of PA extraction found between time 0 and after 3 months (Fig 66). Therefore, it is not necessary to wait a period of time after extraction because no significant amount of PA will be yielded by oak staves.

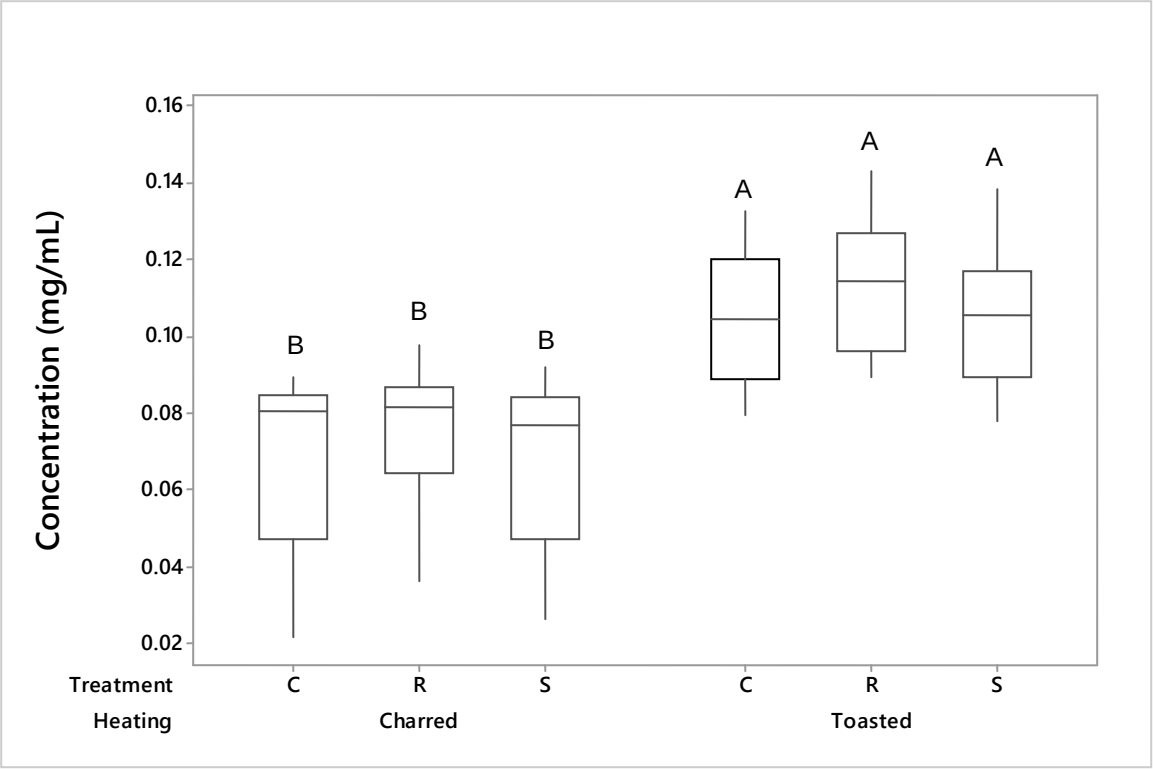


**Figure 67.** Protocatechuic acid extracted by each extraction treatment within each heat treatment.

The amount of PA extracted was not significantly different (α=0.05) across extraction treatments for each heat treatment (Fig. 67). A Welch test was run on extraction treatments within each heat treatment and was found to have at least one significant difference ($p<0.0001$). Results show that while toasted and charred oak staves had a significantly different amount of PA, there was no significant difference among extraction treatments.

While results show there may be an increase in PA extracted by each extraction treatment over time, there was no significant difference found for both time and extraction treatment (Fig 66 and 67). Therefore, the amount of PA extracted was the same across treatments over time (Fig. 68).

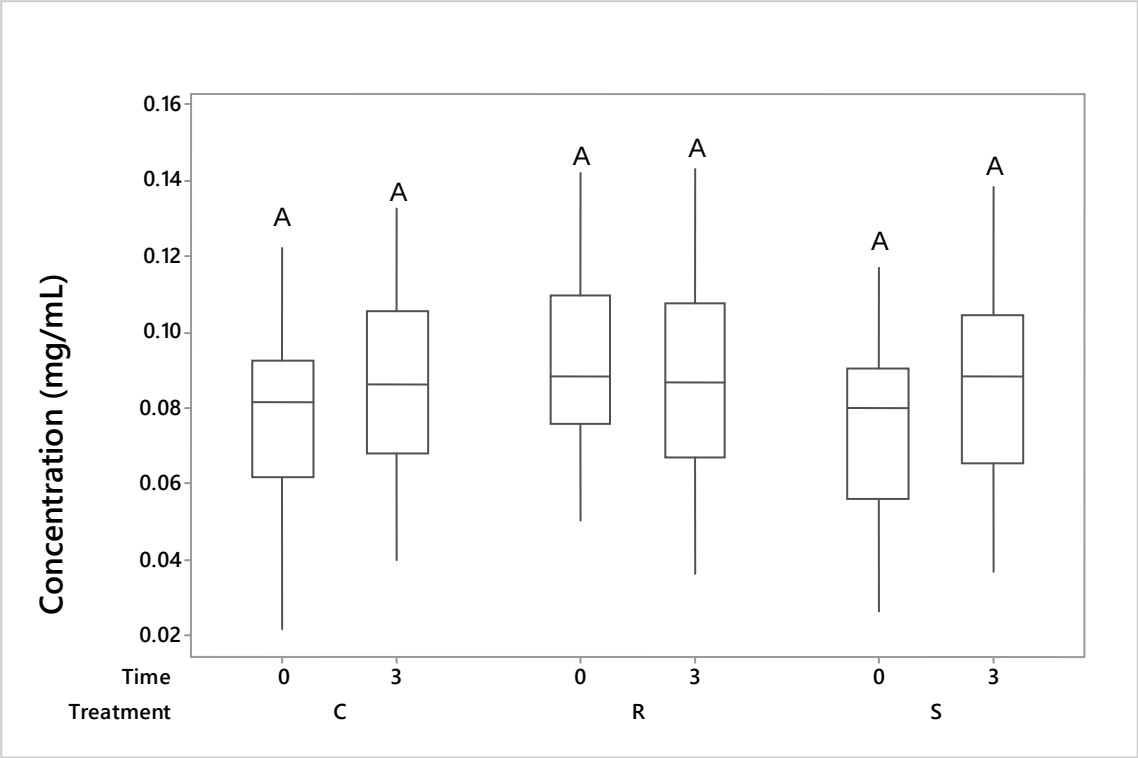


**Figure 68.** Protocatechuic acid extracted by each extraction treatment over time.

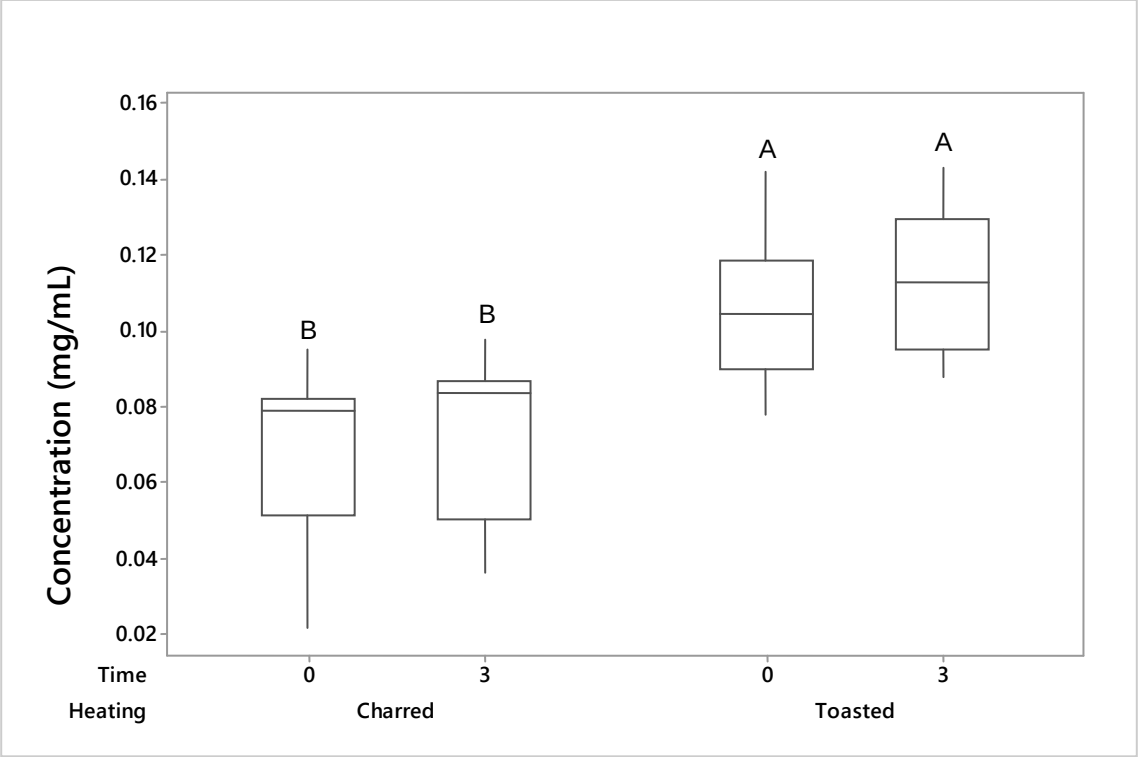


**Figure 69.** Protocatechuic acid extracted from each heat treatment over time.

More PA was extracted from toasted staves than charred staves over time (Fig. 69). A Welch test was run on heat treatments over time and was found to have at least one significant difference ($p<0.0001$). However, there was no significant difference found between PA extracted after three months time and time zero within heating treatments. Results show that time had no effect on the extraction of PA from both charred and toasted staves.

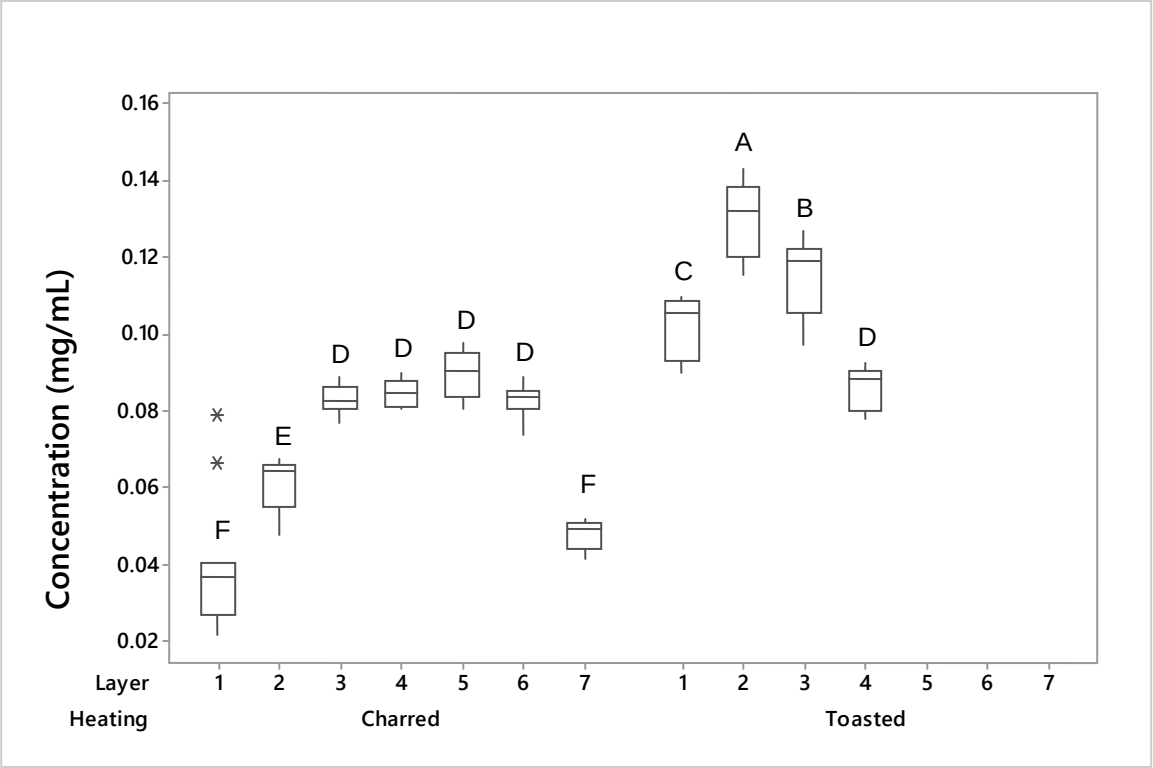


**Figure 70.** Protocatechuic acid extracted from each layer within each heat treatment.

More PA was extracted from toasted layer 2 over all layers within both heat treatments followed by toasted layer 2 and toasted layer 1. Toasted layer 4 had more PA extracted than charred layers 1, 2, and 7, but had a similar amount to charred layers

3, 4, 5, and 6. Charred layer 2 had more PA than charred layers 1 and 7 which both had a similar amount of PA (Fig. 70). A Welch test was run on individual layers within each heat treatment and was found to have at least one significant difference ($p<0.0001$).

Results showed for both heat treatments layers with direct heat treatment and layers further removed from heat treatment did not extract as much PA (charred layers 1, 2, and 7, and toasted layers 1, 3, and 4) as inner layers (charred layers 3, 4, 5, and 6 and toasted layers 2 and 3). Russell states that more compounds should be available from inner toasted layers than from charred and from direct heat treatment and non-treated layers (Russell, 2003).

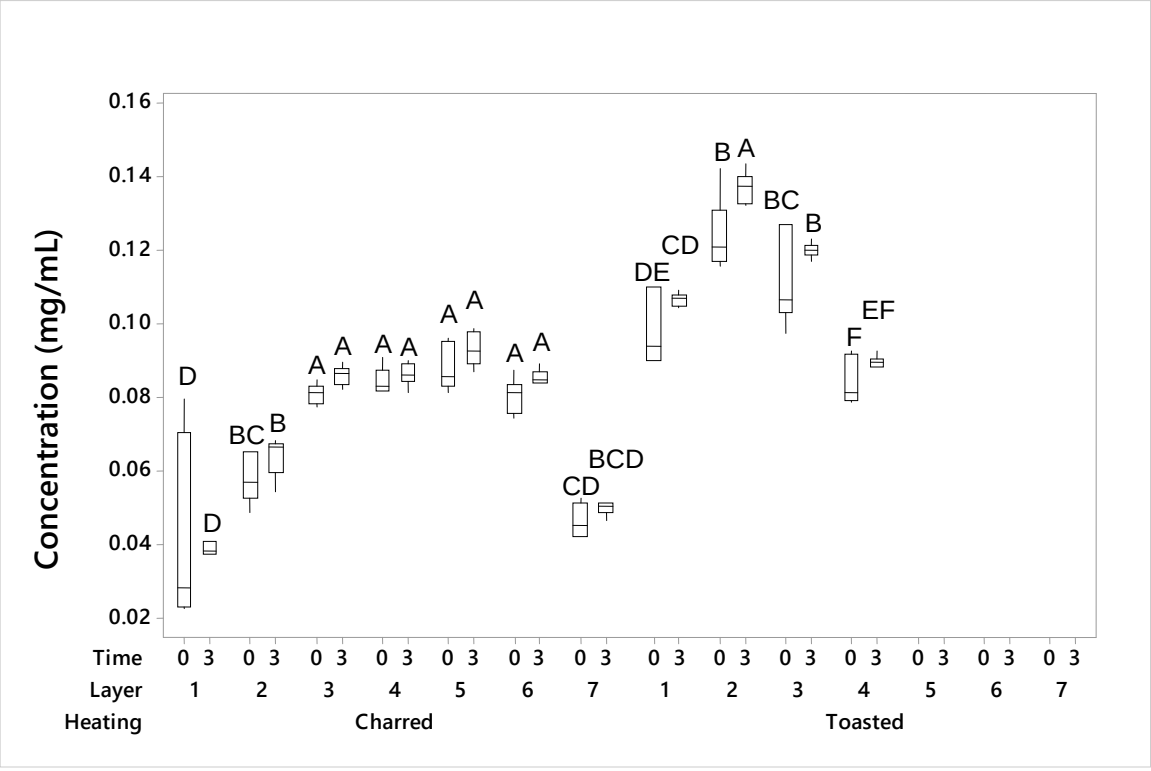


**Figure 71.** Protocatechuic acid extracted from each layer over time.

Charred layers 3, 4, 5, and 6 at time 0 and charred layers 3, 4, 5, and 6 after 3 months had more protocatechuic acid than charred layers 1, 2, and 7 at time 0 and after 3 months. Charred layer 2 after 3 months had more protocatechuic acid than charred layers 1 and 7 at time 0 and charred layer 1 after 3 months, but had a similar amount to charred layer 2 at time 0 and charred layer 7 after 3 months. Charred layer 2 at time 0 had more protocatechuic acid than charred layer 1 at time 0 and after 3 months, but had a similar amount to charred layer 7 at time 0 and after 3 months. Charred layer 7 after 3 months had a similar amount of protocatechuic acid to charred layers 1 and 7 at time 0 and charred layer 1 after 3 months (Fig. 71). A Welch ANOVA determined that at least one value was significantly different ($p<0.0001$). Toasted layer 2 after 3 months had more protocatechuic acid than charred layers 1 through 4 at time 0 and charred layers 1, 3, and 4 after 3 months. Toasted layer 2 at time 0 and toasted layer 3 after 3 months had more protocatechuic acid than toasted layer 1 and 4 at time 0 and after 3 months, but had a similar amount to toasted layer 3 at time 0. Toasted layer 3 at time 0 had more protocatechuic acid than toasted layer 1 and 4 at time 0 and toasted layer 4 after 3 months, but had a similar amount to toasted layer 1 after 3 months. Toasted layer 1 after 3 months had more protocatechuic acid than toasted layer 4 at time 0 and after 3 months, but had a similar amount to toasted layer 1 at time 0. Toasted layer 1 at time 0 had more protocatechuic acid than toasted layer 4 at time 0, but had a similar amount to toasted layer 4 after 3 months. Toasted layer 4 after 3 months had a similar amount to toasted layer 4 at time 0 (Fig. 71). A Welch ANOVA determined that at least one value was significantly different ($p<0.0001$).

### 3.5.3. Ellagic Acid

Significant differences (α=0.05) were found for heat treated charred staves (Fig. 74), across reflux, sonication, and control extraction treatments (Fig.75), between heat treated staves among extraction treatments (Fig.77), among extraction treatments over time (Fig.78), and among layers for charred and toasted staves (Fig.80). However, there was no significant difference found between heat treated charred and toasted staves (Fig. 72), among toasted stave layers (Fig. 73), between time 0 and after 3 months (Fig. 76), between heat treated staves over time (Fig.79), and among layers over time (Fig. 81).

There was no significant difference (α=0.05) found between heat treatments (Fig 72). There was also no significant difference (α=0.05) in the amount of EA extracted from each toasted layer (Fig. 73). Toasted stave layers had a low amount of EA available for extraction.

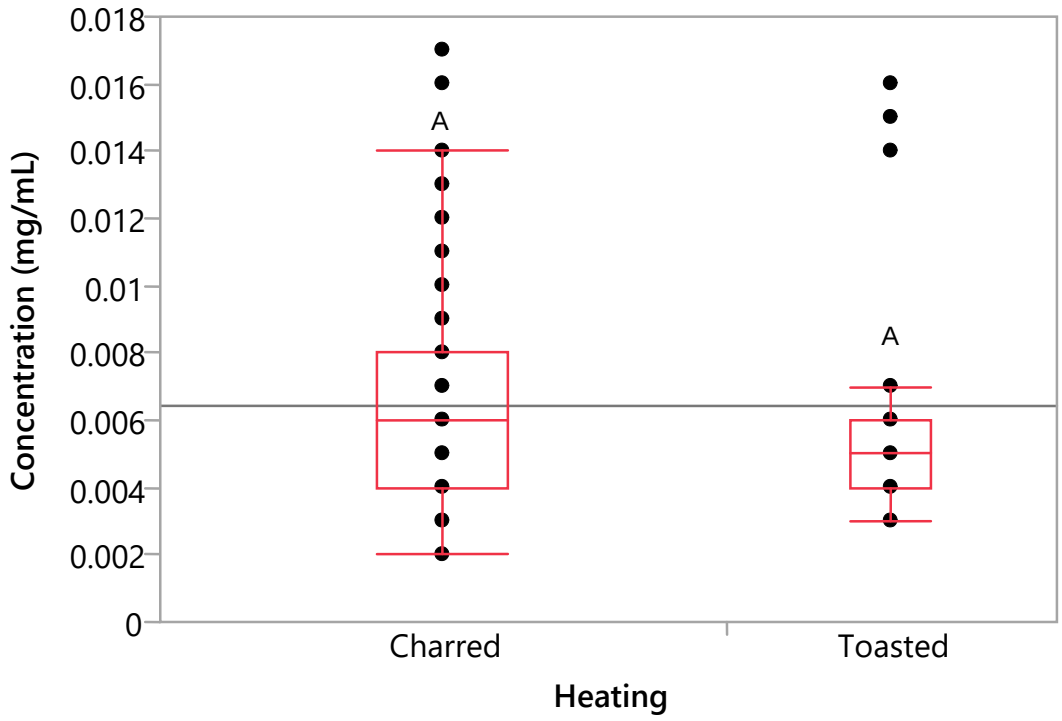


**Figure 72.** Ellagic acid extracted from charred and toasted staves.

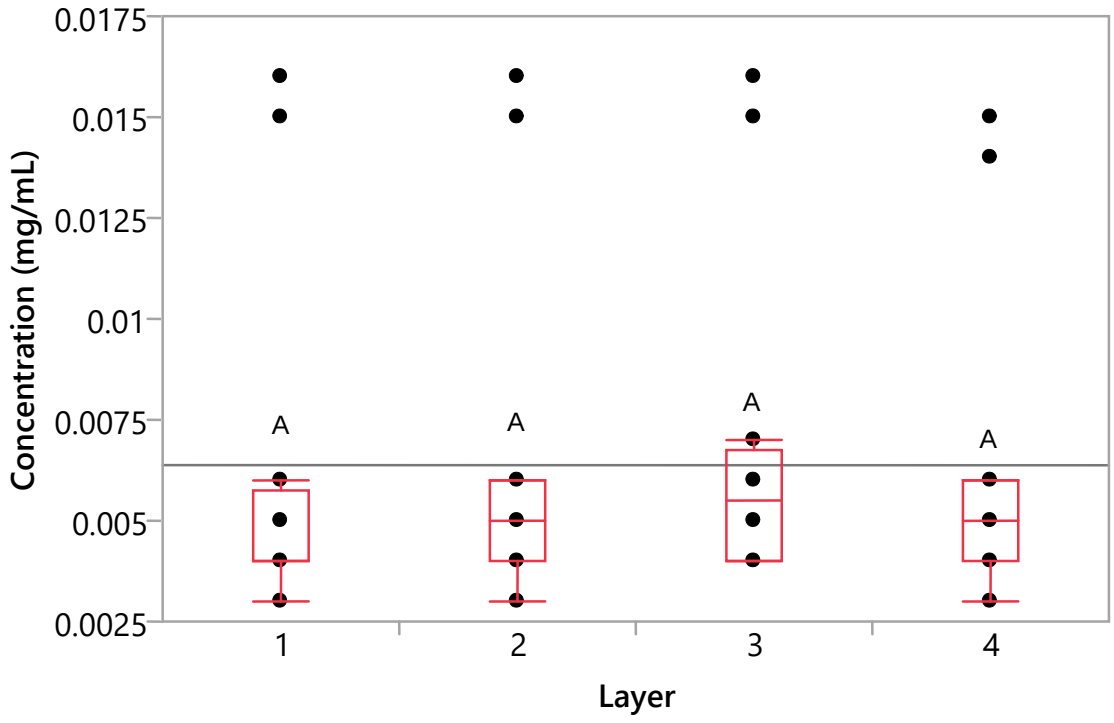


**Figure 73.** Ellagic acid extracted from each toasted layer.

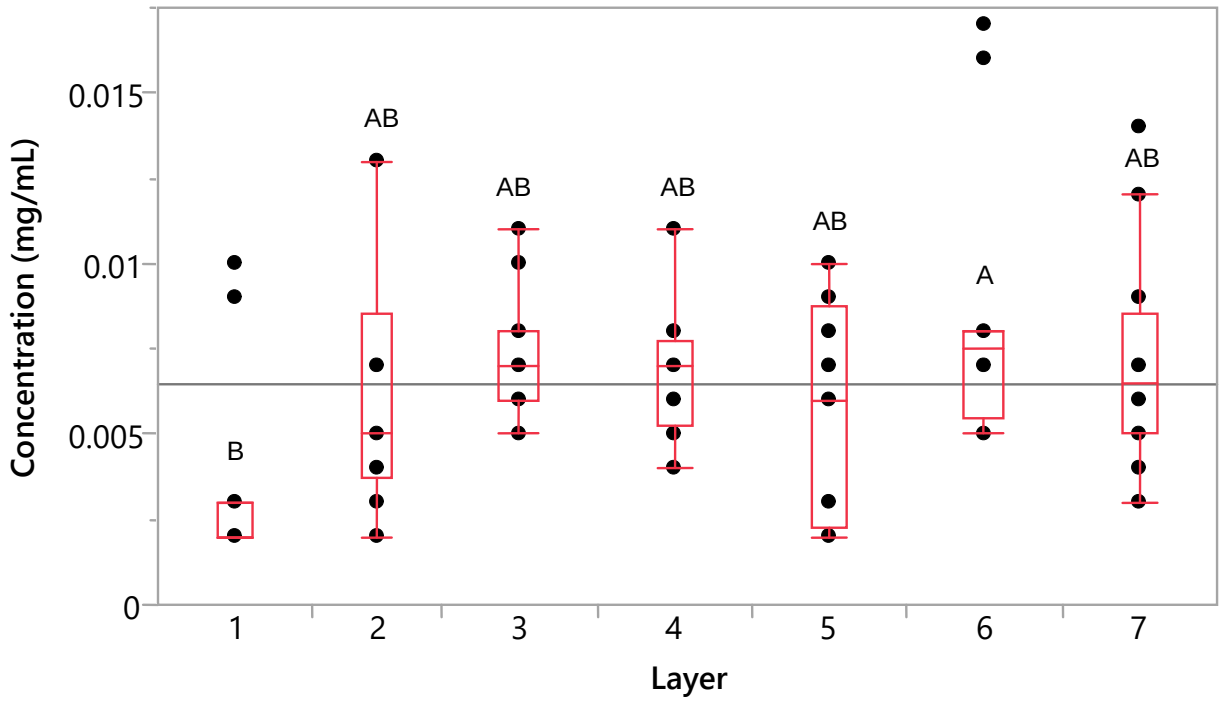


**Figure 74.** Ellagic acid extracted from each charred layer.

Charred layer 6 had more ellagic acid than layer 1, but had a similar amount to charred layers 2, 3, 4, 5, and 7. Charred layers 2, 3, 4, 5, and 7 had more ellagic acid extracted than charred layer 1 (Fig. 74). A Welch test was run on individual charred stave layers and was found to have at least one significant difference (p=0.0218). Results show that charred layer 1 had the least amount of EA available for extraction due to high thermal degradation of oak wood during heat treatment.

Reflux extraction method extracted more ellagic acid than sonication and control methods, while sonication and control extraction methods extracted the same amount of ellagic acid (Fig. 75). A Welch test was run on extraction treatments and was found to

have at least one significant difference ($p<0.0001$). Results show that reflux extraction treatment extracted more EA than sonication and control methods.

There was no significant difference ($\alpha=0.05$) between the amount of ellagic acid extracted over time (Fig. 76). Results show that time had no effect on the extraction of EA.

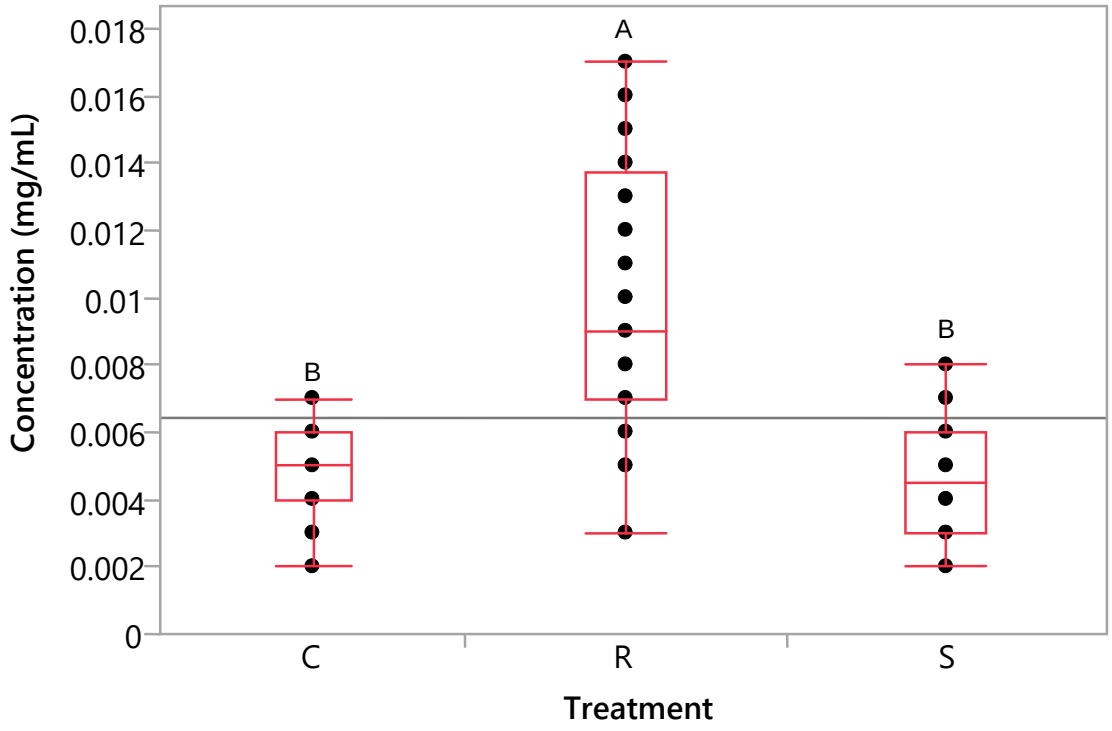


**Figure 75.** Ellagic acid extracted by each extraction treatment.

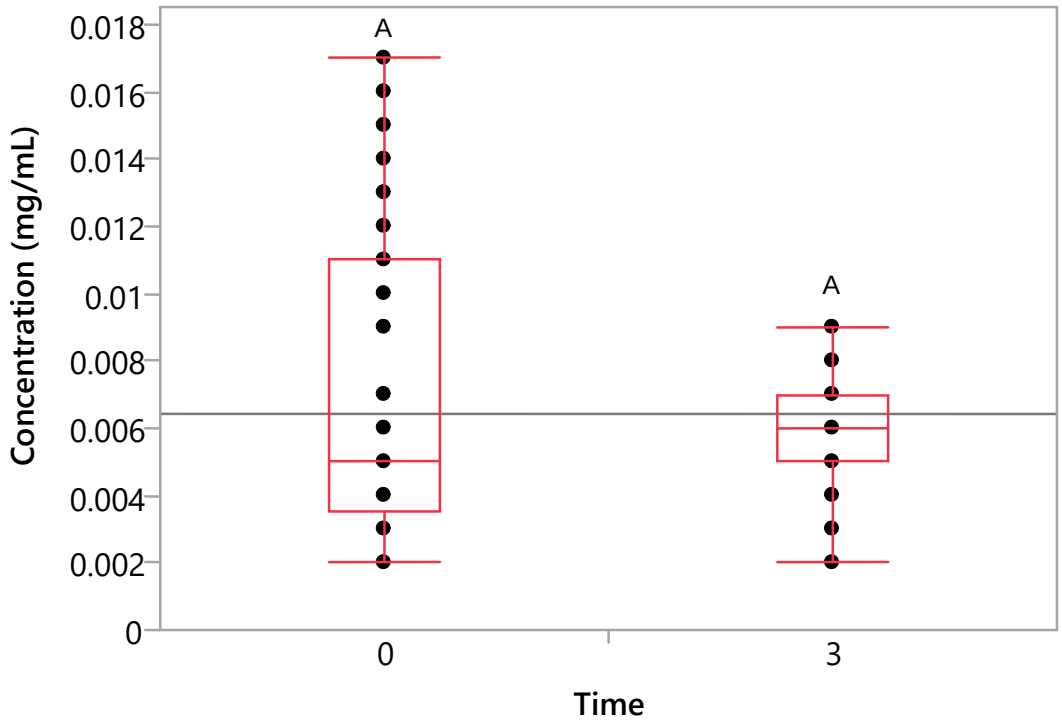


**Figure 76.** Ellagic acid extracted over time.

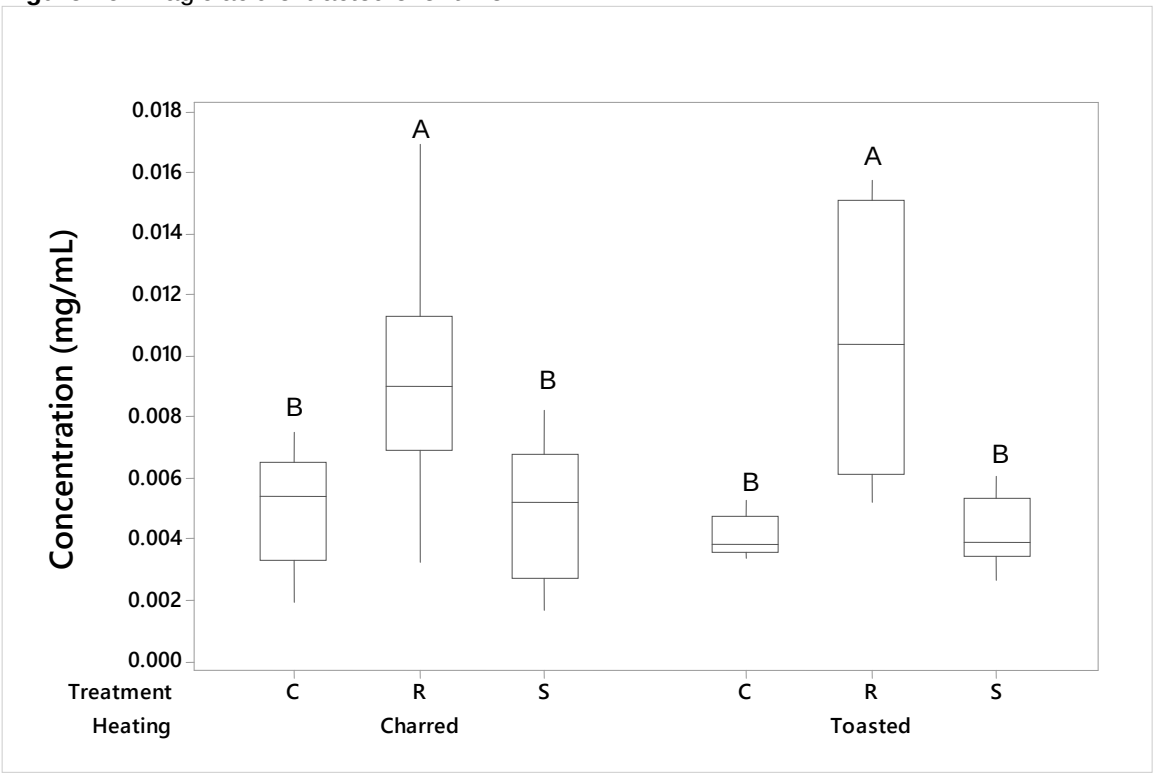


**Figure 77.** Ellagic acid extracted by each extraction treatment within each heat treatment.

Ellagic acid extracted from toasted and charred staves using reflux extraction method was higher than charred and toasted staves using sonication and control methods (Fig. 77). A Welch test was run on extraction treatments within each heat treatment and was found to have at least one significant difference ($p<0.0001$). Results show that reflux was a more effective treatment than sonication and control methods for extracting EA from charred and toasted staves.

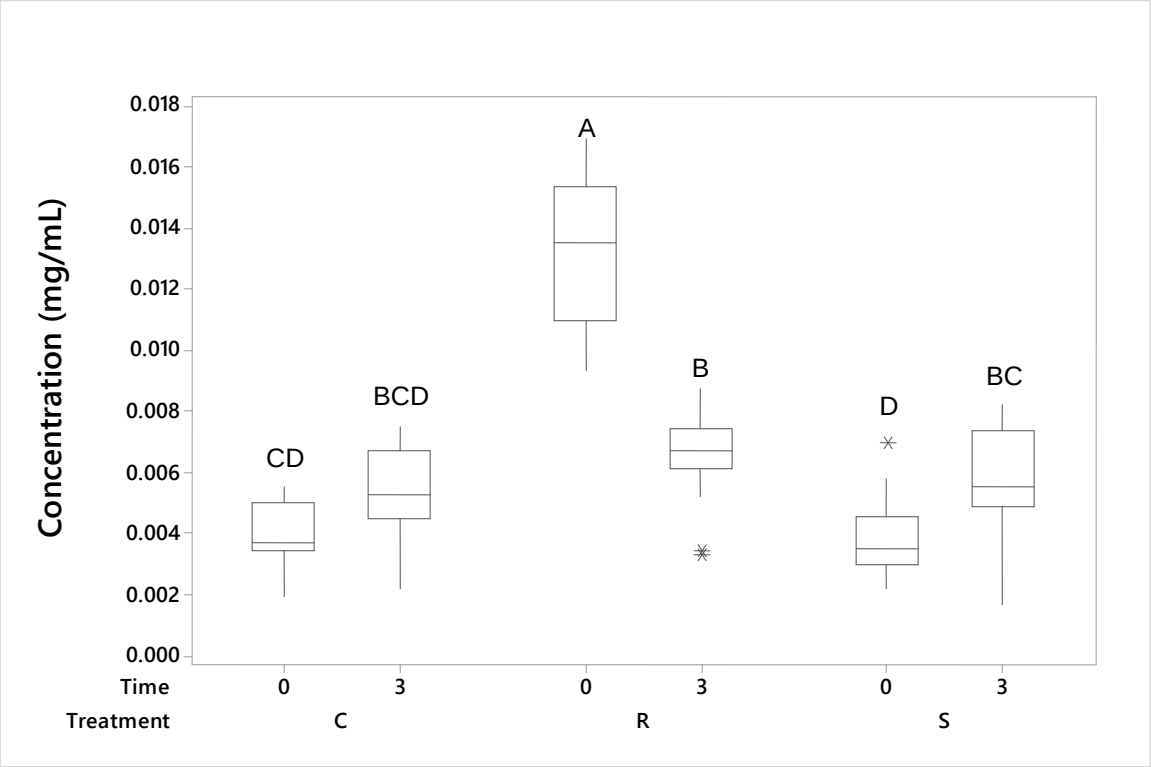


**Figure 78.** Ellagic acid extracted by each extraction treatment over time.

Reflux at time 0 had more ellagic acid than reflux after 3 months, sonication at time 0 and after 3 months, and the control at time 0 and after 3 months. Reflux after 3 months had a similar amount of ellagic acid to sonication and control extraction methods after 3 months, but more than sonication and control methods at time 0. Sonication after 3 months had more ellagic acid extracted than at time 0, but was similar

to the control at time 0 and after 3 months. Control after 3 months had a similar amount extracted to control at time 0 and sonication at time 0. Control at time 0 extracted a similar amount of ellagic acid to the sonication method at time 0 (Fig. 78). A Welch test was run on extraction treatments over time and was found to have at least one significant difference ($p<0.0001$).

Results show reflux extraction method extracted more EA at time 0 compared to all other methods at time 0 and after 3 months. This indicates that the availability of EA for extraction was higher at time 0 for reflux extraction method. However, sonication and control extraction methods extracted more EA after 3 months than at time 0. Ellagic acid is a slowly released and stable ellagitannin which appears in aged spirit from the solubilization of free ellagic acid in wood and from degradation of soluble and insoluble ellagitannins (Viriot et al., 1993). The further extraction of ellagic acid by sonication and control extraction methods indicates the availability of EA to be extracted by these extraction treatments over time.

There was no significant difference ($\alpha=0.05$) in the amount of ellagic acid extracted from toasted and charred staves over time (Fig. 79). A Welch test was run on heat treatments over time and was found to have at least one significant difference ($p=0.0083$). Results show that because both time and heat treatments were not factors in the extraction of EA, there was no interaction between them (Fig. 72 and 76).

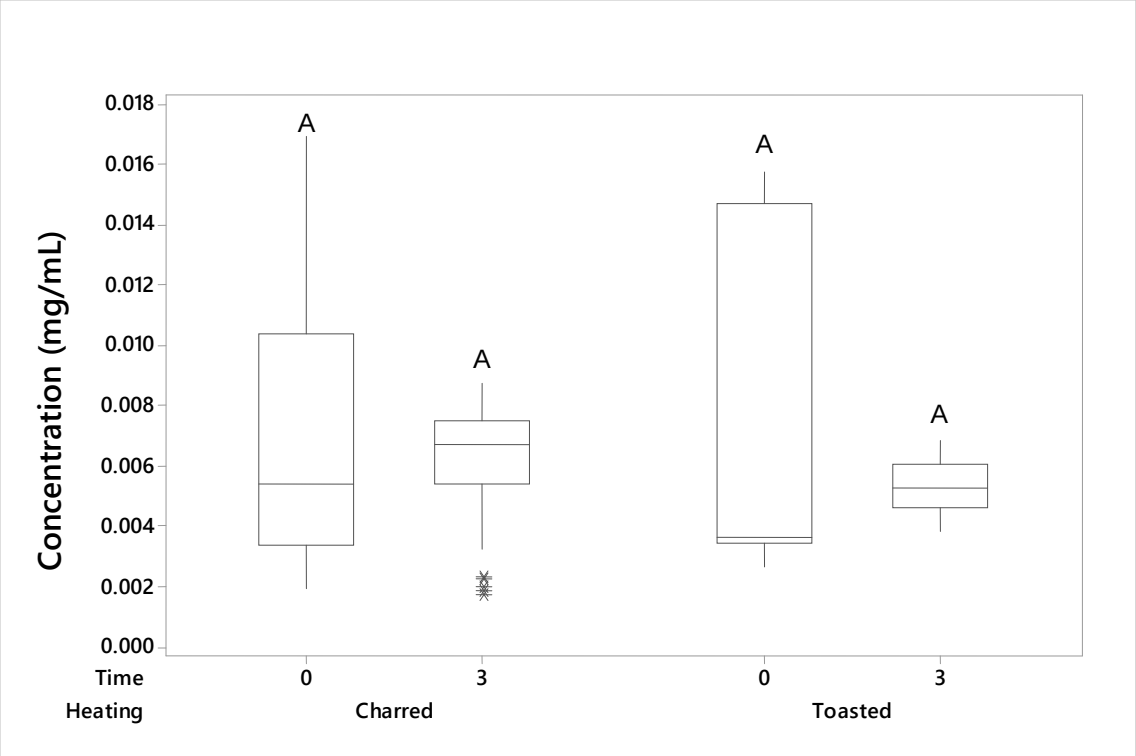


**Figure 79.** Ellagic acid extracted from each heat treatment over time

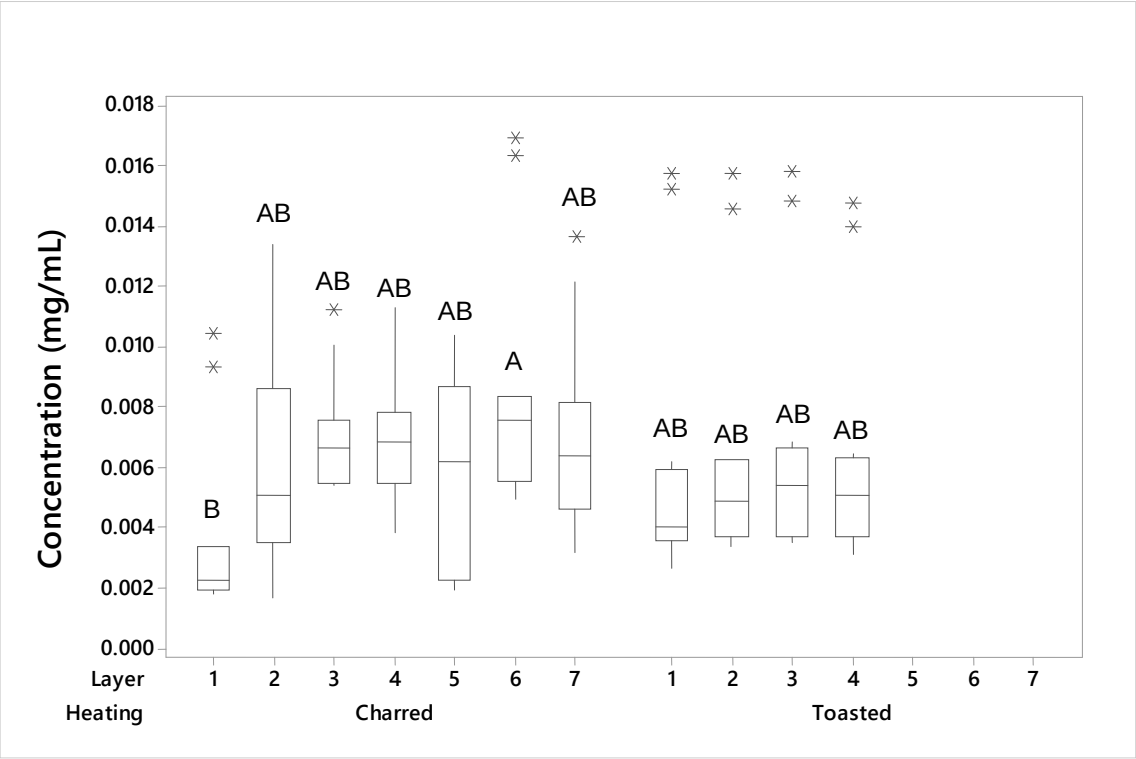


**Figure 80.** Amount of ellagic acid extracted from each layer within each heat treatment.

Charred layer 6 had more ellagic acid extracted than charred layer 1, but had a similar amount to charred layers 2, 3, 4, 5, and 7 and toasted layers 1, 2, 3, and 4. Charred layers 2, 3, 4, 5, and 7 and toasted layer 1 through 4 all had a similar amount of ellagic acid extracted, but had more than charred layer 1 (Fig. 80).

Results show that inner layers had a higher availability of EA for extraction than directly heat treated layers such as charred layer 1 and toasted layer 1. However, inner layers were all similar in the amount of EA extracted from them.

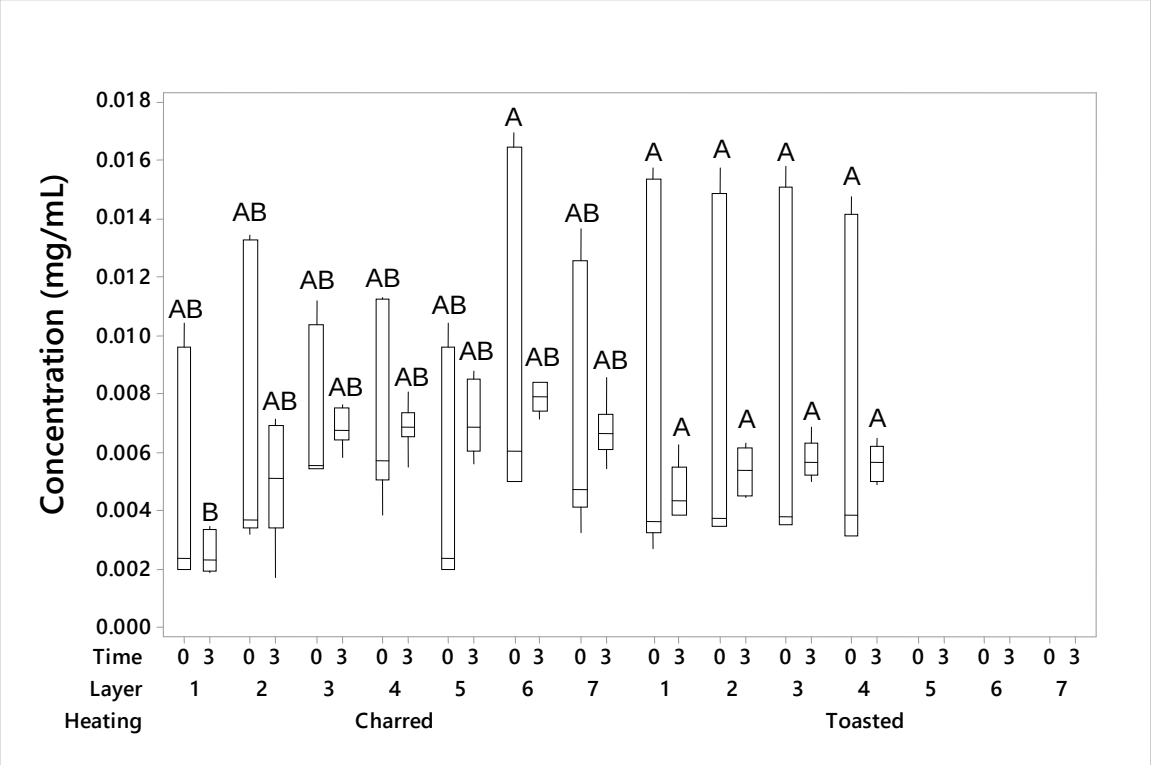


**Figure 81.** Ellagic acid extracted from each layer over time.

Charred layer 6 at time 0 had more ellagic acid than charred layer 1 after 3 months, but had a similar amount to charred layers 1, 2, 3, 4, 5, and 7 at time 0 and 2 through 7 after 3 months. Charred layers 1, 2, 3, 4, 5, and 7 at time 0 and charred

layers 2 through 7 after 3 months had a similar amount of ellagic acid to charred layer 1 after 3 months (Fig. 81). A Welch ANOVA deteremined that at least one value was significantly different ($p<0.0001$). Toasted layers at time 0 and after 3 months all had a similar amount of ellagic acid (Fig. 81). Results indicate that since time was not a factor in the extraction of EA, layers did not differ in the amount of EA extracted between time 0 and after 3 months.

### 3.5.4. Vanillic Acid

Significant differences ($\alpha=0.05$) were found between heat treated charred and toasted staves (Fig. 82), among toasted stave layers (Fig. 83), for heat treated charred staves (Fig. 84), between heat treated staves among extraction treatments (Fig.87), between heat treated staves over time (Fig.89), among layers for charred and toasted staves (Fig.90), and among layers over time (Fig. 91). However, there was no significant difference found across reflux, sonication, and control extraction treatments (Fig.85), between time 0 and after 3 months (Fig. 86), and among extraction treatments over time (Fig.88).

Toasted staves had a higher amount of vanillic acid than charred staves (Fig. 82). A Welch test was run on each heat treatment and was found to have at least one significant difference ($p<0.0001$). Results show that a lighter heat treatment produced a higher availability of vanillic acid.

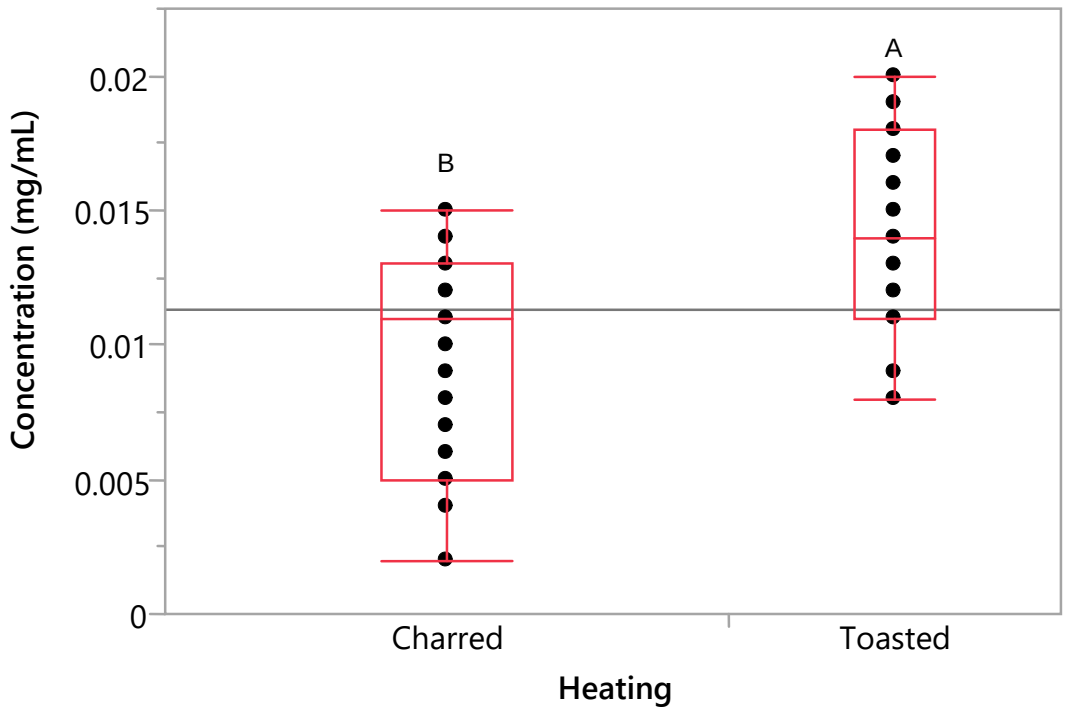


**Figure 82.** Vanillic acid in charred and toasted staves.

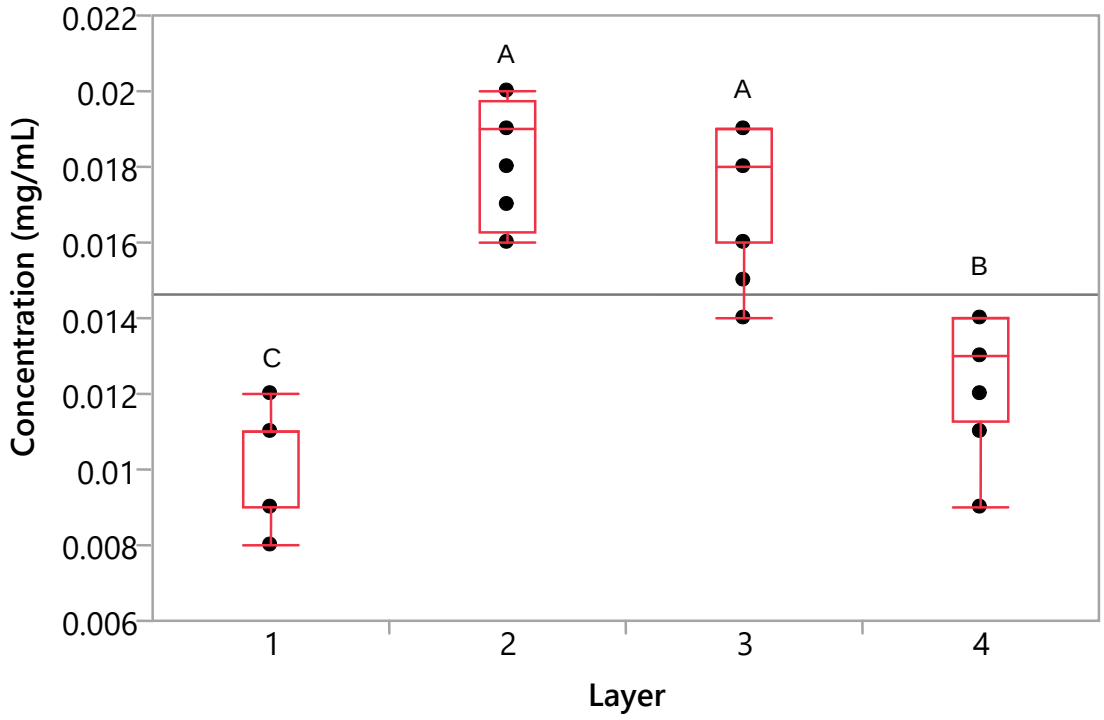


**Figure 83.** Vanillic acid extracted from each toasted layer.

Toasted layers 2 and 3 extracted more vanillic acid than layers 1 and 4. Toasted layer 4 extracted more vanillic acid than toasted layer 1 (Fig. 83). A Welch test was run on individual toasted stave layers and was found to have at least one significant difference ($p<0.0001$). Results show that inner layers had a higher availability of VA than outer layers.

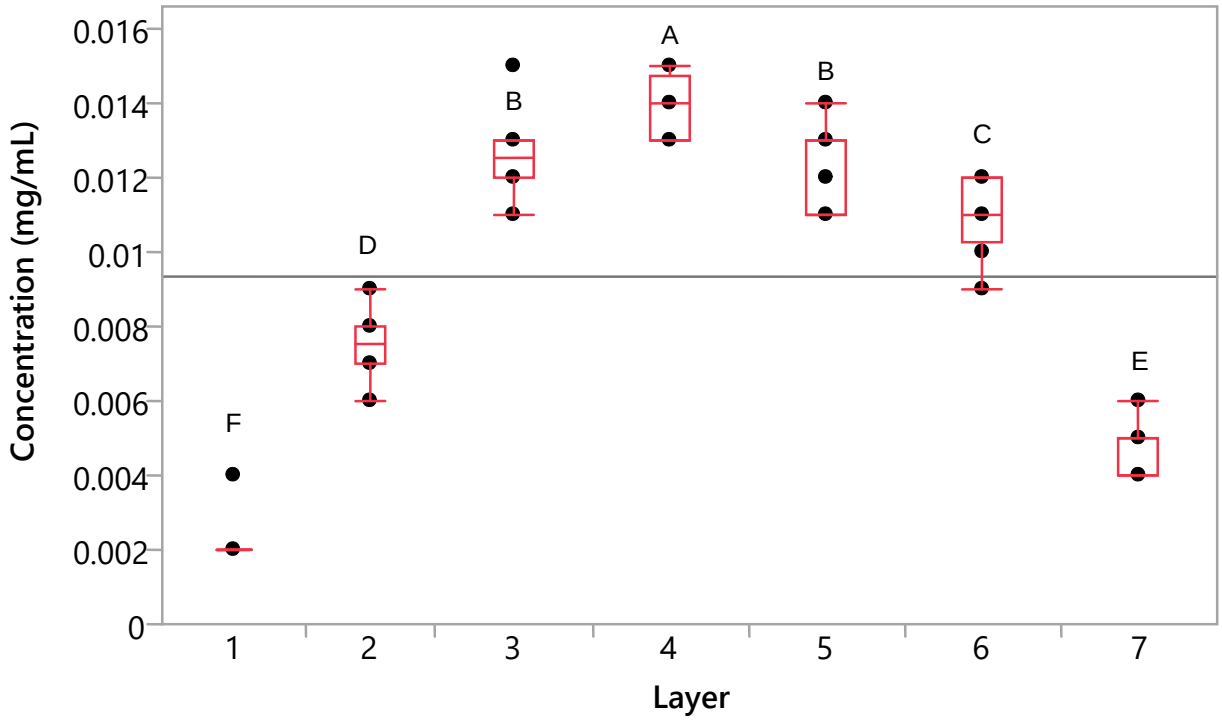


**Figure 84.** Vanillic acid extracted from each charred layer.

Charred layer 4 extracted more vanillic acid than all other layers. Charred layers 3 and 5 had more than layer 1,2, 6, and 7. Charred layer 6 had more vanillic acid extracted than layers 1, 2 and 7. Charred layer 2 had more vanillic acid than layers 1 and 7. Charred layer 7 had more than layer 1 (Fig. 84). A Welch test was run on individual charred stave layers and was found to have at least one significant difference

(p<0.0001). Results show that inner layers had a higher availability of VA than outer most layers such as charred layers 1 and 7.

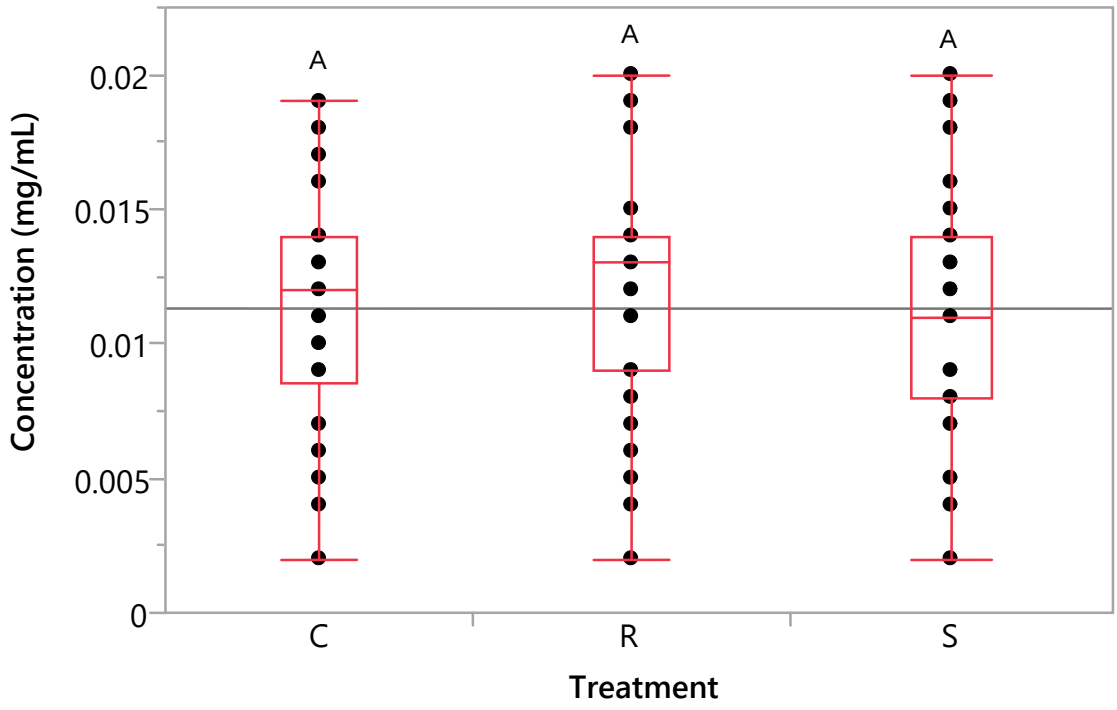


**Figure 85.** Vanillic acid extracted by each extraction treatment.

There is no significant difference (α=0.05) in the amount of vanillic acid extracted among extraction treatments (Fig. 85). Results show that extraction treatment had no effect on the extraction of VA.

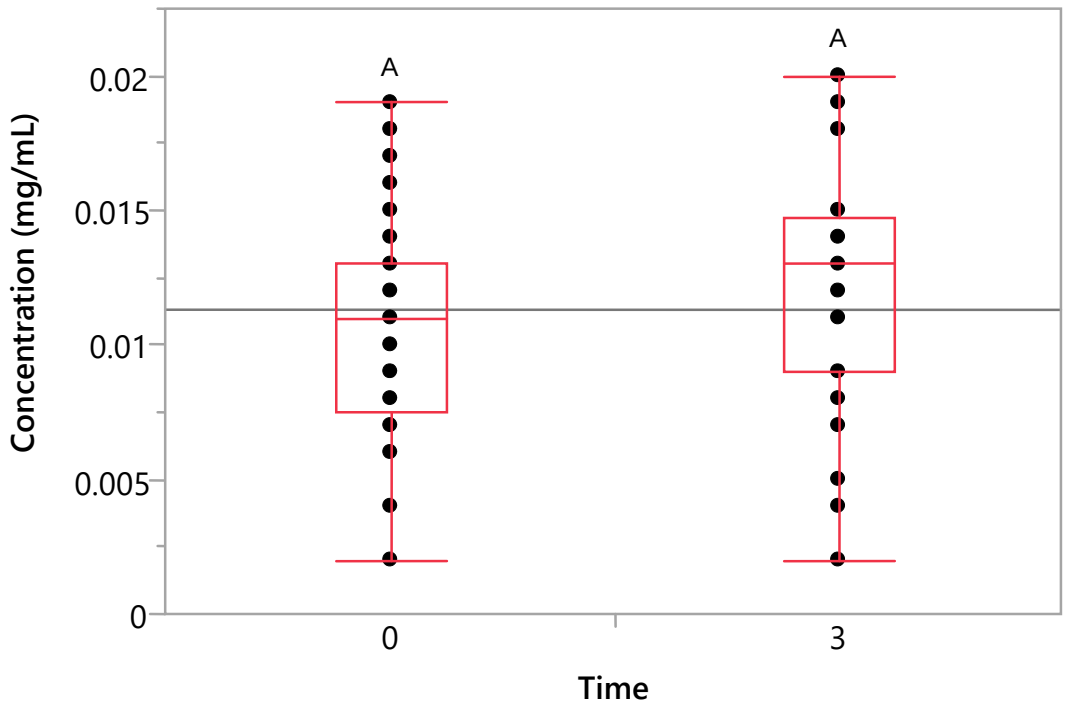


**Figure 86.** Vanillic acid extracted over time.

There was no significant difference (α=0.05) in the amount of vanillic acid extracted over time (Fig. 86). Results show that time had no effect on the extraction of VA.

Toasted staves for all extraction treatments, reflux, sonication, and control, had more vanillic acid extracted than charred staves for all extraction treatments (Fig. 87). A Welch test was run on extraction treatments within each heat treatment and was found to have at least one significant difference ($p<0.0001$). Results show that extraction treatment had no effect on the extraction of VA from toasted and charred staves; however, heat treatment of both charred and toasted staves did have an effect on the extraction of VA (Fig. 82 and 85).

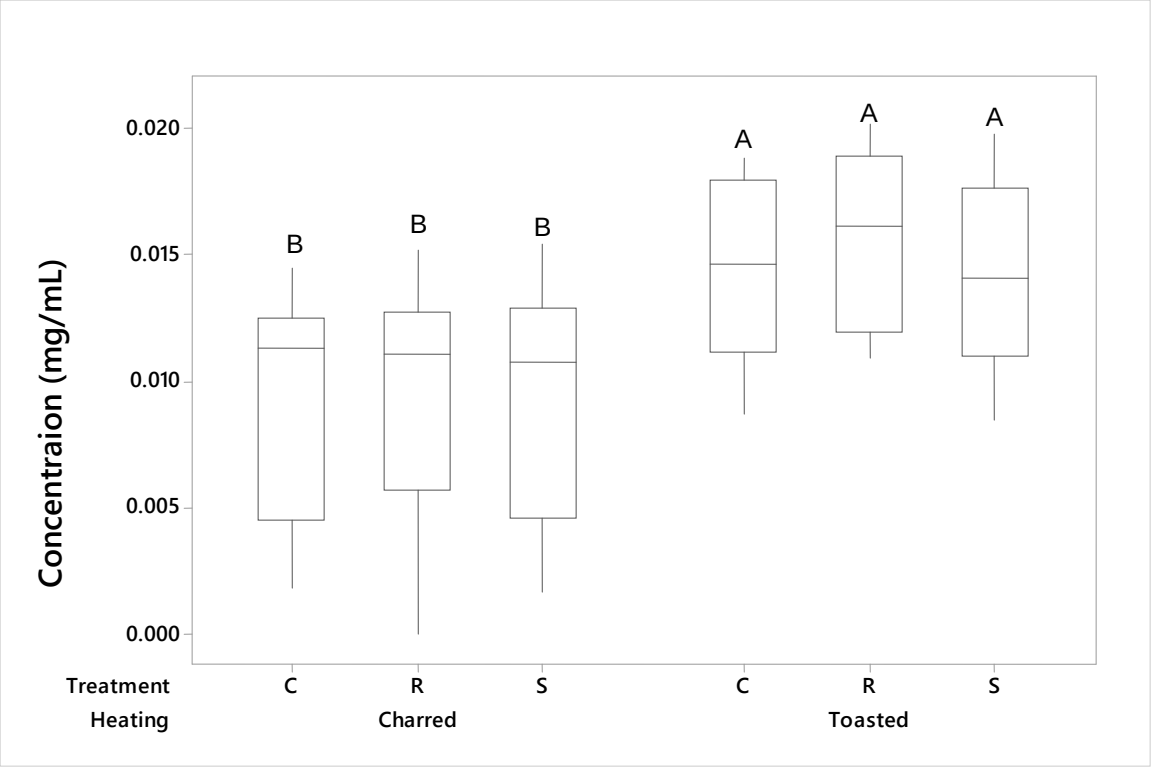


**Figure 87.** Vanillic acid extracted by each extraction treatment within each heat treatment.

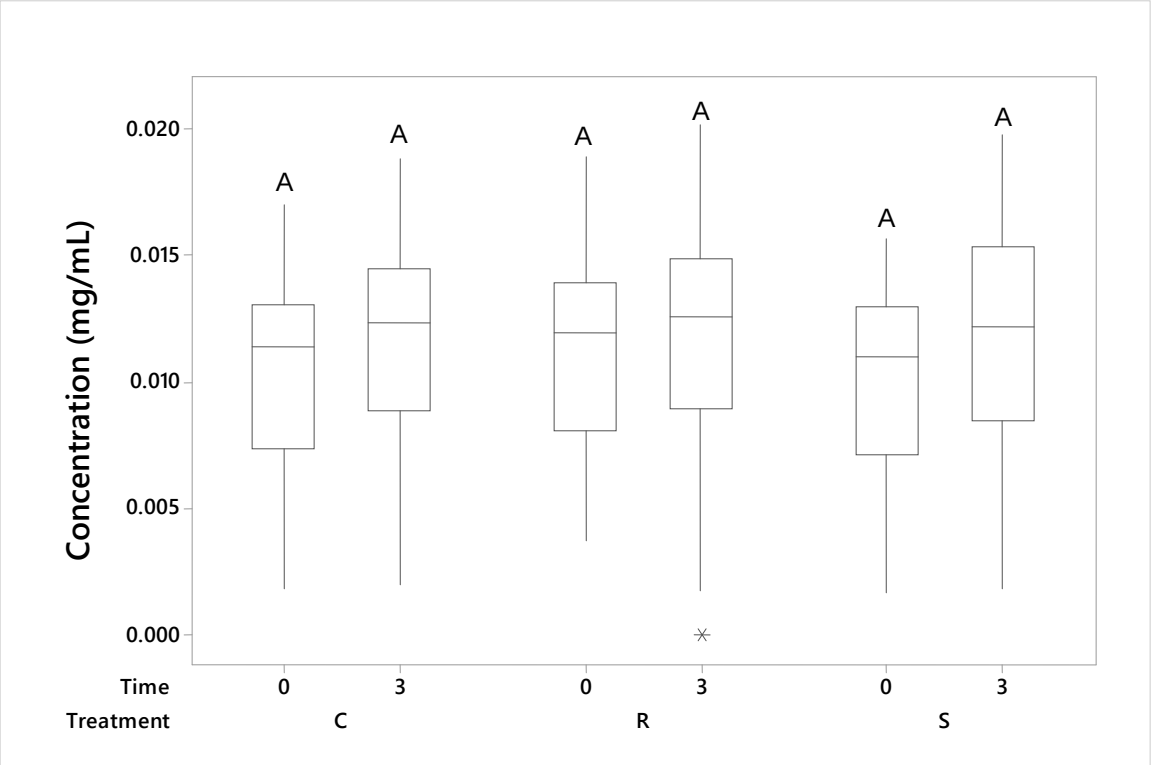


**Figure 88.** Vanillic acid extracted by each extraction treatment over time.

There was no significant difference between extraction treatments over time (Fig. 88). Results show that because time and extraction treatments were both found to be not significant (α=0.05), there interaction also had no effect on the extraction of VA (Fig. 85 and 86).

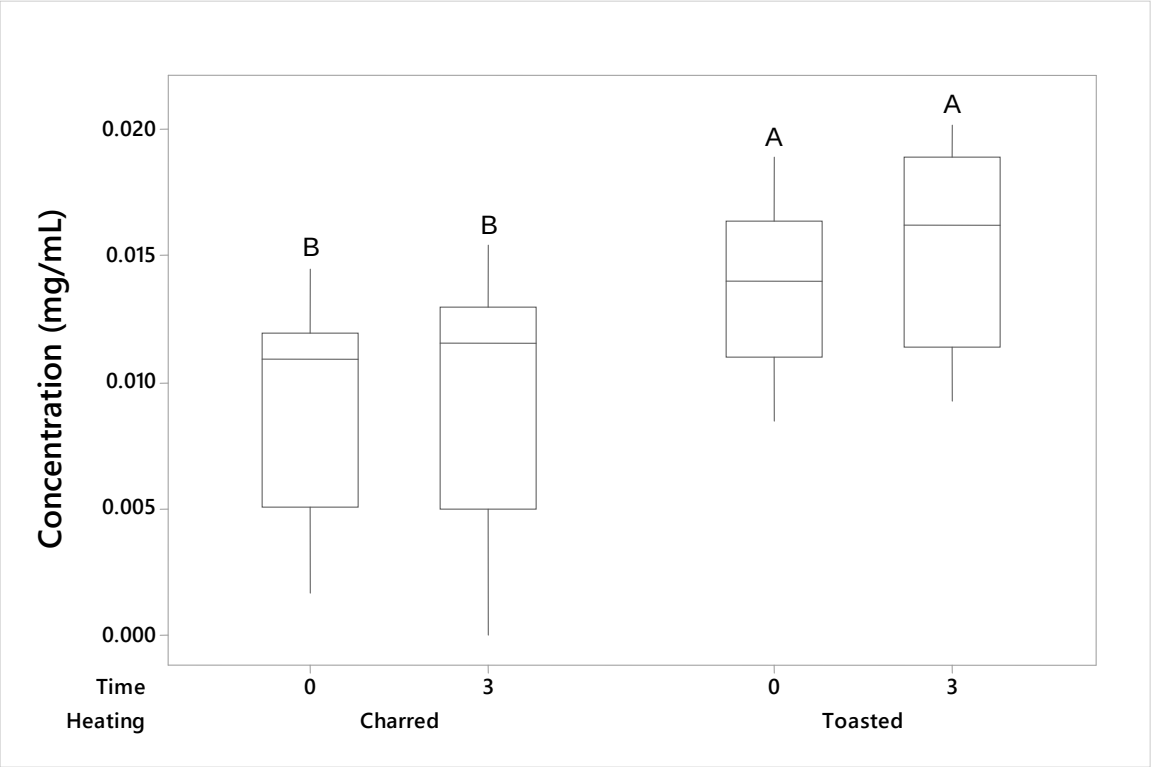


**Figure 89.** Vanillic acid extracted from each heat treatment over time.

Toasted staves at time 0 and after 3 months extracted more vanillic acid than charred staves at time 0 and after 3 months (Fig. 89). A Welch test was run on heat treatments over time and was found to have at least one significant difference ($p<0.0001$). Results show that heat treatment of staves did have an effect on the extraction of VA, but that time did not (Fig. 82 and 86).

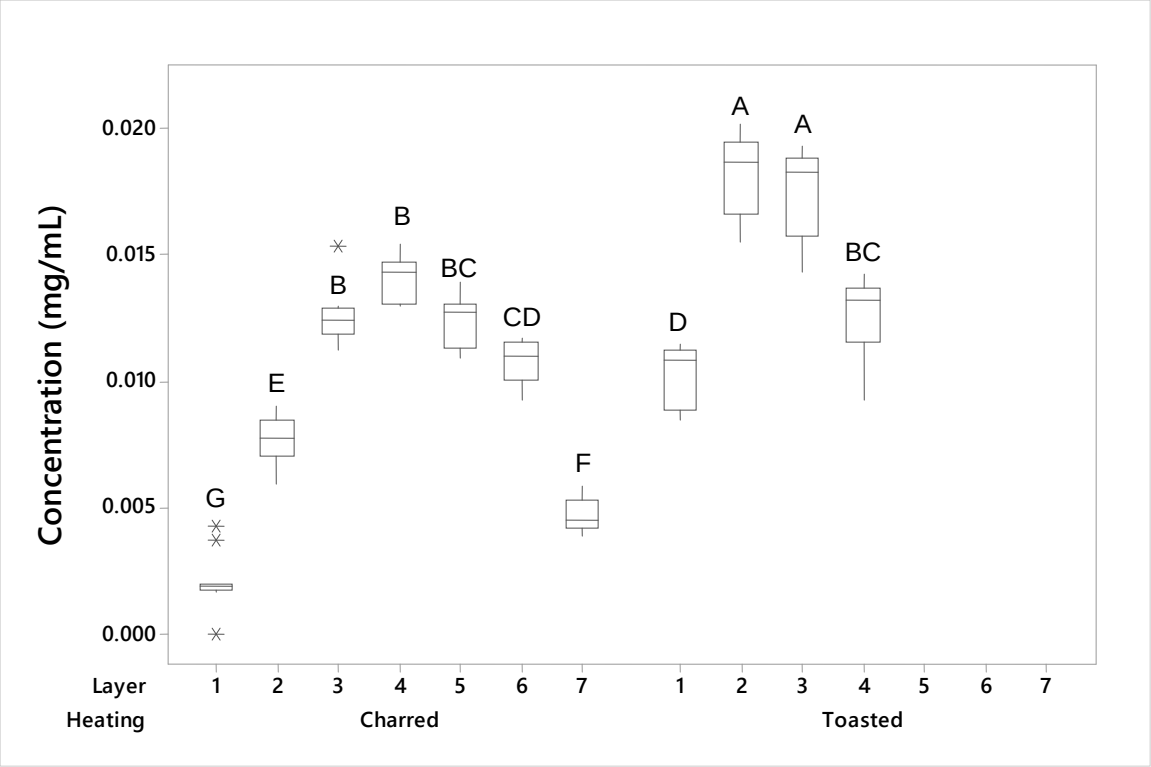


**Figure 90.** Vanillic acid extracted from each layer within each heat treatment.

Toasted layer 2 and 3 had more vanillic acid extracted than charred layers 1 through 7 and toasted layers 1 and 4. Charred layers 3 and 4 had a similar amount of vanillic acid to charred layer 5 and 6 and toasted layer 4, but had more than toasted layer 1 and charred layers 1, 2, and 7. Toasted layer 4 and charred layer 5 had a similar amount to charred layer 6, but had more than toasted layer 1 and charred layers 1, 2, and 7. Charred layer 6 had a similar amount of vanillic acid to toasted layer 1, but had more than charred layers 1, 2, and 7. Toasted layer 1 had more vanillic acid extracted than charred layers 1, 2, and 7. Charred layer 2 had more vanillic acid extracted than charred layers 1 and 7. Charred layer 7 had more vanillic acid extracted than charred

layer 1 (Fig. 90). A Welch test was run on individual layers within each heat treatment and was found to have at least one significant difference ($p<0.0001$).

Results show that inner layers had more VA extracted from both toasted and charred staves than outer layers.

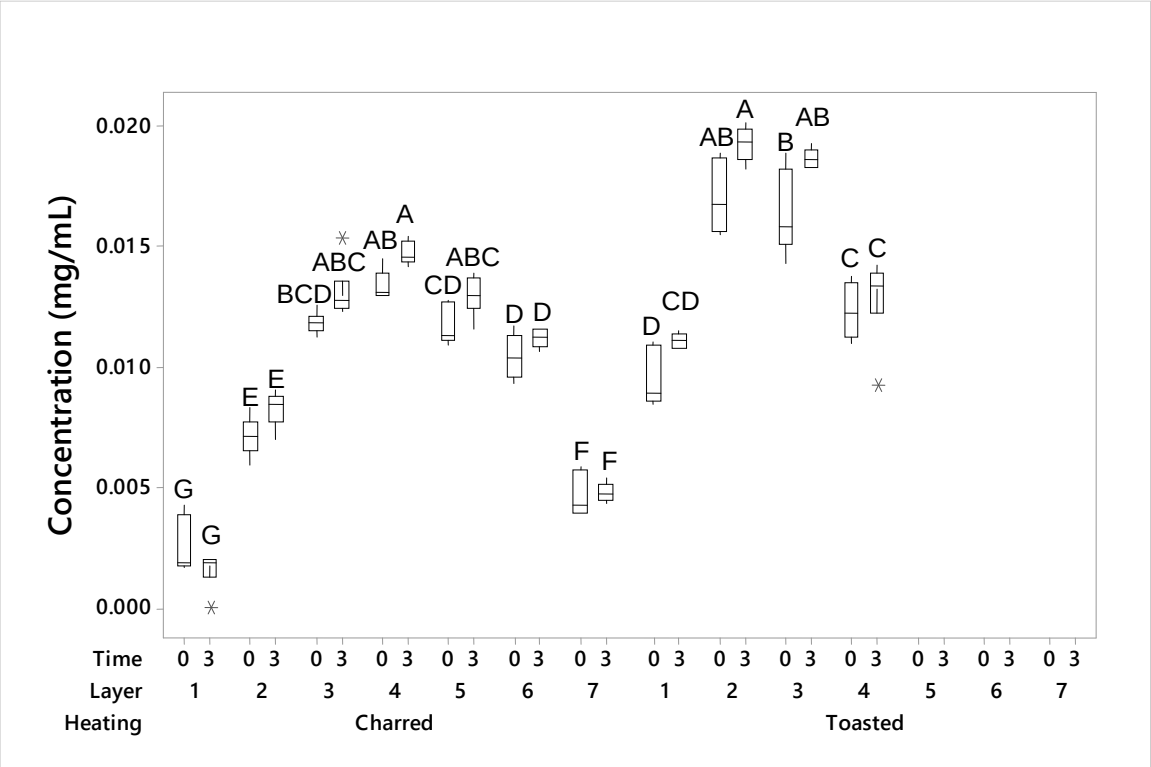


**Figure 91.** Vanillic acid extracted from each layer over time.

Charred layer 4 after 3 months had more vanillic acid than charred layers 1, 2, 3, 5, 6, and 7 at time 0 and charred layers 1, 2, 6, and 7 after 3 months, but had a similar amount to charred layer 4 at time 0 and charred layers 3 and 5 after 3 months. Charred layer 4 at time 0 had more vanillic acid than charred layers 1, 2, 5, 6, and 7 at time 0 and charred layers 1, 2, 6, and 7 after 3 months, but had a similar amount to charred

layer 3 at time 0. Charred layer 3 at time 0 had more vanillic acid than charred layers 1, 2, and 7 at time 0 and after 3 months, but had a similar amount to charred layers 5 and 6 at time 0 and charred layer 6 after 3 months. Charred layer 5 at time 0 had more vanillic acid than charred layers 1, 2, and 7 at time 0 and after 3 months, but had a similar amount to charred layer 6 at time 0 and after 3 months. Charred layer 6 at time 0 and after 3 months had more vanillic acid than charred layers 1, 2, and 7 at time 0 and after 3 months. Charred layer 2 at time 0 and after 3 months had more vanillic acid than charred layers 1 and 7 at time 0 and after 3 months. Charred layer 7 at time 0 and after 3 months had more vanillic acid than charred layer 1 at time 0 and after 3 months. Charred layer 1 at time 0 and after 3 months had a similar amount of vanillic acid (Fig. 91). Toasted layer 2 after 3 months had more vanillic acid than toasted layers 1, 3, and 4 at time 0 and toasted layers 1 and 4 after 3 months, but had a similar amount to toasted layer 2 at time 0 and toasted layer 3 after 3 months. Toasted layer 2 at time 0 and toasted layer 3 after 3 months had more vanillic acid than toasted layers 1 and 4 at time 0 and after 3 months, but had a similar amount to toasted layer 3 at time 0. Toasted layer 3 at time 0 had more vanillic acid than toasted layers 1 and 4 at time 0 and after 3 months. Toasted layer 4 at time 0 and after 3 months had more vanillic acid than toasted layer 1 at time 0, but had a similar amount to toasted layer 1 after 3 months. Toasted layer 1 after 3 months had a similar amount of vanillic acid to toasted layer 1 at time 0 (Fig. 91). A Welch ANOVA deteremined that at least one value was significantly different ($p<0.0001$).

Results show that there was no significant difference (α=0.05) in the extraction of VA from individual layers over time. For example, layer 3 after 3 months had the same amount of VA extracted at time 0. However for toasted oak stave layers, a Welch ANOVA deteremined that for all toasted layer variances, at least one value was significantly different ($p<0.0001$).

### 3.5.5. Syringealdehyde

Significant differences (α=0.05) were found among toasted stave layers (Fig. 93), for heat treated charred staves (Fig. 94), between time 0 and after 3 months (Fig. 96), among extraction treatments over time (Fig.98), between heat treated staves over time (Fig.99), among layers for charred and toasted staves (Fig. 100), among layers over time (Fig. 101). However, there was no significant difference found between heat treated charred and toasted staves (Fig. 92), across reflux, sonication, and control extraction treatments (Fig.95), and between heat treated staves among extraction treatments (Fig.97).

There was no significant difference (α=0.05) in the amount of syringaldehyde extracted from charred and toasted staves (Fig. 92). Results show that heat treatment had no effect on the amount of syringaldehyde extracted.

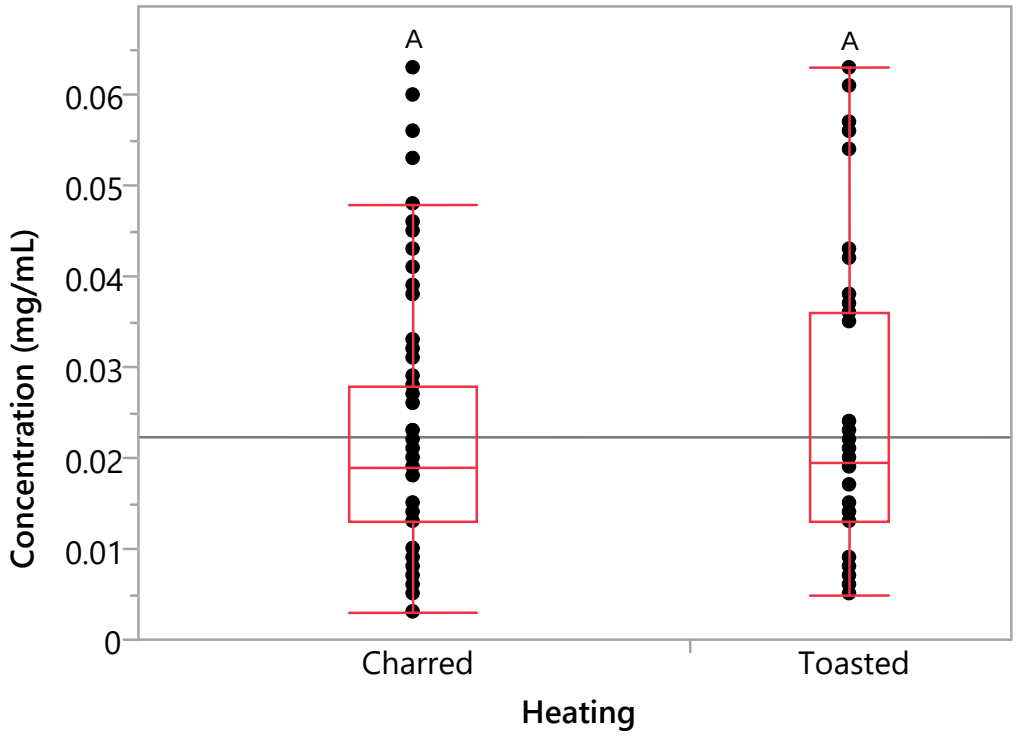


**Figure 92.** Syringaldehyde extracted from charred and toasted staves.

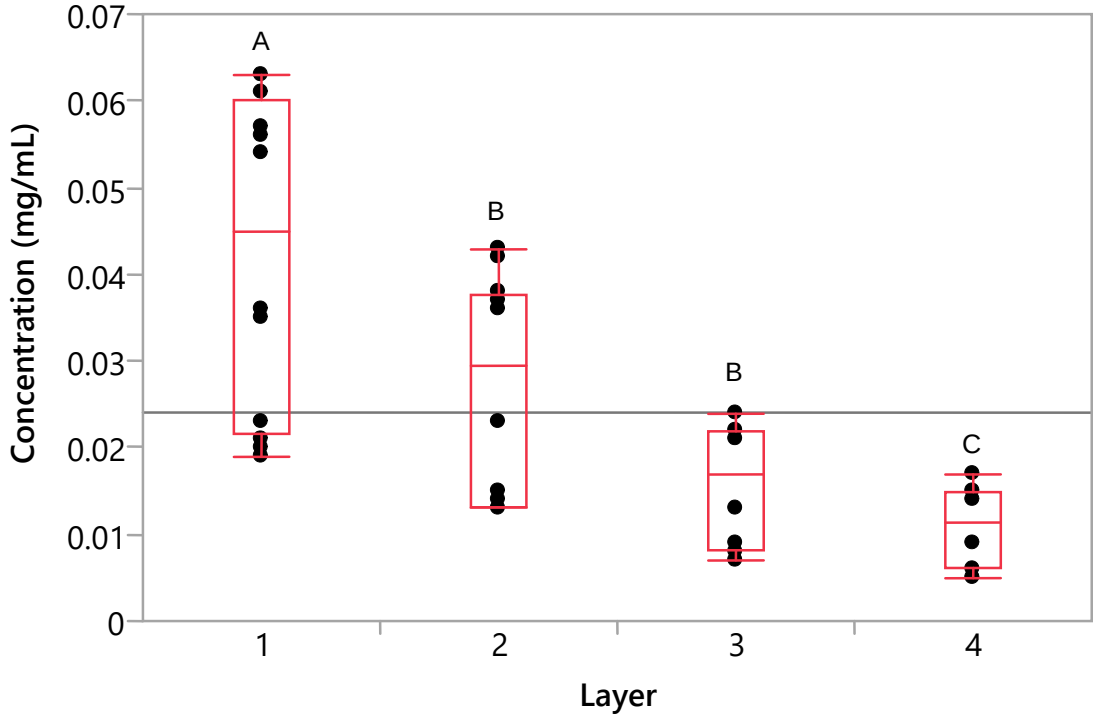


**Figure 93.** Syringaldehyde extracted from each toasted layer.

Toasted layer 1 had the most syringealdehyde extracted. Toasted layer 2 had a similar amount of syringealdehyde extracted to layer 3, but had more than layer 4. Layer 3 had a similar amount to layer 4 (Fig. 93). A Welch test was run on individual toasted stave layers and was found to have at least one significant difference ($p<0.0001$). Results show that the directly heat treated layer, toasted layer 1, had a higher amount of syringealdehyde extracted than other layers. This indicates that syringealdehye is found in higher amounts from more heat treated layers.

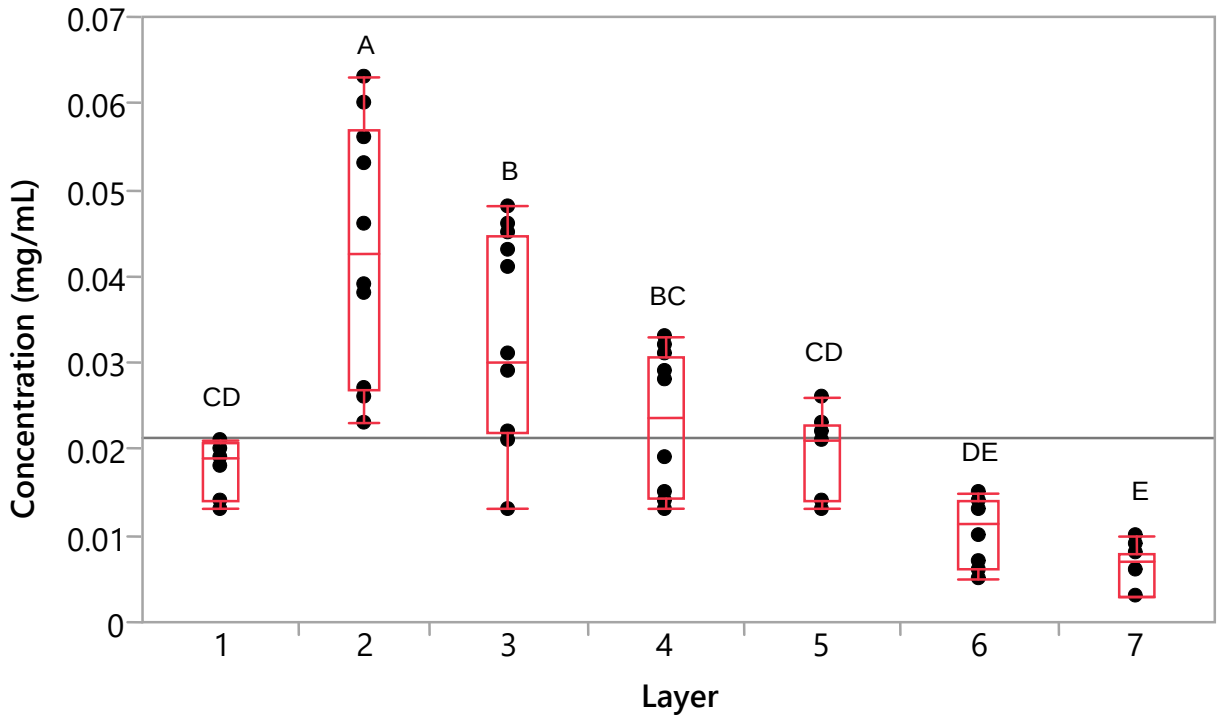


**Figure 94.** Syringaldehyde extracted from each charred layer.

Charred layer 2 had the most syringaldehyde extracted than all other layers. Charred layer 3 had a similar amount extracted to layer 4, but had more than charred layers 1, 5, 6, and 7. Charred layer 4 had a similar amount to charred layers 1 and 5, but had more than charred layers 6 and 7. Charred layers 1 and 5 had a similar amount

of syringealdehyde extracted to charred layer 6, but had more than charred layer 7. Charred layer 6 had a similar amount to charred layer 7 (Fig. 94). A Welch test was run on individual charred stave layers and was found to have at least one significant difference ($p<0.0001$). Results show that charred layers closer to direct heat treatment, charred layers 2 and 3, had a higher availability of syringealdehyde than other layers.

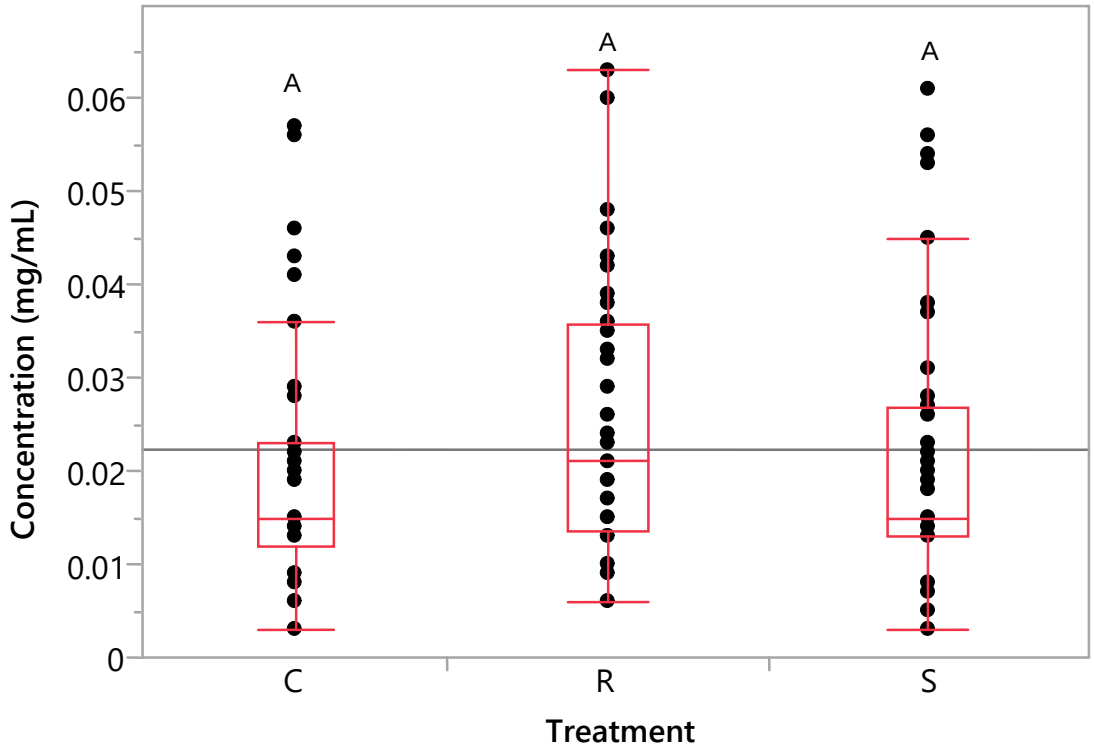


**Figure 95.** Syringaldehyde extracted by each extraction treatment.

There was no significant difference ($\alpha=0.05$) in the amount of syringaldehyde extracted from reflux, sonication, or control extraction methods (Fig. 95). Results show that extraction treatment had no effect on the amount of syringealdehyde extracted from oak staves.

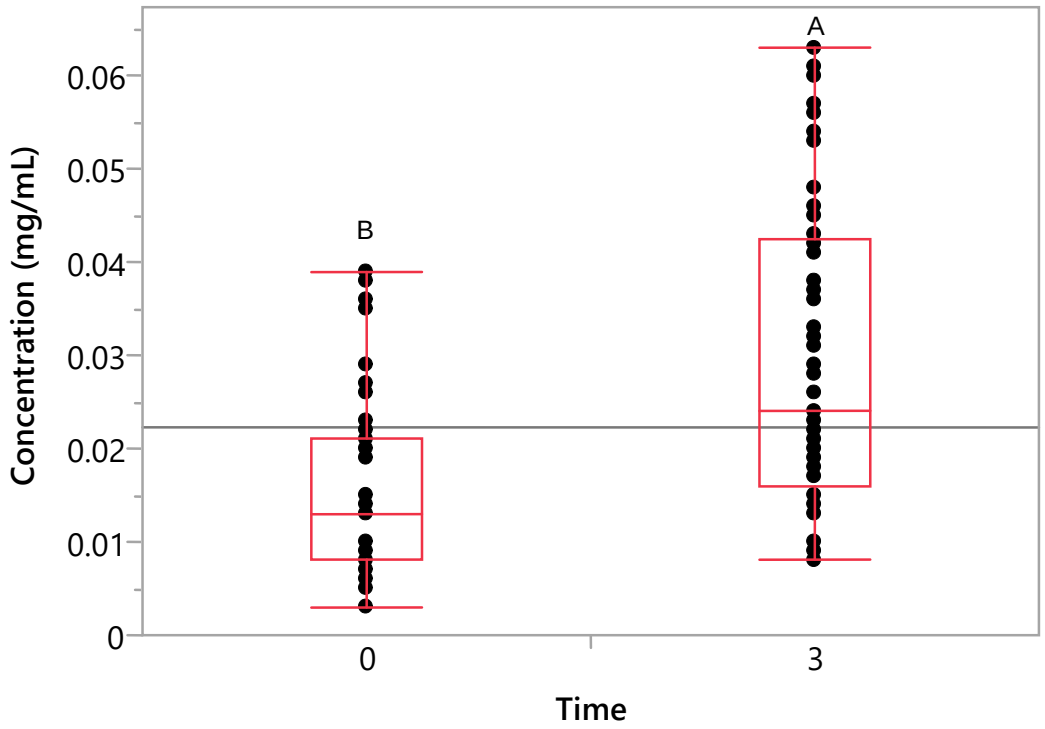


**Figure 96.** Syringealdehyde extracted over time.

More syringealdehyde was extracted after 3 months than at time 0 (Fig. 96). A Welch test was run on over time and was found to have at least one significant difference ($p<0.0001$). Results show that time had an effect on the amount of syringealdehyde extracted.

There was no significant difference ($\alpha=0.05$) between charred and toasted staves for all extraction treatments, reflux, sonication, and control (Fig. 97). Results show that heat treatment and extraction treatments had no effect on the extraction of syringealdehyde from oak staves.

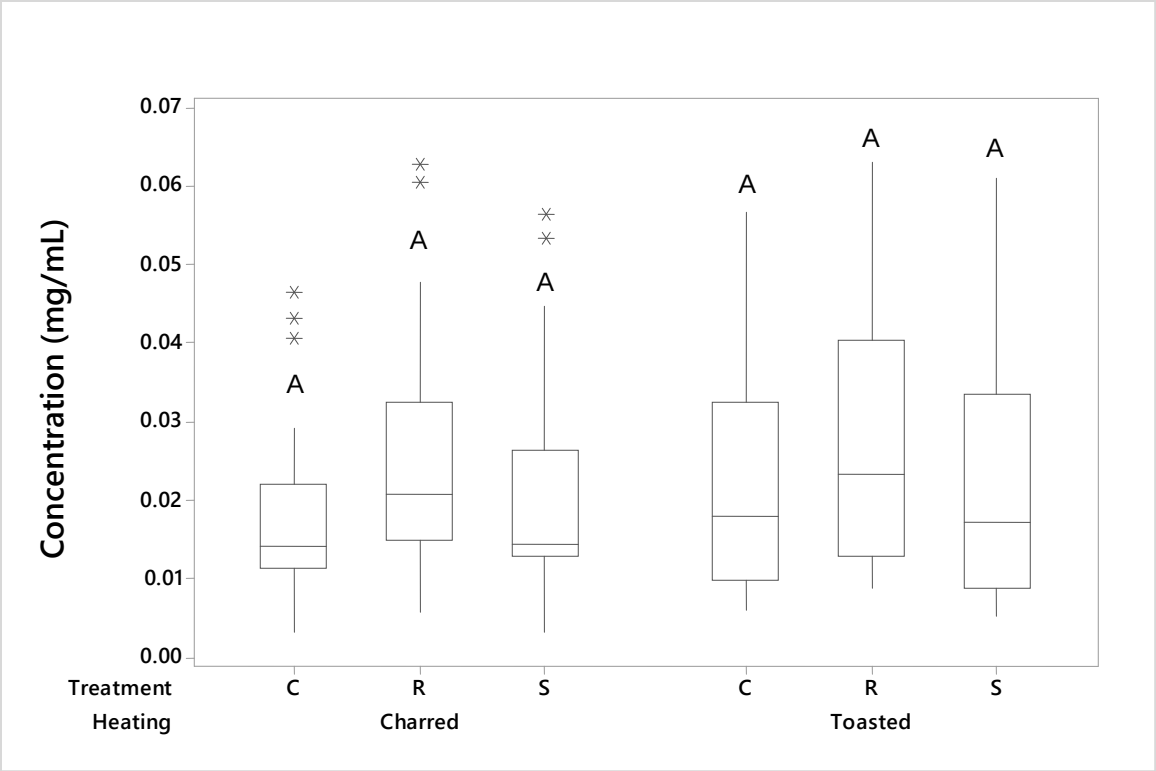


**Figure 97.** Syringealdehyde extracted by each extraction treatment within each heat treatment.

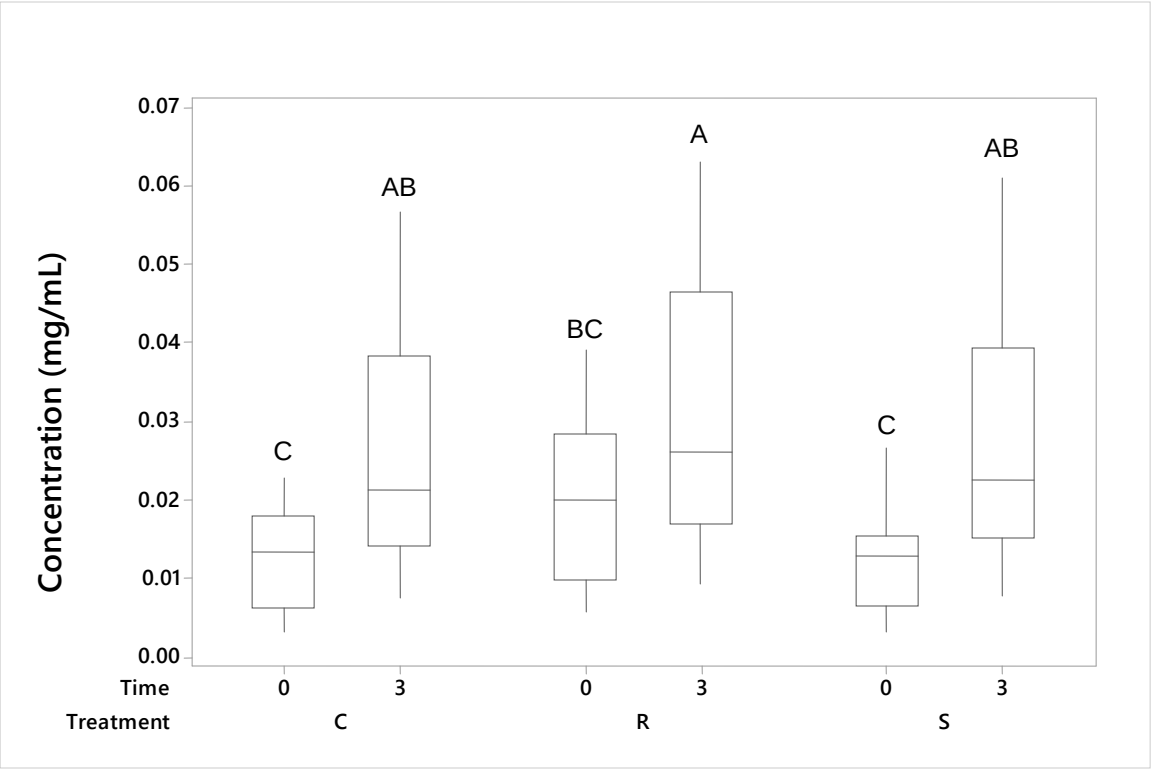


**Figure 98.** Syringealdehyde extracted by each extraction treatment over time.

Reflux extraction treatment extracted more syringealdehyde after 3 months than reflux, control, and sonication at time 0, but was similar to the amount extracted by sonication and control extraction treatments after 3 months. Sonication and control extraction treatments after 3 months extracted more than they did at time 0, but were similar to reflux extraction treatment at time 0. Reflux at time 0 extracted a similar amount to sonication and control extraction treatments. Sonication and control extraction treatments at time 0 extracted the same amount of syringealdehyde (Fig. 98). A Welch test was run on extraction treatments over time and was found to have at least one significant difference ($p<0.0001$).

Results show that extraction treatments all extracted a similar amount of syringealdehyde for time 0 and after 3 months.

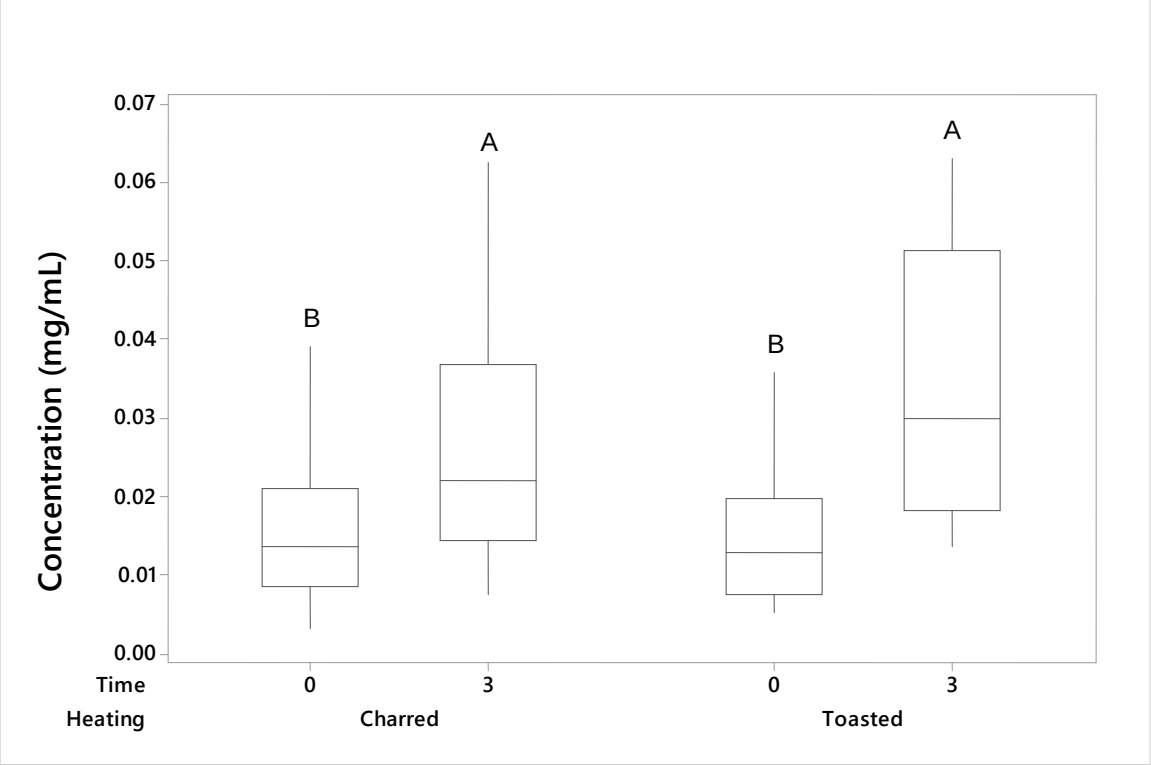


**Figure 99.** Syringealdehyde extracted by each heat treatment over time.

Toasted staves after 3 months extracted the same amount of syringealdehyde to charred staves after 3 months, and both toasted and charred staves after 3 months had more than charred and toasted staves at time 0 (Fig. 99). A Welch test was run on heat treatments over time and was found to have at least one significant difference ($p<0.0001$).

Results show charred and toasted oak staves did not significantly differ ($\alpha=0.05$) in the extraction of syringealdehyde at time 0 or after 3 months. However, the amount of syringealdehyde increased after 3 months for both charred and toasted staves.

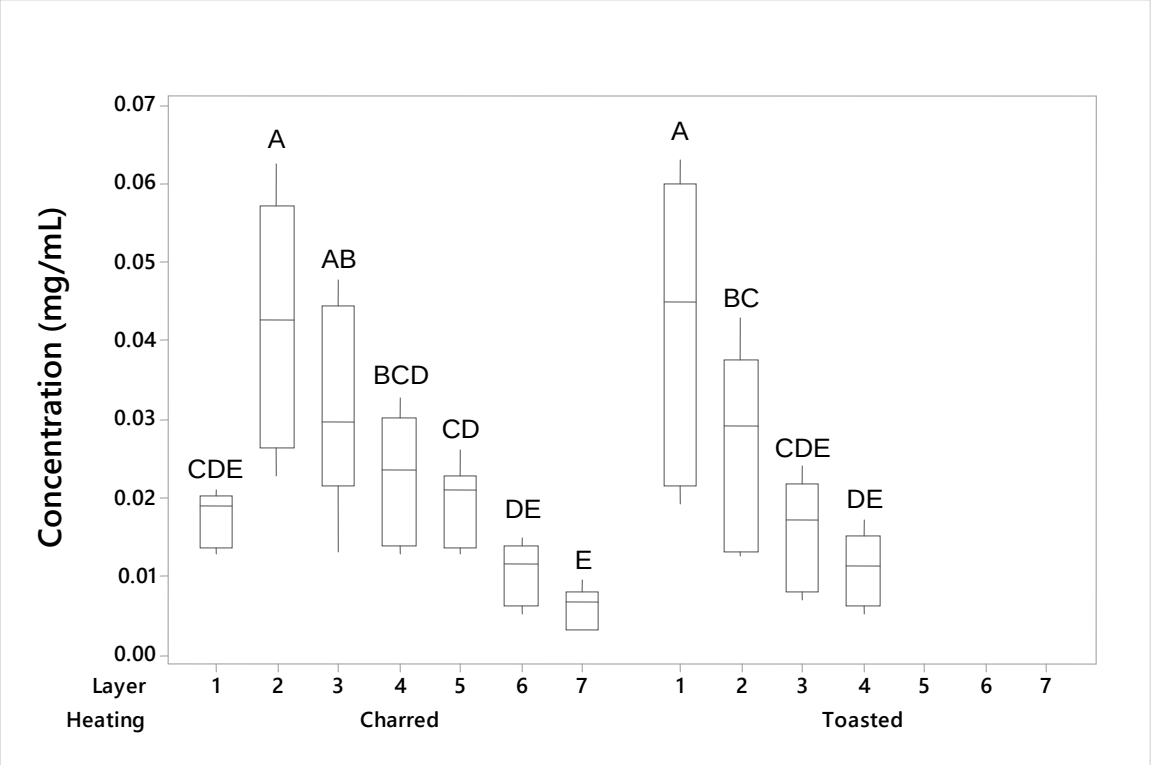


**Figure 100.** Syringealdehyde extracted from each layer within each heat treatment.

Toasted layer 1 and charred layer 2 had a higher amount of syringealdehyde than toasted layers 2, 3, and 4 and charred layers 1, 4, 5, 6, and 7, and were similar to

charred layer 3. Charred layer 3 had a similar amount of syringealdehyde extracted to toasted layer 2 and charred layer 4, but had more than charred layers 1, 5, 6, and 7 and toasted layers 3 and 4. Toasted layer 2 had more syringealdehyde than toasted layer 4 and charred layers 6 and 7, but had a similar amount to charred layers 1, 4, and 5 and toasted layer 3. Charred layer 4 had more syringealdehyde than charred layer 7, but had a similar amount to charred layers 1, 5, and 6 and toasted layers 3 and 4. Charred layer 5 had more syringealdehyde than charred layer 7, but had a similar amount to charred layers 1 and 6 and toasted layers 3 and 4. Charred layer 1 had a similar amount of syringealdehyde to charred layers 6 and 7 and toasted layers 3 and 4. Toasted layer 3 had a similar amount to toasted layer 4 and charred layers 6 and 7. Toasted layer 4 and charred layer 6 had a similar amount to charred layer 7 (Fig. 100). A Welch test was run on individual layers within each heat treatment and was found to have at least one significant difference ($p<0.0001$).

Results show that lightly heat treated layers, toasted layer 1 and charred layers 2 and 3, had the most syringealdehyde available for extraction.

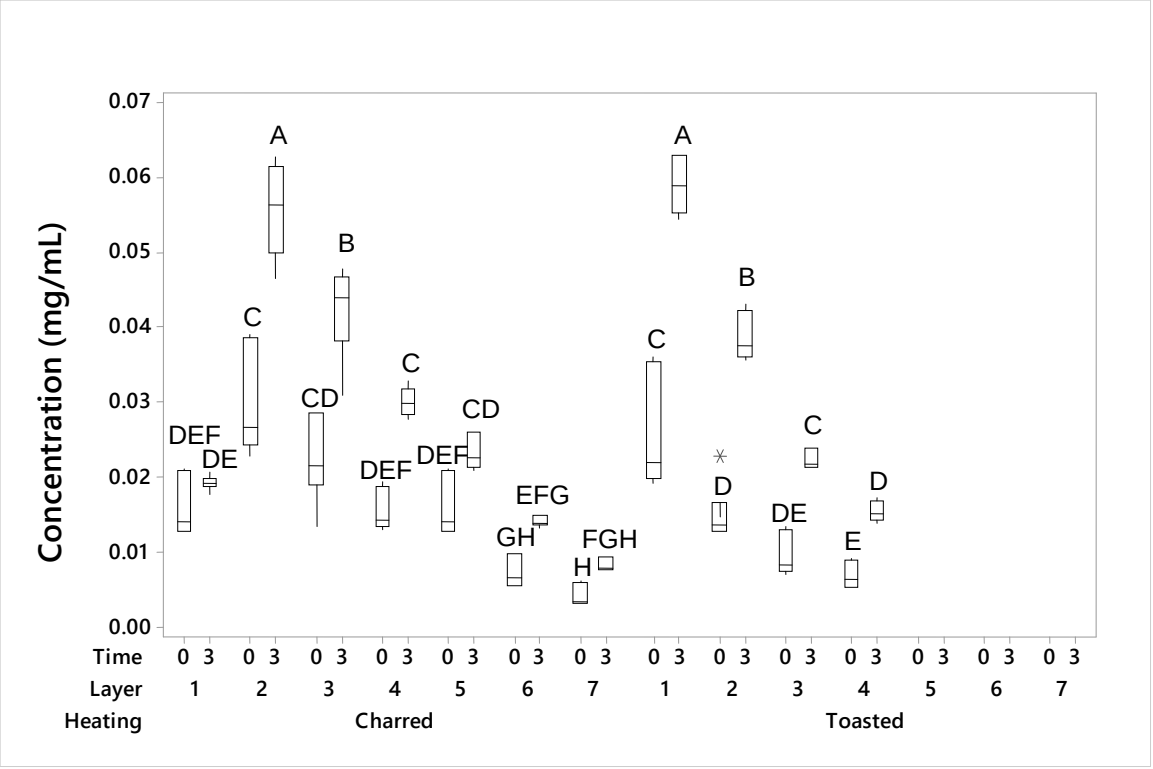


**Figure 101.** Syringealdehyde extracted from each layer over time.

Charred layer 2 after 3 months had more syringealdehyde than charred layers 1 through 7 at time 0 and charred layers 1, 3, 4, 5, 6, and 7 after 3 months. Charred layer 3 after 3 months had more syringealdehyde than charred layers 1 through 7 at time 0 and charred layers 1, 4, 5, 6, and 7 after 3 months. Charred layer 2 at time 0 and charred layer 4 after 3 months had more syringealdehyde than charred layers 1, 4, 5, 6, and 7 at time 0 and charred layers 1, 6, and 7 after 3 months, but had a similar amount to charred layer 3 at time 0 and charred layer 5 after 3 months. Charred layer 3 at time 0 and charred layer 5 after 3 months had more syringealdehyde than charred layers 6 and 7 at time 0 and after 3 months, but had a similar amount to charred layers 1, 4, and 5 at time 0 and charred layer 1 after 3 months. Charred layer 1 after 3 months had more

syringaldehyde than charred layer 6 and 7 at time 0 and charred layer 7 after 3 months, but had a similar amount to charred layers 1, 4, and 5 at time 0 and charred layer 6 after 3 months. Charred layers 1, 4, and 5 at time 0 had more syringealdehyde than charred layers 6 and 7 at time 0, but had a similar amount to charred layers 6 and 7 after 3 months. Charred layer 6 after 3 months had more syringealdehyde than charred layer 7 at time 0, but had a similar amount to charred layer 6 at time 0 and charred layer 7 after 3 months. Charred layer 7 after 3 months had a similar amount of syringealdehyde to charred layers 6 and 7 at time 0 (Fig. 101). A Welch ANOVA determined that at least one value was significantly different ($p<0.0001$). Toasted layer 1 after 3 months had more syringealdehyde than toasted layers 1 through 4 at time 0 and toasted layer 2 through 4 after 3 months. Toasted layer 2 after 3 months had more syringealdehyde than toasted layers 1 through 4 at time 0 and toasted layers 3 and 4 after 3 months. Toasted layer 1 at time 0 and toasted layer 3 after 3 months had more syringealdehyde than toasted layers 2 through 4 at time 0 and toasted layer 4 after 3 months. Toasted layer 2 at time 0 and toasted layer 4 after 3 months had more syringealdehyde than toasted layer 4 at time 0, but had a similar amount to toasted layer 3 at time 0. Toasted layer 3 at time 0 had a similar amount of syringealdehyde to toasted layer 4 at time 0 (Fig. 101). A Welch ANOVA determined that at least one value was significantly different ($p<0.0001$).

Results show that layers closer to direct heat treatment had more syringealdehyde available for extraction after 3 months than at time 0.

# 4. CONCLUSIONS AND RECOMMENDATIONS

## 4.1. Conclusions

The primary objective of this study was to determine if high intensity ultrasound could be used as an alternative, accelerated extraction method which could be utilized in the production of an oak extract to be used for aging wines and whiskies and to compare it to samples extracted by reflux and room-temperature controls. Secondary objectives of this study were to compare the heat treatment of charred and toasted staves, their individual layers, and whether time had an effect on the extraction of color, total soluble phenolics, pH level, and oak compounds identified by gas and liquid chromatography from extraction treatments, heat treated oak staves, and individual oak stave layers.

Reflux extraction treatment was found to be the best method for extracting color, total soluble phenolics, and ellagic acid, while both reflux and control extraction treatments extracted a similar amount of sinapaldehyde; However, the interaction between heat treatment and extraction treatments shows that reflux extracted a similar amount of sinapaldehyde to both the control and sonication extraction treatments from charred oak staves showing that heat treatment of oak staves had more of an effect on the amount of sinapaldehyde than extraction treatment. Reflux extraction treatment used a higher temperature than room-temperature sonication and control treatments. A higher temperature helped to break apart cell walls for the release of more oak compounds darkening the sample color, increasing the amount of total soluble phenolics, and the amount of ellagic acid extracted (Russell, 2003).

Toasted staves had higher amounts of extracted compounds furaldehyde, vanillin, sinapaldehyde, protocatechuic acid, and vanillic acid compared to charred staves. Toasted staves undergo a milder heat treatment than charred oak staves. Because of this, there is a higher availability of oak compounds in toasted staves than in charred (Russell, 2003).

Individual layers from both charred and toasted staves had significant differences in the amount of furaldehyde, vanillin, sinapaldehyde, protocatechuic acid, vanillic acid, and syringealdehyde extracted, while only charred stave layers had significant differences in the extraction of color, total soluble phenolics, pH level, and ellagic acid. The differences of extraction between charred and toasted stave layers depend on the depth of heat treatment. Charred layer 1, for example, is used to bind off-flavor compounds on its carbon layer and does not release as many compounds as inner-layers such as charred layers 2 and 3. Toasted staves go through a milder heat treatment and therefore, have a higher availability of oak compounds for extraction (Russell, 2003).

The amount of time for oak compound extraction was found to be better after 3 months for color intensity, total soluble phenolics, pH level, and for the extraction of vanillin and syringealdehyde. Samples developed more color and reduced the pH level due to the further release of total soluble phenolic compounds from lignin degradation (Zelinka and Stone, 2011). Both vanillin and syringealdehyde are formed from the degradation of lignin during aging indicating that time was a factor in the degradation of

lignin, but was not a factor in the degradation of ellagitannins which were more readily available at time 0.

### 4.2. Interactions

The interaction between charred and toasted heat treated staves and extraction treatments were significantly different for color, total soluble phenolics, and the extraction of vanillin, sinapaldehyde, protocatechuic acid, ellagic acid, and vanillic acid. Reflux was found to extract the most color, total soluble phenolics, vanillin, and ellagic acid for both charred and toasted staves which can be attributed to a high temperature during extraction. However, only heat treatment had an effect on the extraction of protocatechuic acid from charred and toasted staves and not extraction treatment indicating that the formation of protocatechuic acid occurs during the heating process. Sinapaldehyde was extracted more by reflux and control extraction treatments from toasted staves while it was equally extracted by all extraction methods from charred staves.Like protocatechuic acid, sinapaldehyde is also formed during heat treatment/seasoning process. Vanillic acid was extracted more from toasted staves than charred staves for all extraction treatments.  Charred heat treated staves go through a higher heat treatment process which degrades more oak cells thus limiting the amount of vanillin available for extraction compared to toasted staves.

The interaction between extraction treatments and time saw a significant difference for treatments between time 0 and time 3 for color, pH, ellagic acid, and syringealdehyde. For color, reflux extracted a darker color than sonicated and control extraction treatments for time 0 and after 3 months. However, both sonicated and

control extraction treatments increased in color after 3 months due to the release of more oak compounds. Similarly, pH after 3 months became lower than at time 0 indicating the release of phenolic acids, such as ellagic acid. Reflux extraction treatment extracted more ellagic acid at time 0 than after 3 months when compared to all extraction treatments at both time 0 and after 3 months. The higher availability of ellagic acid at time 0 is indicative of the initial degradation of ellagitannins. Lignin derived compounds, such as syringealdehyde, appear over time as degradation of lignin is a longer process than the degradation of ellagitannin (Simón et al., 1999). Extraction treatment had no effect on the extraction of syringealdehyde because the amount was similar for all extraction treatments after 3 months indicating that degradation of lignin due to time was the only factor for the extraction of syringealdehyde in this interaction. Time was a factor for extraction treatments due to the further release of oak compounds increasing color and amount of total soluble phenolics and reducing pH levels.

Degradation of ellagitannins and lignins in heat treated staves over time helped increase color intensity, total soluble phenolics, amount of vanillin, sinapaldehyde, protocatechuic acid, vanillic acid, and syringealdehyde, and reduce pH levels. Toasted and charred staves after 3 months had a darker color, more total soluble phenolics, lower pH, and more syringealdehyde than at time 0 indicating further oak wood degradation. Toasted staves at both time 0 and after 3 months had more vanillin, sinapaldehyde, protocatechuic acid, and vanillic acid extracted than from charred staves at time 0 and after 3 months. The difference in oak compound levels between toasted

and charred staves is due to the milder heat treatment of toasted staves allowing more oak compounds to remain intact for extraction (Russell, 2003).

The interaction between heat treatments among layers was significantly different for color, total soluble phenolics, pH, and the extraction of furaldehyde, vanillin, sinapaldehyde, protocatechuic acid, ellagic acid, vanillic acid, and syringealdehyde. Inner layers of charred and toasted staves had a darker color, more total soluble phenolics, lower pH, and a higher amount of vanillin, sinapaldehyde, protocatechuic acid, ellagic acid, vanillic acid, and syringealdehyde extracted than charred layers 1 and 7. Toasted inner layers had higher levels of vanillin, sinapaldehyde, protocatechuic acid, and ellagic acid than charred inner layers, while charred layer 7 produced a higher amount of furaldehyde than other charred layers and toasted layers. The difference among layers is due to the heat treatment process of charred and toasted staves and the degradation of wood (Russell, 2003). Inner-layers will have a higher availability of oak compounds because of retention of compounds which will be released during aging. Outside layers, such as charred layer 1, has direct heat treatment and will be more degraded than inner-layers limiting the availability of compounds for extraction. Similarly, charred layer 7, which is furthest away from direct heat treatment, is not degraded enough to extract as many compounds as inner-layers. Furthermore, the depth of each layer and the size of the grain must be considered for the extraction of oak compounds (Russell, 2003; Pracomtal et al., 2014).

The interaction among extraction treatments over time was significantly different for color, total soluble phenolics, pH, furaldehyde, vanillin, sinapaldehyde,

protocatechuic acid, vanillic acid, and syringealdehyde. Extraction of color, total soluble phenolics, pH, vanillin, and syringealdehyde among layers was better after 3 months than at time 0 while the extraction of furaldehyde, sinapaldehyde, protocatechuic acid, and vanillic acid had no significant difference over time among layers. Each layer was affected by each heat treatment process and was also affected by time over if it was an ellagitannin or lignin derived compound. For example, lignin derived compounds, such as vanillin, appeared more after 3 months compared to ellagitannin derived compounds, like ellagic acid, which was found in higher amounts initially (Russell, 2003; Viriot et al., 1999).

Overall for each analysis, reflux was found to be the better extraction treatment to sonication and control extraction methods. However, after 3 months, sonication and control methods were not found to be significantly different to reflux for extraction of color, total soluble phenolics, pH, and various oak compounds indicating that both sonication and control methods are viable accelerated extraction treatments. Of the three extraction treatments, sonication is the most controllable method of extraction as the parameters can be altered to produce desired results in the amount of color, total soluble phenolics, pH level, and oak compound extraction.

Toasted staves extracted more oak compounds than charred staves for specific oak compounds. However, for overall color, total soluble phenolics, and pH level, charred and toasted staves equally extracted more after 3 months than at time 0. Individual layers saw no significant difference over time, but did differ between toasted and charred oak staves.

### 4.3. Recommendations

The production of an oak extract to be used in the acceleration of aging of wines and whiskies would depend upon the desirability of compounds in the oak extract. While reflux was found to be a better extraction treatment overall, after 3 months, high intensity ultrasound and control methods were found to be comparable extraction treatments and could similarly be used in the production of an oak extract. However of the three extraction treatments, HIUS extraction treatment is the most controllable method as parameters can easily change depending on the desired level of compound extraction.

Further study must be done on individual compounds and their desirability in order to determine which accelerated method is a more effective and purposeful method for production of an oak extract to be used in accelerating the aging of wines and whiskies. Similarly, the study of individual compounds and their desirability would also help determine whether toasted or charred oak barrels are preferred for aging of wines and whiskies. Many oak compounds are commonly known components of wines and whiskies; however, their levels differ from batch to batch and it is unknown at what level these components become desirable or undesirable. A sensory study introducing variations in the amount of each oak compound found in wines and whiskies could help determine the level of desirability for each oak compound and would provide information towards accelerated extraction methods and heat treatment selection when producing an oak extract to be used wines and whiskies.

# LIST OF REFERENCES

Álamo, M.., Nevares, I., Gallego, L., Martin, C., Merino, S. (2008). Aging markers from bottled red wine aged with chips, staves, and barrels. *Analytica Chimica Acta*. 621, 86-99.

Álamo, M., Nevares, I., Gallego, L., Fernández de Simón, B., Cadahía, E. (2010). Micro-oxygenation strategy depends on origin and size of oak chips or staves during accelerated red wine aging. *Analytica Chimica Acta*. 660, 92-101.

Alcohol, Tobacco Products and Firearms. 27 C.F.R. 5 §5.11 (2015).

Arzeni, C., Martínez, K., Zema, P., Arias, A., Pérez, O.E., Pilosof, A.M.R. (2012). Comparative study of high intensity ultrasound effects on food proteins functionality. *Journal of Food Engineering*, 108, 463-72.

Ashokkumar,M., Mason, T.J. (2007). *Sonochemistry*. Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley and Sons, Ltd.

Barrera-García, V.D., Gougeon, R.D., Majo, D.D., Aguirre, C., Voilley, A., Chassagne, D. (2008). Different sorption behaviors for wine polyphenols in contact with oak wood. *Journal of Agricultural and Food Chemistry*. 55, 7021-7.

Bermúdez-Aguirre, D., Mobbs, T., Barbosa-Cánovas, G.V. (2011). Ultrasound applications in food processing. *Ultrasound Technologies for Food and Bioprocessing*. DOI: 10.1007/978-1-4419-7472-3_3, 65-105.

Bucur, V. (2006). High-power ultrasonic treatment for wood processing. *Acoustics of Wood* (2nd Ed.). 333-45.

Caldeira, I., Anjos, O., Portal, V., Belchior, A.P., Canas, S. (2010). Sensory and chemical modifications of wine-brandy aged with chestnut and oak wood fragments in comparison to wooden barrels. *Analytica Chimica Acta*. 660, 43-52.

Caldeira, I., Pereira, R., Clímaco, M.C., Belchior, A.P., Bruno de Sousa, R. (2004). Improved method of extraction of aroma compounds in aged brandies and aqueous alcoholic wood extracts using ultrasound. *Analytica Chimica Acta*. 513, 125-34.

Caldeira, M., Rodrigues, F., Perestrelo, R., Marques, J.C., Camara, J.S. (2007). Comparison of two extraction methods for evaluation of volatile constituent patterns in commercial whiskeys elucidation of the main odour-active compounds. *Talanta*. 74, 78-90.

Campbell, I. (2003). Yeast and fermentation. *Whisky: Technology, Production and Marketing*.115-50.

Clyne, J., Conner, J.M., Paterson, A., Piggott, J.R. (1993). The effect of cask charring on Scotch whisky maturation. *International Journal of Food Science and Technology*. 28, 69-81.

Conner, J.M., Paterson, A., Piggott, J.R. (1992). Analysis of lignin from oak casks used for the maturation of scotch whisky. *Journal of the Science of Food and Agriculture*. 60, 349-53.

Conner, J.M., Paterson, A., Piggott, J.R. (1994). Agglomeration of ethyl esters in model spirit solutions and malt whiskies. *Journal of the Science of Food and Agriculture*. 66, 45-53.

Conner, J.M., Paterson, A., Piggott, J.R. (1994). Interactions between ethyl esters and aroma compounds in model spirit solutions. *Journal of Agricultural and Food Chemistry*. 42, 2231-4.

Conner, J.M.., Paterson, A., Piggott, J.R. (1999). Release of distillate flavor compounds in Scotch malt whisky. *Journal of the Science of Food and Agriculture*. 79, 1015-20.

Delgado de la Torre, M. P., Priego-Capote, F., Luque de Castro, M.D. (2013). Comparative profiling analysis of woody flavoring from vine-shoots and oak chips. *Journal of the Science of Food and Agriculture*. 94, 504-14.

Doussot, F., Jéso, B.D., Quideau, S., Pardon, P. (2002). Extractives content in cooperage oak wood during natural seasoning and toasting; influence of tree species, geographic location, and single-tree effects. *Journal of Agricultural and Food Chemistry*, 50, 5955-61.

Folin, 0., Ciocalteu,V. (1927). On tyrosine and tryptophan determinations in proteins. *The Journal of Biological Chemistry*, 73, 627-50.

Gibson, M. (2014, Nov. 4). 5 Things You Need to Know About Japanese Whisky. *Time*. Retrieved from: http://time.com/3556449/japanese-whisky-best-in-world/.

Glabasnia, A., Hofmann, T. (2006). Sensory-directed identification of taste-active ellagitannins in American (*Quercus alba* L.) and European oak wood (*Quercus robur* L.) and quantitative analysis in bourbon whiskey and oak-matured red wines. *Journal of Agricultural and Food Chemistry*. 54, 3380-90.

González-Arjona, D., González-Gallero, V., Pablos, F., González, A.G. (1999). Authentication and differentiation of irish whiskeys by higher-alcohol congener analysis. *Analytica Chimica Acta*. 381, 257-64.

H. 1084. 108th General Assembly. (2013).

Jarauta, I., Cacho, J., Ferreira, V. (2005). Concurrent phenomena contributing to the formation of the aroma of wine during aging in oak wood: an analytical study. *Journal of Agricultural and Food Chemistry*. 53, 4166-77.

Jackson, Michael. (2004). Completed Guide to Single Malt Scotch. (5th ed). Philidelphia, PA: Running Press Book Publishers.

Jeffery, J.D.E. (2012). Aging of whiskey spirits in barrels of non-traditional volume (master's thesis). Retrieved from: Michigan State University.

Kim, D.O., Lee, C.Y. (2002). HPLC Separation of Polyphenolics. *Current Protocols in Food Analytical Chemistry*. I1.3.1-I1.3.16.

Kulkarni, V.M., Rathod, V.K. (2014). Mapping of an ultrasonic bath for ultrasound assisted extraction of mangiferin from *Mangifera indica* leaves. *Ultrasonics Sonochemistry*, 21, 606-11.

Lapointe, F.J., Legendre, P. (1994). A classification of pure malt scotch whiskies. *Journal of the Royal Statistical Society.* 43.1, 237-57.

Lee, H., Feng, H. (2011). Effect of power ultrasound on food quality. *Ultrasound Technologies for Food and Bioprocessing*.DOI: 10.1007/978-1-4419-7472-3_22, 559-82.

Locke, M. (2015). Irish whiskey is making a comeback. *News Bank, INC.* DOI: 502.0.918335892, 7C.

Luque-García, J.L., Luque de Castro, M.D. (2003). Ultrasound: a powerful tool for leaching. *Trends in Analytical Chemistry*, 22(1), 41-7.

Martini, S. (2013). An overview of ultrasound. *Sonocrystallization of Fats*. DOI: 10.1007/978-1-4614-7693-1_2. 7-16.

Martini, S. (2013). Common uses of power ultrasound in the food industry. *Sonocrystallization of Fats*. DOI: 10.1007/978-1-4614-7693-1_4, 27-33.

Moreno, N.J., Marco, A.G., Azpilicueta, C.A. (2007). Influence of wine turbidity on the accumulation of volatile compounds from the oak barrel. *Journal of Agricultural and Food Chemistry*. 55, 6244-51.

Mosedale, J.R., Puech, J.L. (1998). Wood maturation of distilled beverages. *Trends in Food Science and Technology*, 9, 95-101.

Mosedale, J.R., Puech, J.L. (2003). Wines, spirits, and other beverages. *Barrels*. 393-403.

Natali, N., Chinnici, F., Riponi, C. (2006). Characterization of volátiles in extracts from oak chips obtained by accelerated solvent extraction (ASE). *Journal of Agricultural and Food Chemistry*. 54, 8190-8.

Nevares, I., Álamo, M. (2008). Measurement of dissolved oxygen during red wines tank aging with chips and micro-oxygenation. *Analytica Chimica Acta*. 621, 68-78.

Pandey, K.K. (2005). A note on the influence of extractives on the photo-discoloration and photo-degradation of wood. *Polymer Degradation and Stability*. 87, 375-9.

Perez-Prieto, L.J., Lopez-Roca, J.M., Martínez-Cutillas, A., Mínguez, F.P., Gómez-Plaza, E. (2002). Maturing wines in oak barrels, effects of origin, volume, and age of the barrel on the wine volatile composition. *Journal of Agricultural and Food Chemistry*, 50(11), 3272-6.

Piggott, J.R., Conner, J.M., Paterson, A., Clyne, J. (1993). Effects on scotch whisky composition and flavor of maturation in oak casks with varying histories. *International Journal of Food Science and Technology*. 28, 303-18.

Piggott, J.R., Conner, J.M. (2003). Composition and analysis of whisky. *Whisky, Whiskey, and Bourbon*. 6178-83.

Piggott, J.R., Conner, J.M. (2003). Whiskies. *Fermented Beverage Production*. 239-62.

Pizarro, C., Rodrígez-Tecedor, S., Esteban-Díez, I., Pérez-del-Notario, N., González-Sáiz, J.M. (2014). Experimental design approach to evaluate the impact of oak chips and micro-oxygenation on the volatile profile of red wines. *Food Chemistry*, 148, 357-66.

Prida, A., Puech,J.L. (2006). Influence of geographical origin and botanical species on the content of extractives in American, French and East European oak woods. *Journal of Agricultural and Food Chemistry*. 54, 8115-26.

Puckette, M., Hammack, J. (2013). The surprising truth about oaking wine. Retrieved from: http://winefolly.com/review/oaking-wine/.

Rodríguez-Bencomo, J.J., Ortega-Heras, M., Pérez-Magariño, S., González-Huerta, C. (2009). Volatile compounds of red wines macerated with Spanish, American, and French oak chips. *Journal of Agricultural and Food Chemistry*. 57, 6383-91.

Reusch, W. (2013). Visible and Ultraviolet Spectroscopy. Retrieved from: https://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/UV-Vis/spectrum.htm.

Russell, I. (2003). *Whisky: Technology, Production, and Marketing*. San Diego, CA: Academic Press.

Santos, H.M., Lodeiro, C., Capelo-Martinez J.L.(2009). The Power of Ultrasound. *Ultrasound in Chemistry: Analytical Applications*, 1-16.

Sanza, M.A., Domínguez, I.N. (2006). Wine aging in bottle from artificial systems (staves and chips) and oak woods anthocyanin composition. *Analytica Chimica Acta*. 563, 255-63.

Scotch Whisky Association. (2015). Retrieved from: http://www.scotch-whisky.org.uk/.

Sonderegger, W., Kránitz, K., Bues, C.T., Niemz, P. (2015). Aging effects on physical and mechanical properties of spruce, fir, and oak wood. *Journal of Cultural Heritage*. 16(6), 883-9.

Tao, Y., Zhang, Z., Sun, D.W. (2014). Experimental and modeling studies of ultrasound-assisted release of phenolics from oak chips into model wine. *Ultrasonics Sonochemistry*, 21, 1839-48.

Tesfaye,W., Morales, M.L., Benítez,B., García-Parrilla, M.C., Troncoso, A.M. (2004). *Analytica Chimica Acta*. 513, 239-45.

Vilkhu, K., Mawson, R., Simons, L., Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry-a review. *Food Science and Emerging Technologies*, 9(2), 161-9.

Viriot, C., Scalbert, A., Lapierre, C., Moutounet, M. (1993). Ellagitannins and lignins in aging of spirits in oak barrels. *Journal of Agricultural and Food Chemistry*. 41, 1872-9.

Waterhouse, A.L., Towey, J.P. (1994). Oak lactone isomer ratio distinguishes between wines fermented in American and French oak barrels. *Journal of Agricultural and Food Chemistry*. 42, 1971-4.

Weiss, J., Kristbergsson, K., Kjartansson, G.T. (2011). Engineering food ingredients with high-intensity-ultrasound. *Ultrasound Technologies for Food and Bioprocessing*. DOI: 10.1007/978-1-4419-7472-3_10, 239-85.

White, C., Zainasheff, J. (2010). *Yeast: The Practical Guide to Beer Fermentation*. Boulder, CO: Brewers Publications.

Zelinka, S.L., Stone, D.S. (2011). The effect of tannins and pH on the corrosion of steel in wood extracts. *Materials and Corrosion*. 62.8, 739-44.

Zhang, B., Zeng, X.A., Lin, W.T., Sun, D.W., Cai, J.L. (2013). Effects of electric field treatments on phenol compounds of brandy aging in oak barrels. *Innovative Food Science and Emerging Technologies*, 20, 106-14.

# APPENDICES

## Appendix A.

**Table 2.** Stave width and length measurements (millimeters) prior to shaving.

| Measurements of Staves (mm) | | | | |
|---|---|---|---|---|
| **Stave** | **Width** | | | **Length** |
| | **Top** | **Middle** | **Bottom** | |
| **Toasted** | | | | |
| **1** | 58 | 76 | 63 | 909 |
| **2** | 57 | 69 | 59 | 914 |
| **3** | 56 | 66 | 56 | 905 |
| **4** | 101 | 109 | 105 | 915 |
| **5** | 59 | 69 | 60 | 913 |
| **Charred** | | | | |
| **1** | 68 | 76 | 68 | 910 |
| **2** | 63 | 72 | 65 | 912 |
| **3** | 65 | 74 | 65 | 904 |
| **4** | 76 | 85 | 77 | 913 |
| **5** | 66 | 76 | 66 | 913 |
| **6** | 59 | 69 | 60 | 913 |

**Table 3.** Oak shavings were sifted for uniformity using mesh sieves and were weighed (grams). Medium sized oak shavings were used for sample extractions.

| Amount of Oak Shavings (g) | | | |
|---|---|---|---|
| **Layer** | **Large (g)** **(>0.028 mm diameter mesh)** | **Medium (g)** **(>0.028 mm diameter mesh>0.63 µm diameter mesh)** | **Small (g)** **(>0.63 µm diameter mesh)** |
| **Toasted** | | | |
| **1** | 49.12 | 78.77 | 44.57 |
| **2** | 50.35 | 84.08 | 20.58 |
| **3** | 48.57 | 89.99 | 17.69 |
| **4** | 70.22 | 107.82 | 17.57 |
| **Charred** | | | |
| **1** | 16.21 | 32.53 | 23.82 |
| **2** | 3.82 | 24.38 | 22.85 |
| **3** | 13.04 | 47.99 | 20.81 |
| **4** | 9.59 | 36.99 | 8.67 |
| **5** | 35.32 | 106.86 | 22.38 |
| **6** | 73.01 | 112.92 | 20.66 |
| **7** | 129.1 | 169.69 | 14.06 |

**Table 4.** Randomization of layers for each extraction treatment generated from random.org.

| Layers | | |
|---|---|---|
| **Toasted** | | |
| **HIUS** | **Reflux** | **Control** |
| 4 | 1 | 4 |
| 1 | 3 | 2 |
| 3 | 4 | 1 |
| 1 | 1 | 4 |
| 2 | 3 | 1 |
| 4 | 2 | 3 |
| 2 | 4 | 2 |
| 3 | 2 | 3 |
| **Charred** | | |
| 7 | 6 | 3 |
| 1 | 1 | 7 |
| 3 | 2 | 1 |
| 6 | 6 | 5 |
| 4 | 7 | 5 |
| 3 | 5 | 1 |
| 1 | 7 | 6 |
| 5 | 5 | 2 |
| 6 | 2 | 4 |
| 7 | 1 | 2 |
| 2 | 3 | 6 |
| 5 | 4 | 7 |
| 4 | 4 | 3 |
| 2 | 3 | 4 |

## Standard Curves

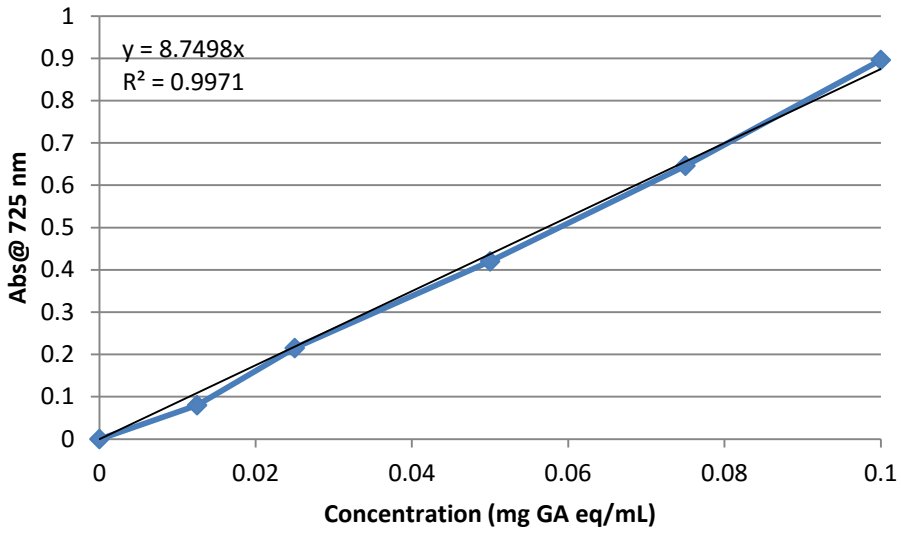


**Figure 102.** Standard curve of gallic acid for concentrations 0- 0.1 mg/mL used to calculate total soluble phenolics.

**Table 5.** Gallic acid concentrations used to calculate total soluble phenolics.

| Total Soluble Phenolics Standard Curve | |
|---|---|
| **Concentration (mg GA eq/mL)** | **Abs (725 nm)** |
| 0 | 0 |
| 0.0125 | 0.08 |
| 0.025 | 0.215 |
| 0.05 | 0.42 |
| 0.075 | 0.646 |
| 0.1 | 0.896 |

## HPLC Standards

**Table 6.** Retention times of standards identified by high performance liquid chromatography at 280 nanometers.

| Retention Times | Standards |
|---|---|
| 7.3 | Gallic Acid |
| 10.5 | Protocatechuic Acid |
| 13.2 | Protocatechuic Aldehyde |
| 14.3 | Methyl Gallate |
| 16.5 | Vanillic Acid |
| 24.5 | Syringealdehyde |
| 27.6 | Scopoletin |
| 31.1 | Ellagic Acid |
| 32.8 | Sinapaldehyde |

**Table 7.** Average areas of peaks from gallic acid concentrations found by high performance liquid chromatography at 280 nanometers.

| Gallic Acid Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 7.3 | 13,419,886 |
| **0.25** | 7.3 | 7,275,966 |
| **0.1** | 7.3 | 2,934,042 |
| **0.05** | 7.3 | 1,488,201 |
| **0.025** | 7.3 | 685,954 |
| **0.01** | 7.3 | 276,270 |
| **0.005** | 7.3 | 134,702 |

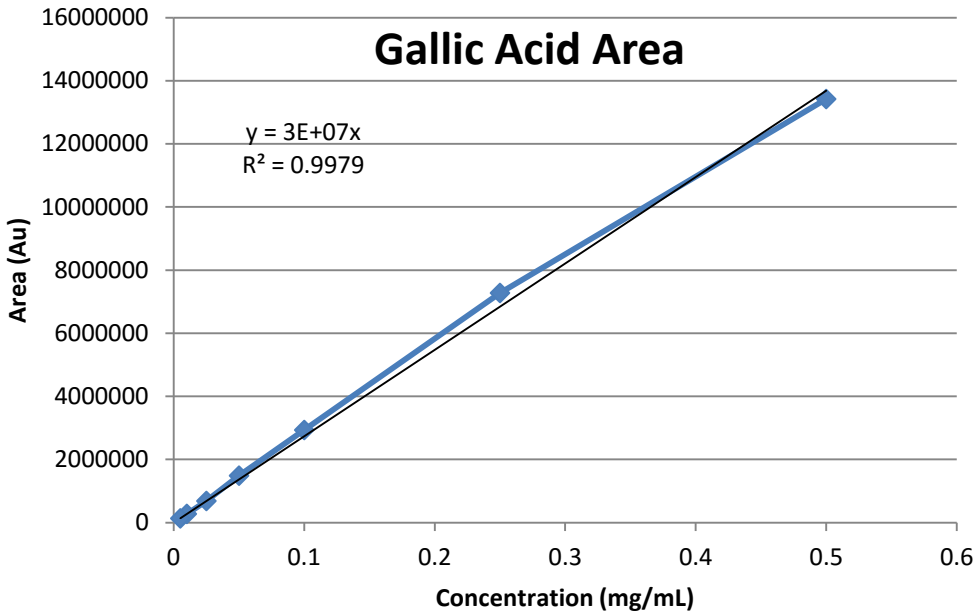


**Figure 103.** Standard curve of gallic acid from high performance liquid chromatography at 280 nanometers.

**Table 8.** Average areas of peaks from protocatechuic acid concentrations found by high performance liquid chromatography at 280 nanometers.

| Protocatechuic Acid Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 10.5 | 7,477,600 |
| **0.25** | 10.5 | 4,043,537 |
| **0.1** | 10.5 | 1,628,089 |
| **0.05** | 10.5 | 825,829 |
| **0.025** | 10.5 | 379,887 |
| **0.01** | 10.5 | 152,745 |
| **0.005** | 10.5 | 74,039 |

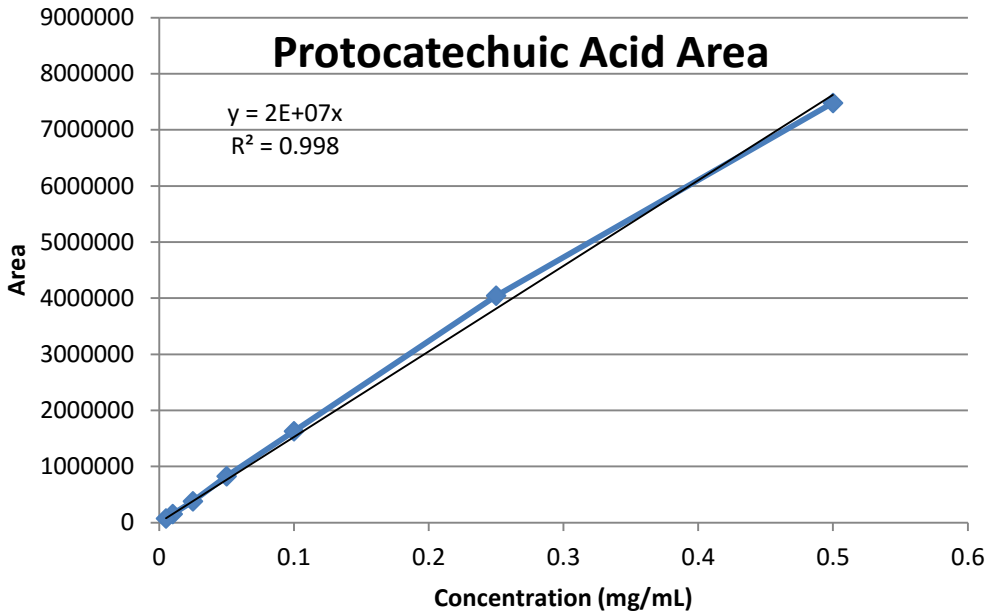


**Figure 104.** Standard curve of protocatechuic acid from high performance liquid chromatography at 280 nanometers.

**Table 9.** Average areas of peaks from protocatechuic aldehyde concentrations found by high performance liquid chromatography at 280 nanometers.

| Protocatechuic Aldehyde Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 13.2 | 19,104,227 |
| **0.25** | 13.2 | 10,356,189 |
| **0.1** | 13.2 | 4,175,781 |
| **0.05** | 13.2 | 2,122,076 |
| **0.025** | 13.2 | 983,673 |
| **0.01** | 13.2 | 403,251 |
| **0.005** | 13.2 | 201,259 |

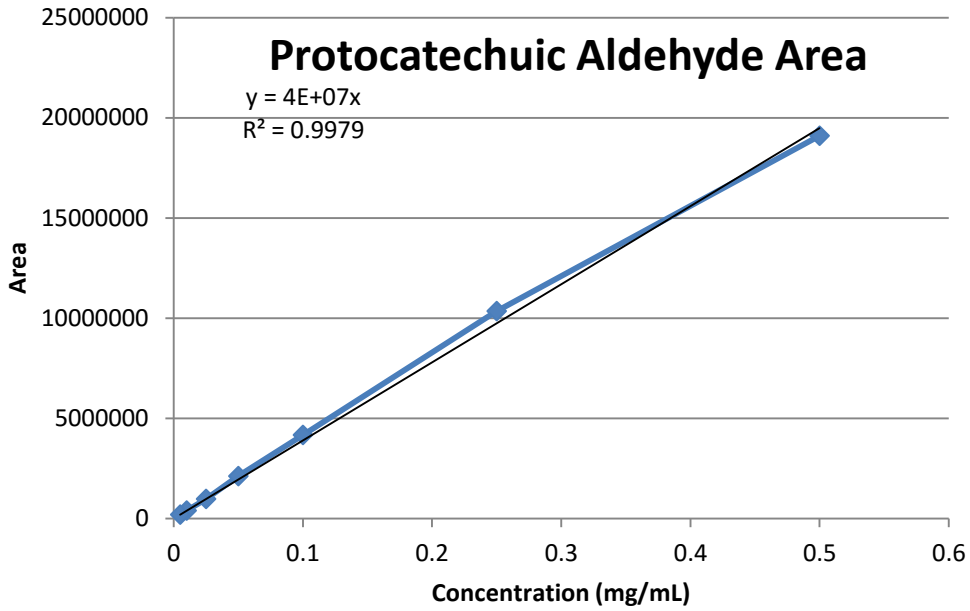


**Figure 105.** Standard curve of protocatechuic aldehyde from high performance liquid chromatography at 280 nanometers.

**Table 10.** Average areas of peaks from methyl gallate concentrations found by high performance liquid chromatography at 280 nanometers.

| Methyl Gallate Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 14.3 | 14,248,548 |
| **0.25** | 14.3 | 7,723,397 |
| **0.1** | 14.3 | 3,121,264 |
| **0.05** | 14.3 | 1,594,455 |
| **0.025** | 14.3 | 748,446 |
| **0.01** | 14.3 | 314,222 |
| **0.005** | 14.3 | 158,385 |

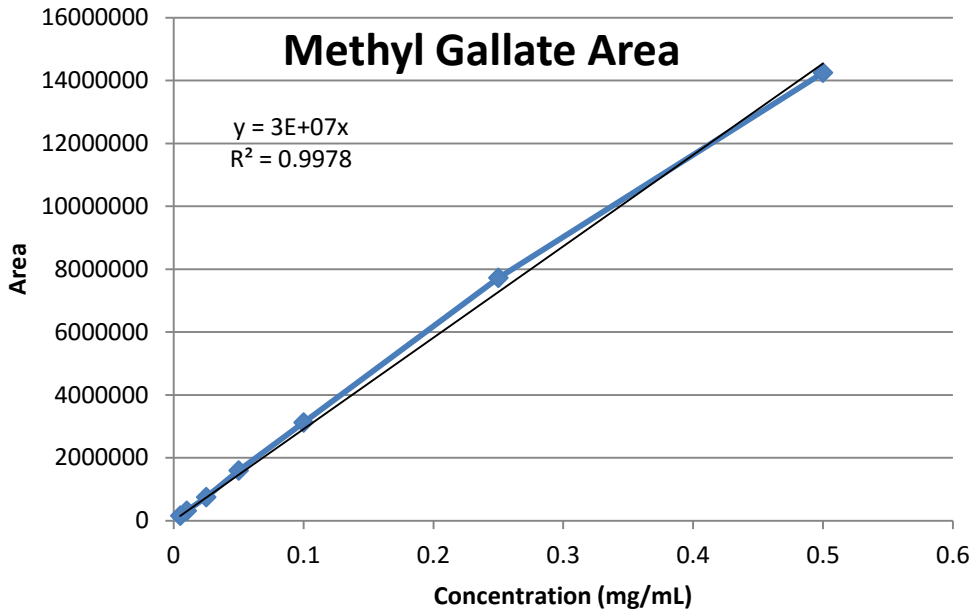


**Figure 106.** Standard curve of methyl gallate from high performance liquid chromatography at 280 nanometers.

**Table 11.** Average areas of peaks from vanillic acid concentrations found by high performance liquid chromatography at 280 nanometers.

| Vanillic Acid Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 16.5 | 7,927,524 |
| **0.25** | 16.6 | 4,283,352 |
| **0.1** | 16.5 | 1,721,152 |
| **0.05** | 16.5 | 871,638 |
| **0.025** | 16.5 | 399,356 |
| **0.01** | 16.5 | 159,402 |
| **0.005** | 16.6 | 77,112 |

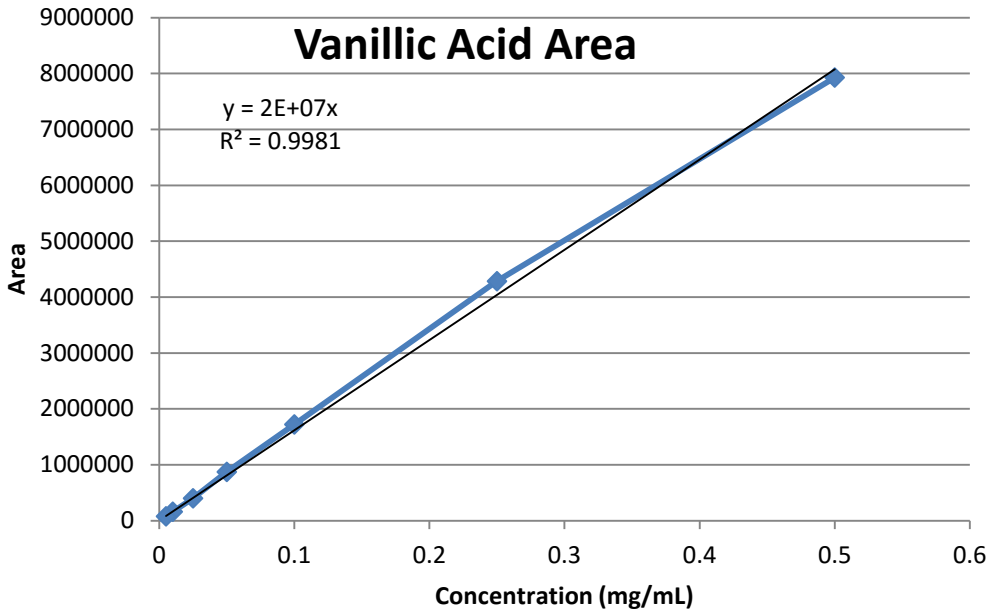


**Figure 107.** Standard curve of vanillic acid from high performance liquid chromatography at 280 nanometers.

**Table 12.** Average areas of peaks from syringealdehyde concentrations found by high performance liquid chromatography at 280 nanometers.

| Syringealdehyde Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 24.5 | 8,798,128 |
| **0.25** | 24.5 | 4,746,948 |
| **0.1** | 24.5 | 1,907,776 |
| **0.05** | 24.5 | 963,201 |
| **0.025** | 24.5 | 440,915 |
| **0.01** | 24.4 | 173,285 |
| **0.005** | 24.5 | 82,281 |

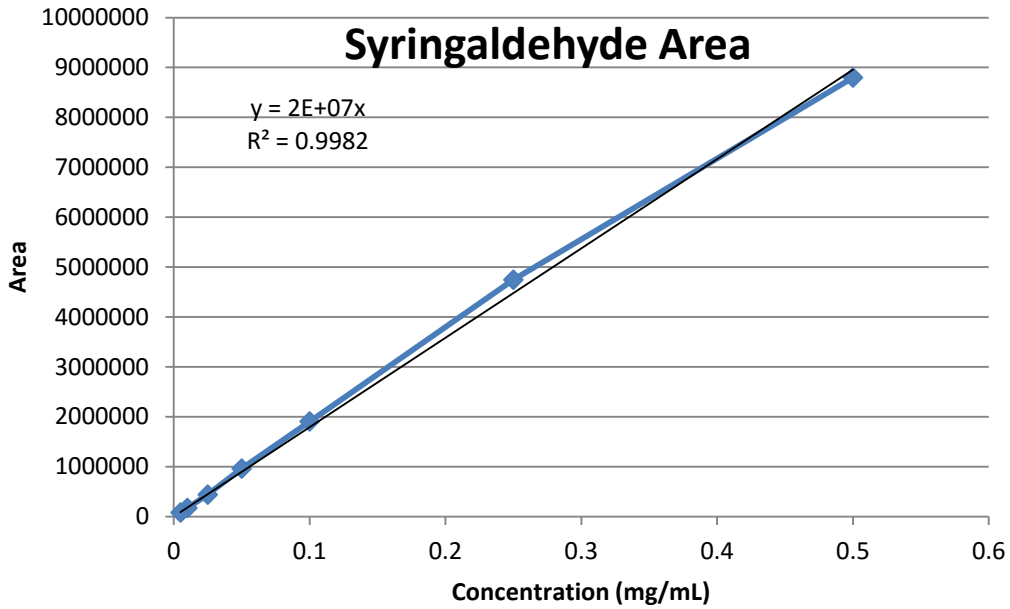


**Figure 108.** Standard curve of syringealdehyde from high performance liquid chromatography at 280 nanometers.

**Table 13.** Average areas of peaks from scopoletin concentrations found by high performance liquid chromatography at 280 nanometers.

| Scopoletin Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 27.6 | 2,317,722 |
| **0.25** | 27.7 | 1,248,169 |
| **0.1** | 27.6 | 499,581 |
| **0.05** | 27.6 | 251,774 |
| **0.025** | 27.6 | 113,142 |
| **0.01** | 27.5 | 42,167 |
| **0.005** | 27.7 | 18,403 |

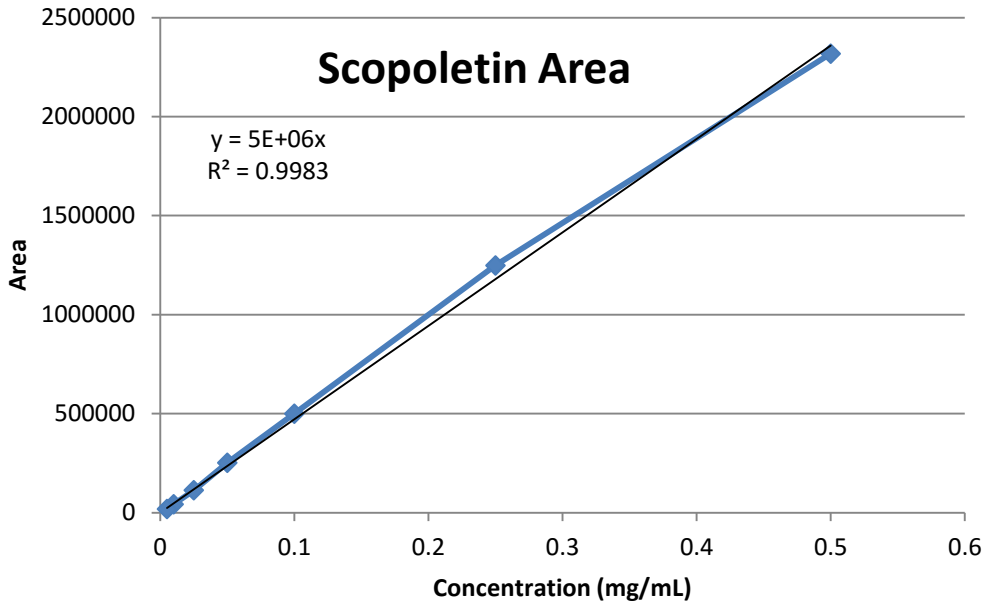


**Figure 109.** Standard curve of scopoletin from high performance liquid chromatography at 280 nanometers.

**Table 14.** Average areas of peaks from ellagic acid concentrations found by high performance liquid chromatography at 280 nanometers.

| Ellagic Acid Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 31.0 | 8,917,881 |
| **0.25** | 31.1 | 5,356,767 |
| **0.1** | 31.1 | 2,153,383 |
| **0.05** | 31.2 | 1,082,164 |
| **0.025** | 31.2 | 492,945 |
| **0.01** | 31.2 | 193,425 |
| **0.005** | 31.3 | 89,504 |

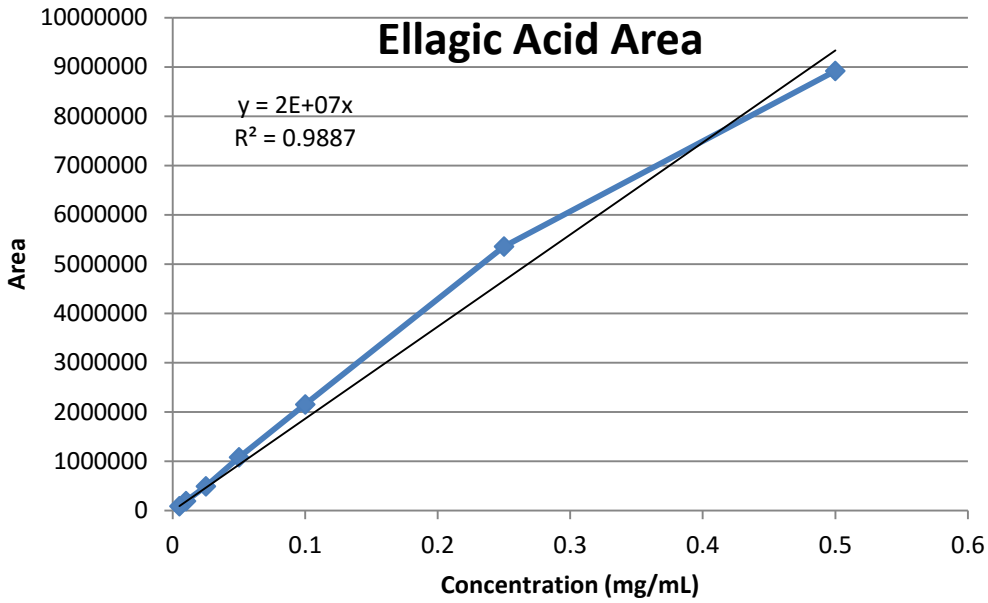


**Figure 110.** Standard curve of ellagic acid from high performance liquid chromatography at 280 nanometers.

**Table 15.** Average areas of peaks from sinapaldehyde concentrations found by high performance liquid chromatography at 280 nanometers.

| Sinapaldehyde Average | | |
|---|---|---|
| **mg/mL** | **RT** | **Area** |
| **0.5** | 32.8 | 3,014,553 |
| **0.25** | 32.8 | 1,635,533 |
| **0.1** | 32.8 | 663,934 |
| **0.05** | 32.8 | 337,104 |
| **0.025** | 32.8 | 154,393 |
| **0.01** | 32.8 | 61,743 |
| **0.005** | 32.8 | 29,424 |

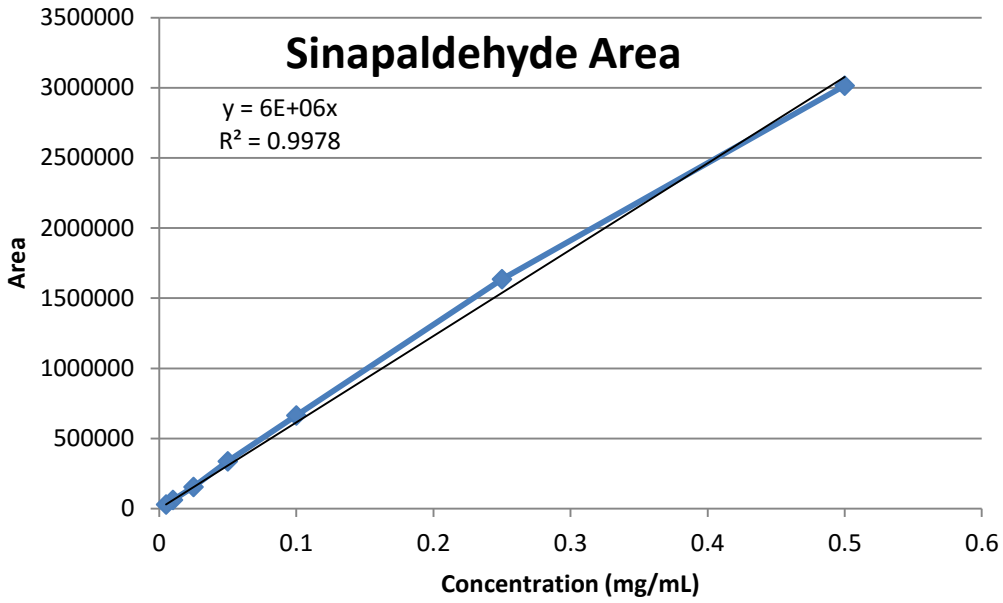


**Figure 111.** Standard curve of sinapaldehyde from high performance liquid chromatography at 280 nanometers.

## GC Standards

**Table 16.** Retention times of standards identified by gas chromatography.

| Retention Times | Standards |
|---|---|
| 15.3 | Furaldehyde |
| 20.6 | Butyric Acid |
| 32.6 | Octanoic Acid |
| 36.6 | Ferulic Acid |
| 44.2 | Gallic Aldehyde |
| 44.5 | Vanillin |
| 45.07 | Sinapic Acid |
| 57 | Caffeic Acid |
| 59.2 | B-Resorcylic Acid |
| 60.5 | B-Resorcylic Aldehyde |
| 60.7 | Coniferaldehyde |

**Table 17.** Average areas of peaks from furaldehyde found by gas chromatography.

| Furaldehyde Average | | |
|---|---|---|
| mg/mL | RT | Area |
| 0.05 | 15.3 | 26.8 |
| 0.04 | 15.3 | 17.8 |
| 0.03 | 15.3 | 12.4 |
| 0.02 | 15.3 | 5.01 |
| 0.01 | 15.3 | 3.79 |
| 0.005 | 15.3 | 1.49 |

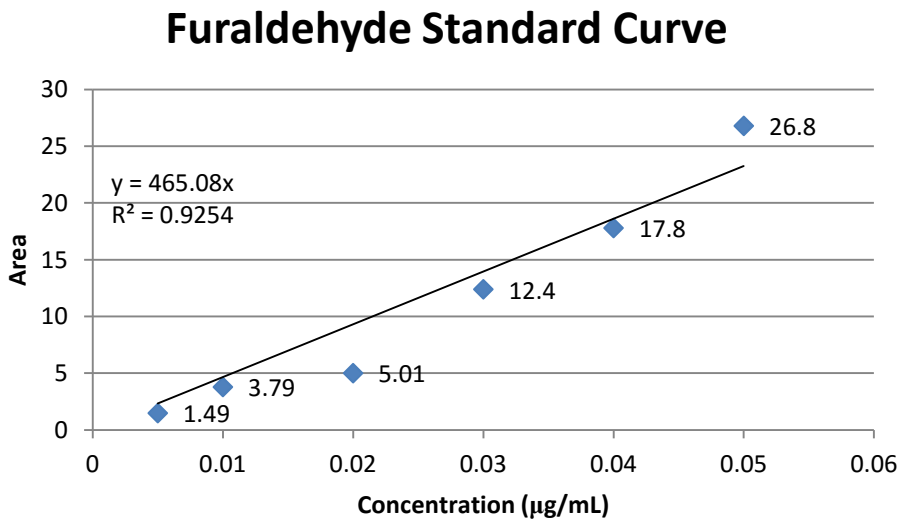


**Figure 112.** Standard curve of furaldehyde from gas chromatography.

**Table 18.** Average areas of peaks from vanillin found by gas chromatography.

| Vanillin Average | | |
|---|---|---|
| mg/mL | RT | Area |
| 0.05 | 44.5 | 24.2 |
| 0.04 | 44.5 | 20.95 |
| 0.03 | 44.5 | 14.43 |
| 0.02 | 44.5 | 9.65 |
| 0.01 | 44.5 | 4.18 |
| 0.005 | 44.5 | 3.91 |
| 0.0025 | 44.5 | 2.31 |

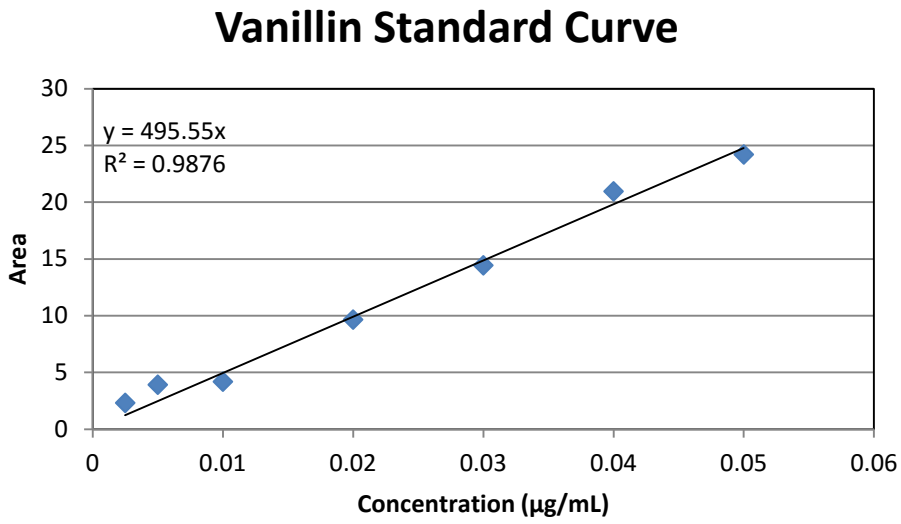


**Figure 113.** Standard curve of vanillin from gas chromatography.

# Appendix B.

## Results

**Table 19.** Weight (grams) of each extracted sample measured at time 0 and after 3 months.

| | Weight of Vials (g) | | | | | |
|---|---|---|---|---|---|---|
| | HIUS | | Reflux | | Control | |
| Layers | 0 | 3 | 0 | 3 | 0 | 3 |
| **Charred Rep 1** | | | | | | |
| **1** | 86.6 | 86.2 | 81.4 | 81.3 | 92.1 | 91.5 |
| **2** | 87.7 | 86.6 | 83.7 | 83.6 | 90.9 | 90.3 |
| **3** | 90.0 | 89.8 | 85.9 | 85.7 | 93.1 | 91.8 |
| **4** | 89.0 | 88.8 | 88.5 | 88.5 | 90.7 | 90.3 |
| **5** | 89.0 | 89.0 | 88.6 | 88.4 | 93.1 | 92.9 |
| **6** | 95.7 | 87.8 | 85.2 | 85.2 | 93.9 | 93.5 |
| **7** | 87.7 | 87.3 | 81.1 | 81.0 | 93.6 | 93.2 |
| **Charred Rep 2** | | | | | | |
| **1** | 89.2 | 88.1 | 87.1 | 87.1 | 92.4 | 92.3 |
| **2** | 89.3 | 89.0 | 91.1 | 90.4 | N/A | |
| **3** | 89.3 | 88.4 | 91.6 | 91.2 | 90.3 | 90.2 |
| **4** | 89.7 | 88.7 | 87.7 | 87.6 | 91.4 | 91.1 |
| **5** | 92.8 | 92.6 | 89.0 | 88.1 | 94.2 | 93.9 |
| **6** | 87.5 | 87.3 | 85.8 | 85.8 | 93.8 | 93.5 |
| **7** | 89.1 | 89.0 | 88.8 | 88.1 | 93.5 | 93.3 |
| **Toasted Rep 1** | | | | | | |
| **1** | 88.8 | 88.4 | 88.7 | 88.7 | 91.7 | 91.3 |
| **2** | 88.5 | 88.5 | 86.0 | 86.0 | 92.6 | 92.6 |
| **3** | 86.4 | 86.2 | 87.0 | 87.0 | 91.5 | 91.1 |
| **4** | 86.5 | 86.5 | 88.7 | 88.2 | 92.5 | 92.4 |
| **Toasted Rep 2** | | | | | | |
| **1** | 88.3 | 88.1 | 84.9 | 84.6 | 92.4 | 92.0 |
| **2** | 86.4 | 86.4 | 85.6 | 85.5 | 92.7 | 92.2 |
| **3** | 86.4 | 85.3 | 85.0 | 85.0 | 92.4 | 92.0 |
| **4** | 90.3 | 90.3 | 84.0 | 84.0 | 93.4 | 93.4 |

**Table 20.** Color intensity (measured as absorbance at 420 nanometers) of each extracted sample measured at time 0 and after 3 months.

| | **Color** (Absorbance 420 nm) | | | | | |
|---|---|---|---|---|---|---|
| | **HIUS** | | **Reflux** | | **Control** | |
| **Layers** | 0 | 3 | 0 | 3 | 0 | 3 |
| **Charred Rep 1** | | | | | | |
| **1** | 0.83 | 1.66 | 2.33 | 2.36 | 0.74 | 1.68 |
| **2** | 1.62 | 2.21 | 2.24 | 2.58 | 1.53 | 2.18 |
| **3** | 1.95 | 2.49 | 2.40 | 2.51 | 1.92 | 2.33 |
| **4** | 1.41 | 2.18 | 2.39 | 2.09 | 1.41 | 1.92 |
| **5** | 1.02 | 2.07 | 2.33 | 2.41 | 0.92 | 1.86 |
| **6** | 1.26 | 1.82 | 2.30 | 2.27 | 0.88 | 1.67 |
| **7** | 0.58 | 1.26 | 2.06 | 1.58 | 0.58 | 1.19 |
| **Charred Rep 2** | | | | | | |
| **1** | 0.86 | 1.80 | 2.29 | 2.33 | 0.77 | 1.70 |
| **2** | 1.38 | 2.30 | 2.31 | 2.59 | N/A | |
| **3** | 2.42 | 2.49 | 1.90 | 2.37 | 2.42 | 2.49 |
| **4** | 2.40 | 2.06 | 1.44 | 1.95 | 2.40 | 2.06 |
| **5** | 2.32 | 2.52 | 1.00 | 1.87 | 2.32 | 2.52 |
| **6** | 2.27 | 2.13 | 0.89 | 1.66 | 2.27 | 2.13 |
| **7** | 2.13 | 1.69 | 0.60 | 1.23 | 2.13 | 1.69 |
| **Toasted Rep 1** | | | | | | |
| **1** | 2.36 | 2.58 | 1.21 | 2.05 | 2.36 | 2.58 |
| **2** | 2.31 | 2.54 | 1.36 | 2.07 | 2.31 | 2.54 |
| **3** | 2.41 | 2.38 | 1.11 | 1.77 | 2.41 | 2.38 |
| **4** | 2.41 | 1.96 | 0.83 | 1.50 | 2.41 | 1.96 |
| **Toasted Rep 2** | | | | | | |
| **1** | 2.33 | 2.51 | 1.20 | 2.03 | 2.33 | 2.51 |
| **2** | 2.34 | 2.51 | 1.33 | 2.07 | 2.34 | 2.51 |
| **3** | 2.40 | 2.23 | 1.10 | 1.75 | 2.40 | 2.23 |
| **4** | 2.39 | 1.99 | 0.95 | 1.49 | 2.39 | 1.99 |

**Table 21.** Amount of total soluble phenolics (measured in milligram gallic acid equivalents per milliliter at an absorbance of 725 nanometers) of each extracted sample measured at time 0 and after 3 months.

| | **Total Soluble Phenolics** (mg GA eq./mL) | | | | | |
|---|---|---|---|---|---|---|
| | **HIUS** | | **Reflux** | | **Control** | |
| **Layers** | 0 | 3 | 0 | 3 | 0 | 3 |
| **Charred Rep 1** | | | | | | |
| **1** | 0.36 | 0.39 | 0.86 | 0.70 | 0.34 | 0.59 |
| **2** | 0.81 | 1.08 | 1.14 | 1.22 | 0.79 | 1.07 |
| **3** | 0.92 | 1.22 | 1.14 | 1.23 | 0.97 | 1.16 |
| **4** | 0.79 | 1.14 | 1.10 | 1.12 | 0.83 | 1.07 |
| **5** | 0.67 | 1.17 | 1.14 | 1.18 | 1.12 | 1.16 |
| **6** | 0.92 | 1.22 | 1.18 | 1.29 | 0.69 | 1.20 |
| **7** | 0.65 | 1.05 | 1.17 | 1.27 | 0.61 | 1.18 |
| **Charred Rep 2** | | | | | | |
| **1** | 0.36 | 0.61 | 0.85 | 0.72 | 0.36 | 0.57 |
| **2** | 0.76 | 1.10 | 1.15 | 1.12 | N/A | |
| **3** | 0.74 | 1.14 | 1.14 | 1.18 | 0.98 | 1.18 |
| **4** | 0.65 | 1.09 | 1.09 | 1.12 | 0.82 | 1.09 |
| **5** | 0.58 | 1.16 | 1.16 | 1.20 | 0.67 | 1.16 |
| **6** | 0.67 | 1.18 | 1.17 | 1.25 | 0.72 | 1.19 |
| **7** | 0.55 | 1.17 | 1.15 | 1.29 | 0.58 | 1.13 |
| **Toasted Rep 1** | | | | | | |
| **1** | 0.67 | 0.93 | 1.26 | 0.99 | 0.64 | 1.05 |
| **2** | 0.61 | 0.97 | 1.28 | 1.03 | 0.73 | 1.10 |
| **3** | 0.50 | 0.95 | 1.23 | 1.01 | 0.68 | 1.07 |
| **4** | 0.51 | 0.88 | 1.23 | 1.00 | 0.60 | 1.04 |
| **Toasted Rep 2** | | | | | | |
| **1** | 0.57 | 0.94 | 1.27 | 1.03 | 0.66 | 1.07 |
| **2** | 0.62 | 0.99 | 1.25 | 1.02 | 0.72 | 1.08 |
| **3** | 0.58 | 0.96 | 1.24 | 0.98 | 0.73 | 1.05 |
| **4** | 0.49 | 0.93 | 1.23 | 0.95 | 0.70 | 1.03 |

**Table 22.** PH level of each extracted sample measured at time 0 and after 3 months.

| Layers | pH | | | | | |
|---|---|---|---|---|---|---|
| | HIUS | | Reflux | | Control | |
| | 0 | 3 | 0 | 3 | 0 | 3 |
| **Charred Rep 1** | | | | | | |
| **1** | 5.55 | 4.96 | 4.46 | 4.9 | 4.76 | 4.94 |
| **2** | 4.34 | 3.92 | 4.16 | 3.94 | 4.2 | 3.93 |
| **3** | 4.28 | 3.87 | 4.07 | 3.9 | 4.24 | 3.98 |
| **4** | 4.4 | 3.88 | 4.03 | 3.79 | 4.18 | 3.84 |
| **5** | 4.23 | 3.84 | 4.07 | 3.89 | 4.28 | 3.84 |
| **6** | 4.28 | 3.93 | 3.99 | 3.85 | 4.2 | 3.89 |
| **7** | 4.22 | 3.91 | 3.97 | 3.85 | 4.21 | 3.89 |
| **Charred Rep 2** | | | | | | |
| **1** | 5.5 | 4.97 | 4.63 | 4.86 | 4.7 | 4.93 |
| **2** | 4.31 | 3.94 | 4.12 | 3.93 | N/A | |
| **3** | 4.2 | 3.83 | 4.03 | 3.73 | 4.26 | 3.97 |
| **4** | 4.23 | 3.75 | 4 | 3.77 | 4.19 | 3.86 |
| **5** | 4.21 | 3.85 | 4.04 | 3.81 | 4.23 | 3.89 |
| **6** | 4.24 | 3.94 | 4.01 | 3.89 | 4.22 | 3.93 |
| **7** | 4.21 | 3.82 | 4.02 | 3.91 | 4.18 | 3.9 |
| **Toasted Rep 1** | | | | | | |
| **1** | 4.41 | 3.92 | 4.13 | 3.9 | 4.35 | 3.97 |
| **2** | 4.32 | 3.91 | 4.09 | 3.9 | 4.45 | 3.94 |
| **3** | 4.24 | 3.93 | 4.06 | 3.91 | 4.2 | 3.94 |
| **4** | 4.35 | 3.87 | 4.02 | 3.89 | 4.15 | 3.96 |
| **Toasted Rep 2** | | | | | | |
| **1** | 4.39 | 3.95 | 4.13 | 3.95 | 4.37 | 3.97 |
| **2** | 4.32 | 3.85 | 4.08 | 3.9 | 4.24 | 3.93 |
| **3** | 4.28 | 3.88 | 4.07 | 3.91 | 4.2 | 3.94 |
| **4** | 4.19 | 3.88 | 4.05 | 3.89 | 4.17 | 3.95 |

# Appendix C.

## HPLC Chromatograms Time 0 (280 nanometers)

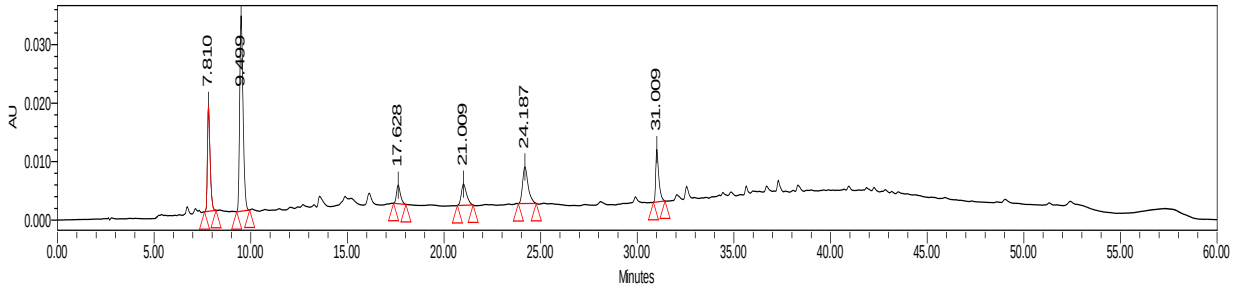


**Figure 114.** HPLC @ 280 nm Charred layer 1, Sonicated rep. 1, run 1

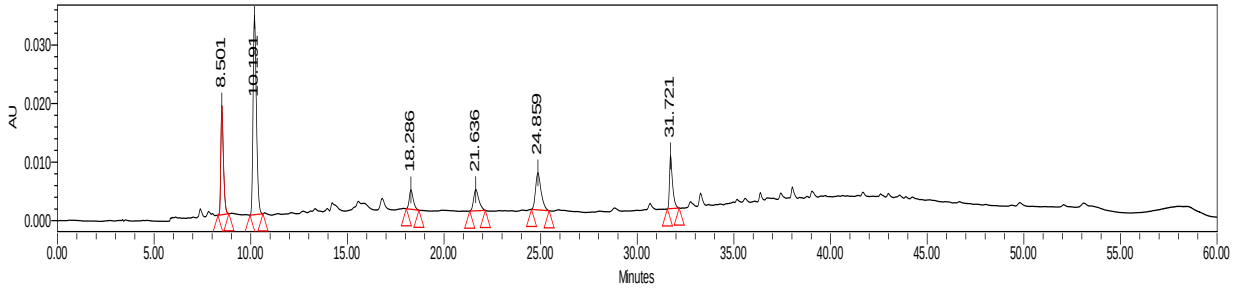


**Figure 115.** HPLC @280 nm charred layer 1, sonicated rep.1, run 2

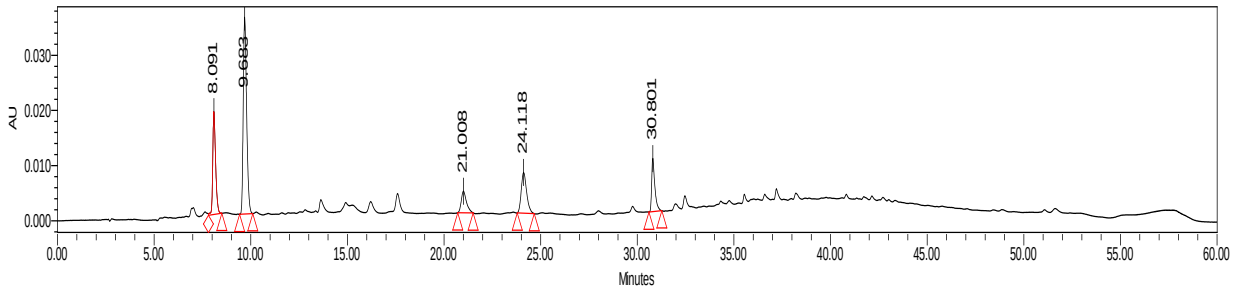


**Figure 116.** HPLC @280 nm charred layer 1, sonicated rep. 2, run 1

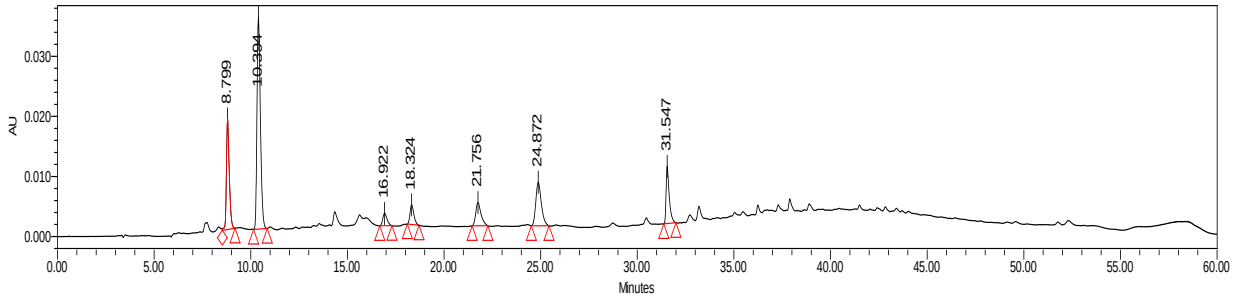


**Figure 117.** HPLC @ 280 nm charred layer 1, sonicated rep 2, run 2

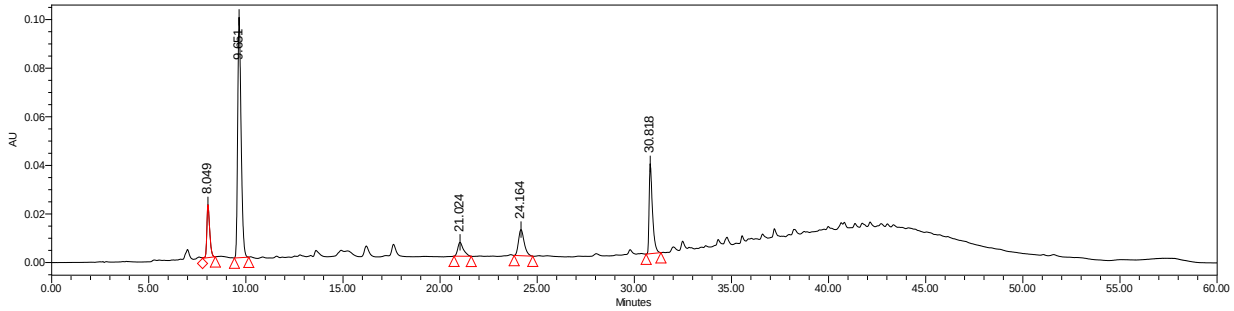


**Figure 118.** HPLC @280 nm, charred layer 1, reflux rep 1, run 1

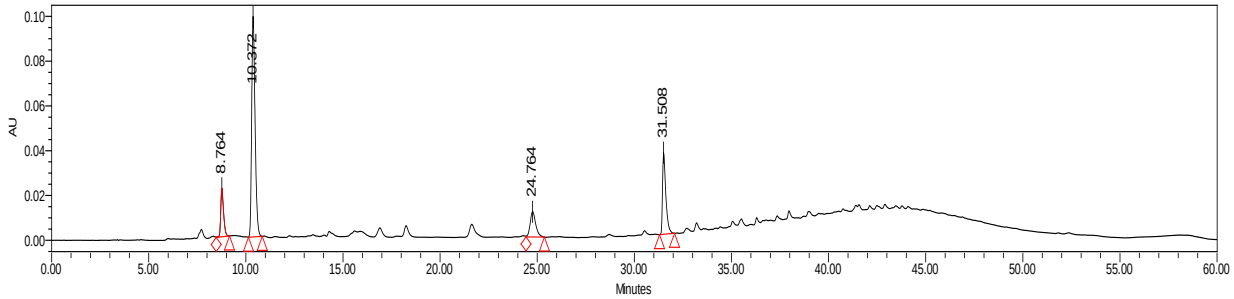


**Figure 119.** HPLC @ 280, charred layer 1, reflux rep 1, run 2

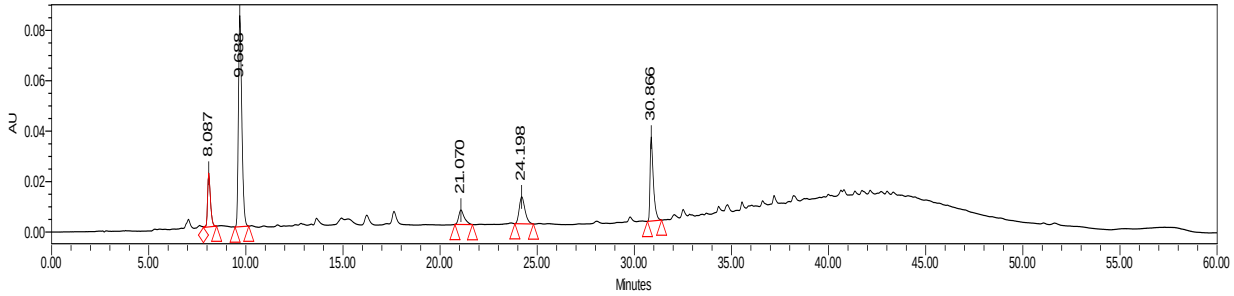


**Figure 120.** HPLC @280 nm, charred layer 1, reflux rep 2, run 1

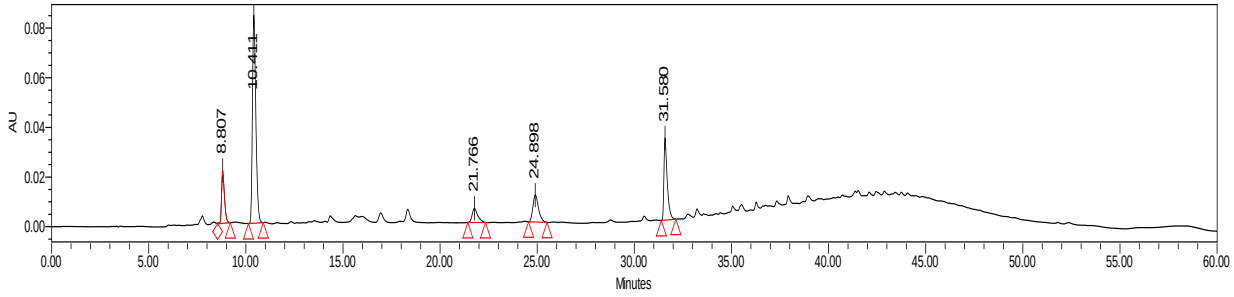


**Figure 121.** HPLC @280 nm, charred layer 1, reflux rep 2, run 2

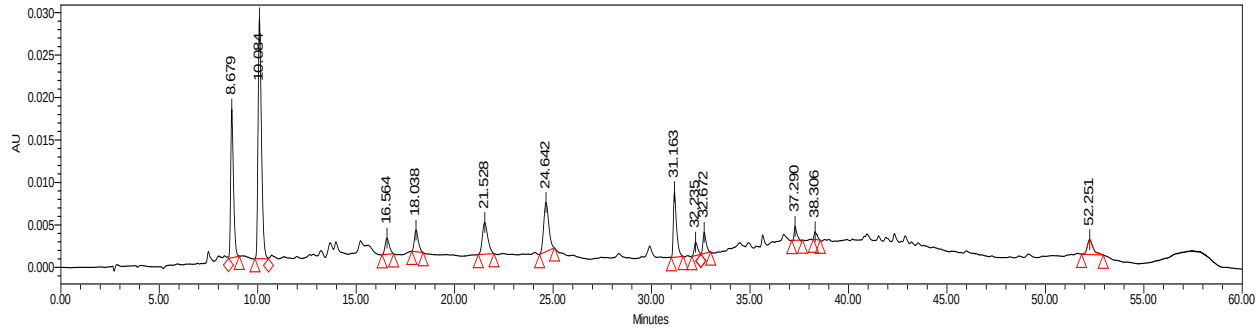


**Figure 122.** HPLC @280 nm, charred layer 1, control rep 1, run 1

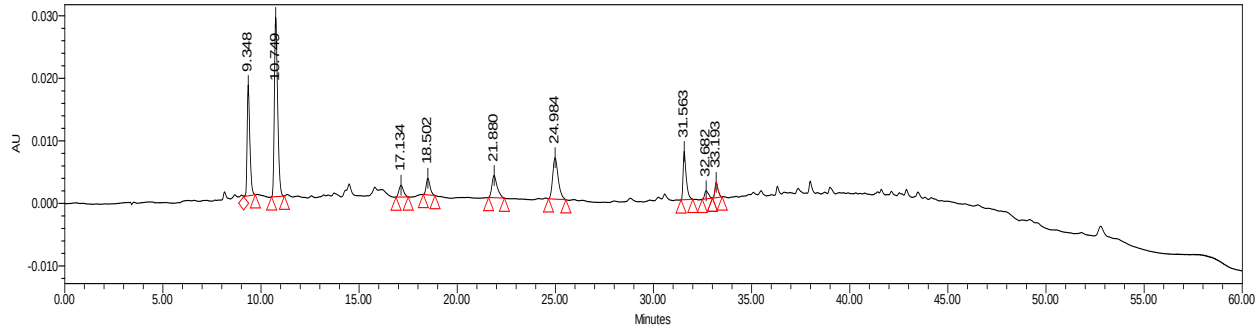


**Figure 123.** HPLC @280 nm, charred layer 1, control rep 1, run 2

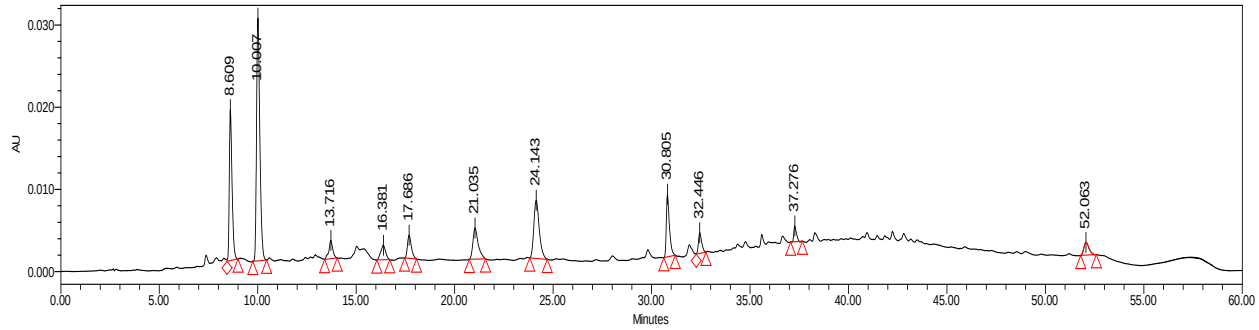


**Figure 124.** HPLC@280 nm, charred layer 1, control rep 2, run 1

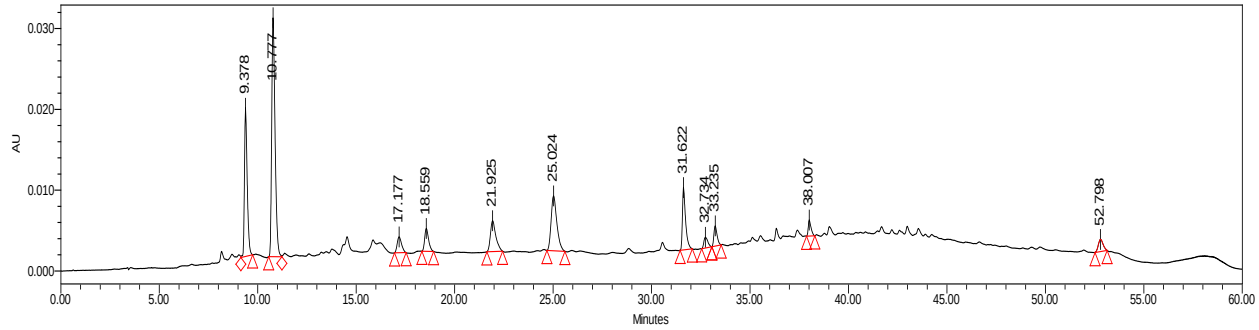


**Figure 125.** HPLC@280 nm, charred layer 1, control rep 2, run 2

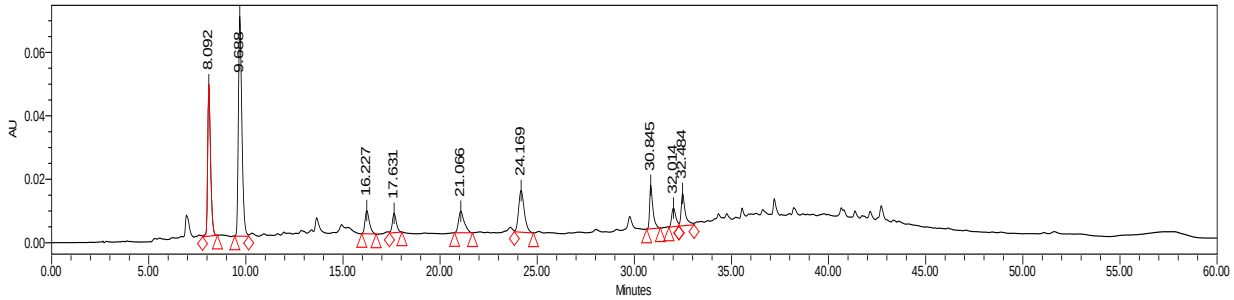


**Figure 126.** HPLC @280 nm, charred layer 2, sonicated rep 1, run 1

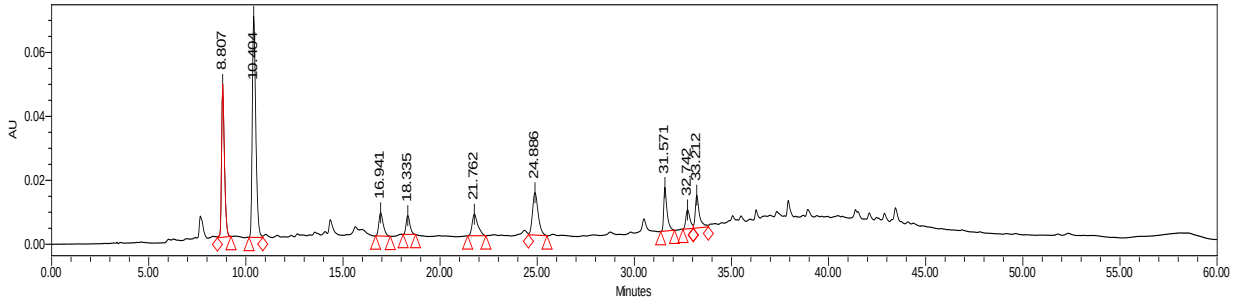


**Figure 127.** HPLC @280 nm, charred layer 2, sonicated rep 1, run 2

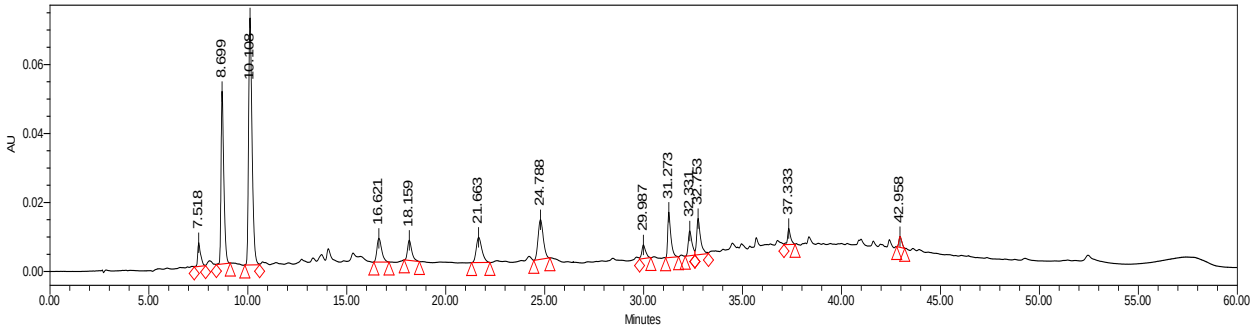


**Figure 128.** HPLC@280 nm, charred layer 2, sonicated rep 2, run 1

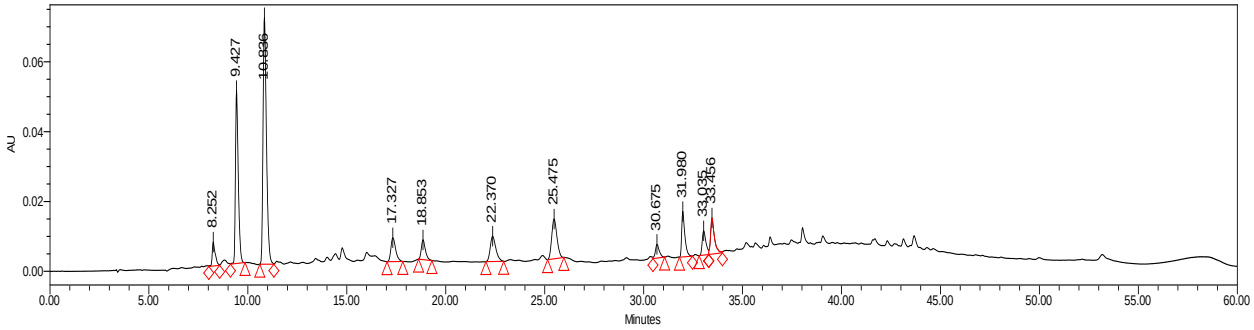


**Figure 129.** HPLC@280 nm, charred layer 2, sonicated rep 2, run 2

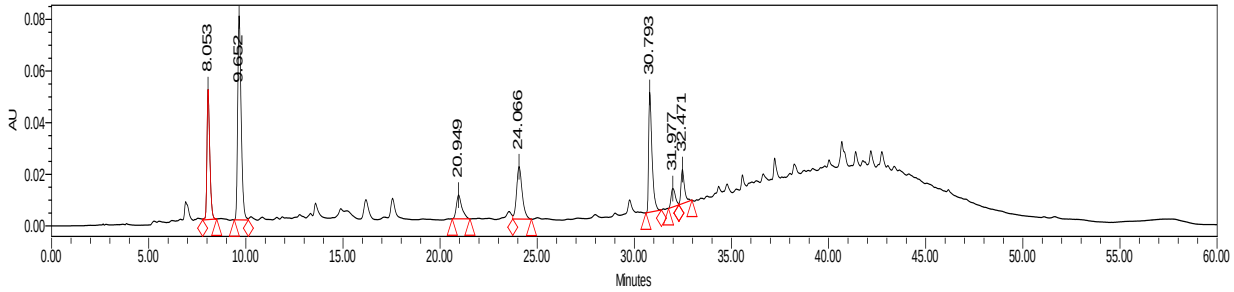


**Figure 130.** HPLC @280 nm, charred layer 2, reflux rep 1, run 1

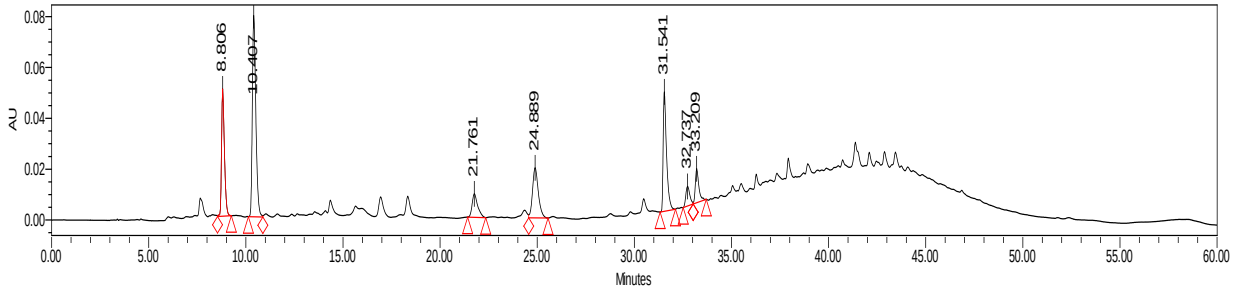


**Figure 131.** HPLC @280 nm, charred layer 2, reflux rep 1, run 2

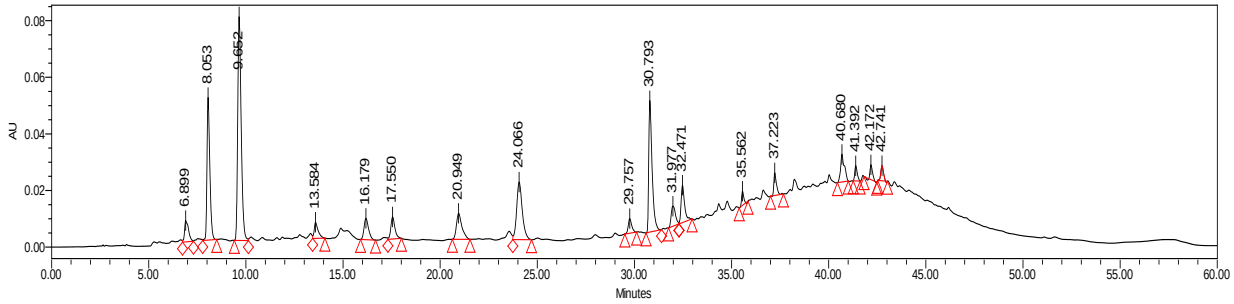


**Figure 132.** HPLC@280 nm, charred layer 2, reflux rep 2, run 1

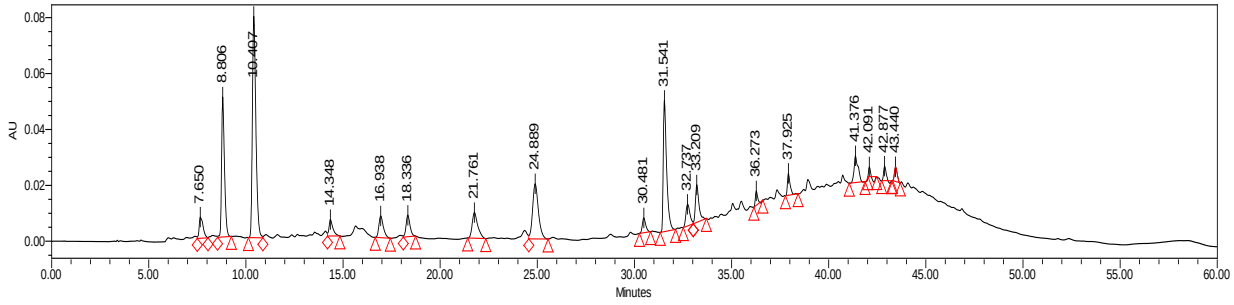


**Figure 133.** HPLC@280 nm, charred layer 2, reflux rep 2, run 2

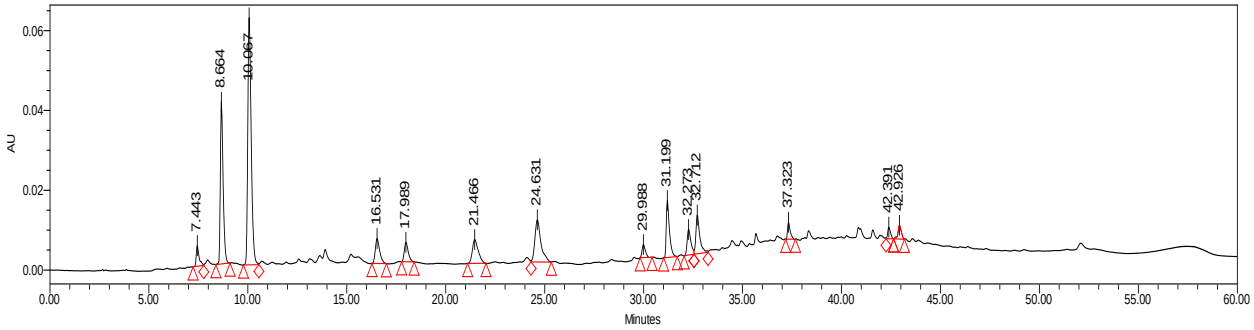


**Figure 134.** HPLC @280 nm, charred layer 2, control rep 1, run 1

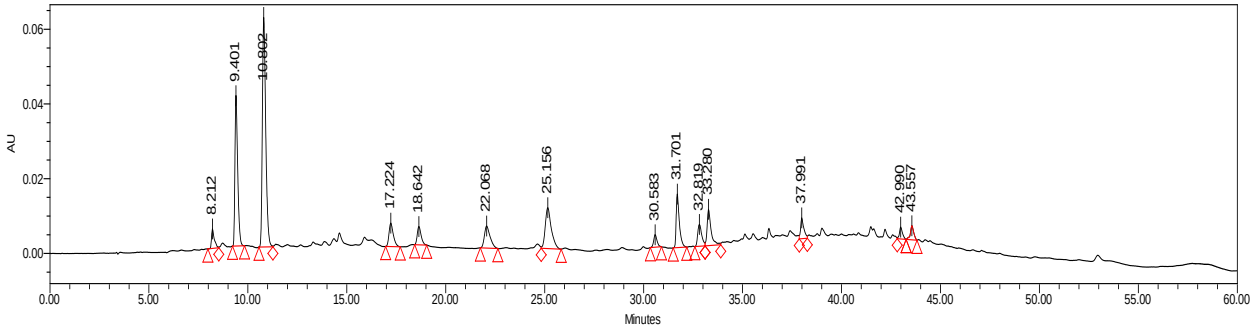


**Figure 135.** HPLC@280 nm, charred layer 2, control rep 1, run 2

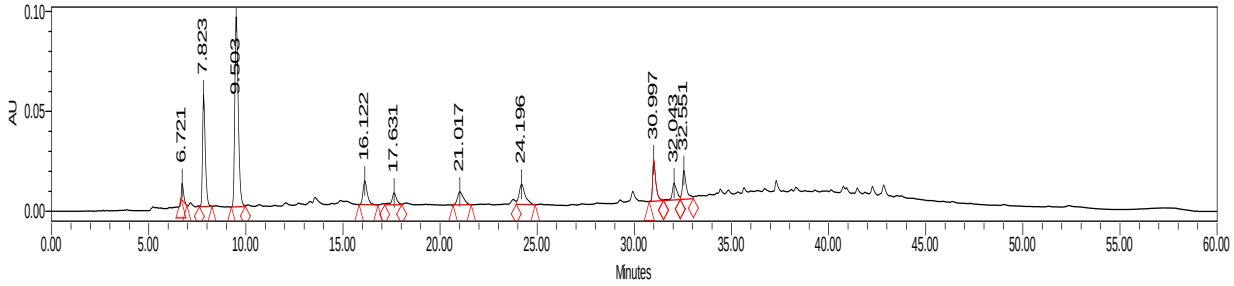


**Figure 136**. HPLC @280 nm, charred layer 3, sonicated rep 1, run 1

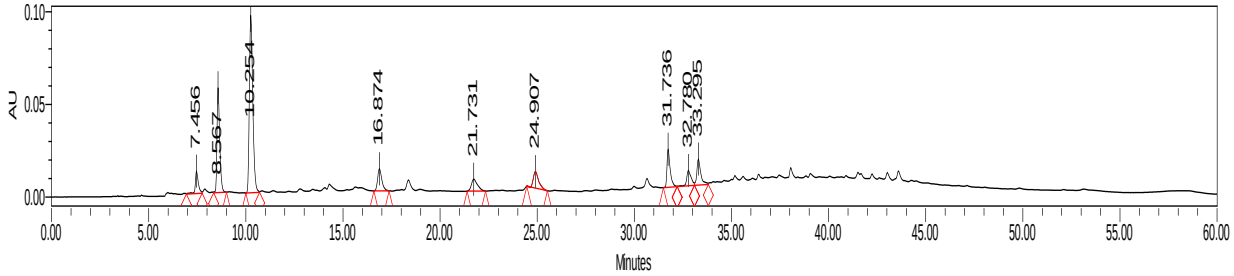


**Figure 137.** HPLC @280 nm, charred layer 3, sonicated rep 1, run 2

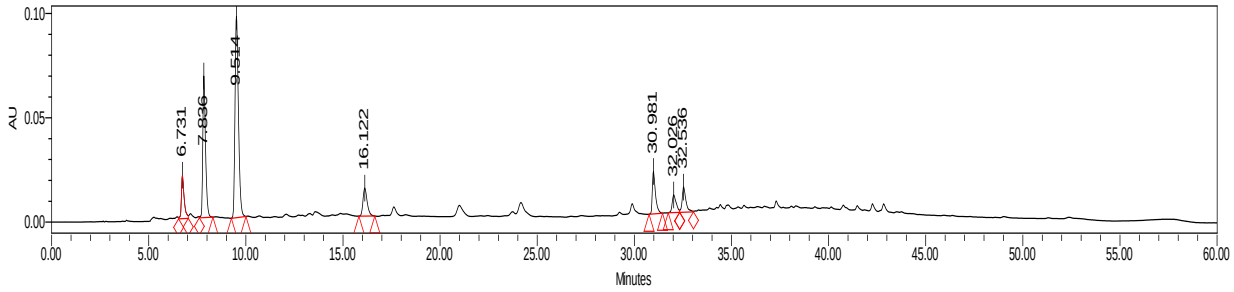


**Figure 138.** HPLC @280 nm, charred layer 3, sonicated rep 2, run 1

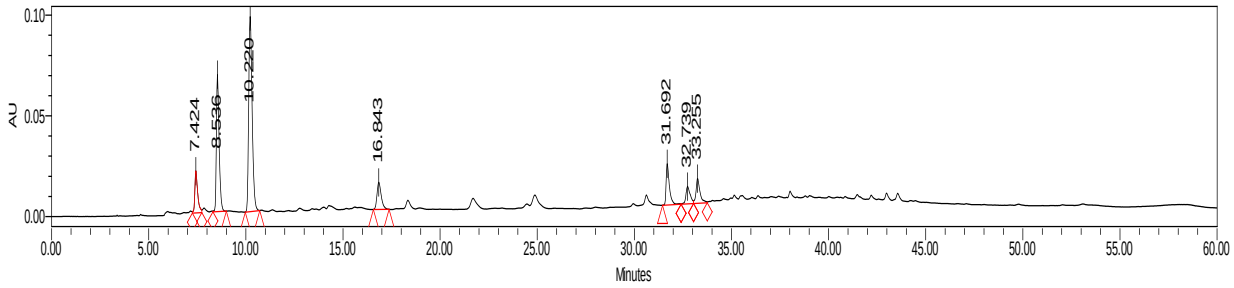


**Figure 139.** HPLC @280 nm, charred layer 3, sonicated rep 2, run 2

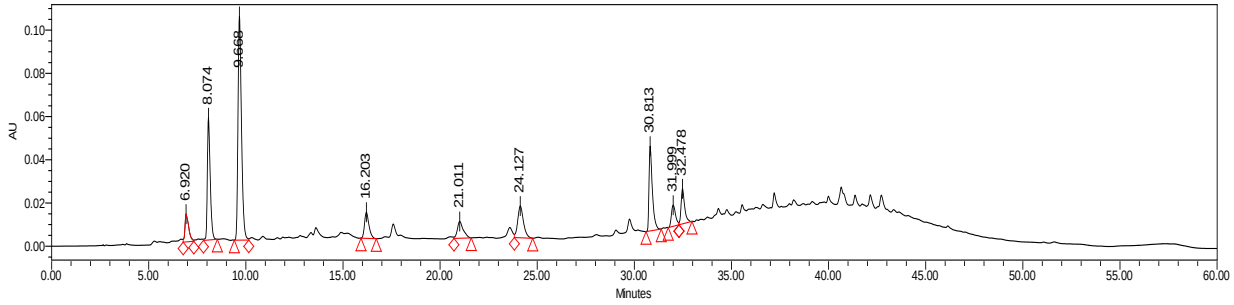


**Figure 140.** HPLC @280 nm, charred layer 3, reflux rep 1, run 1

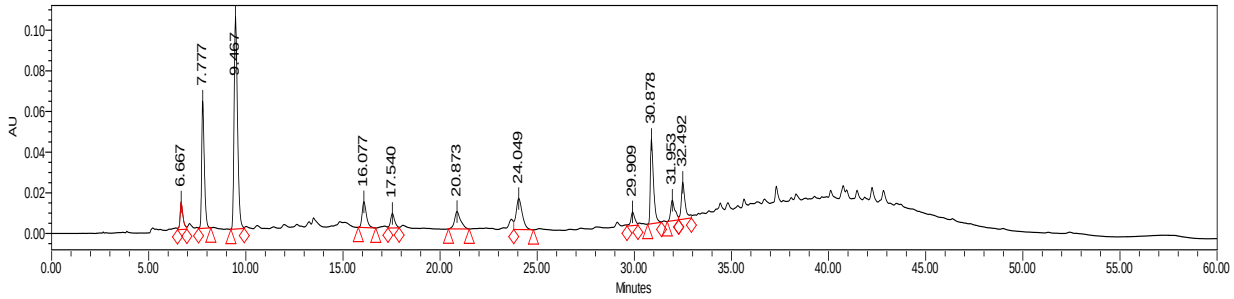


**Figure 141.** HPLC @280 nm, charred layer 3, reflux rep 1, run 2

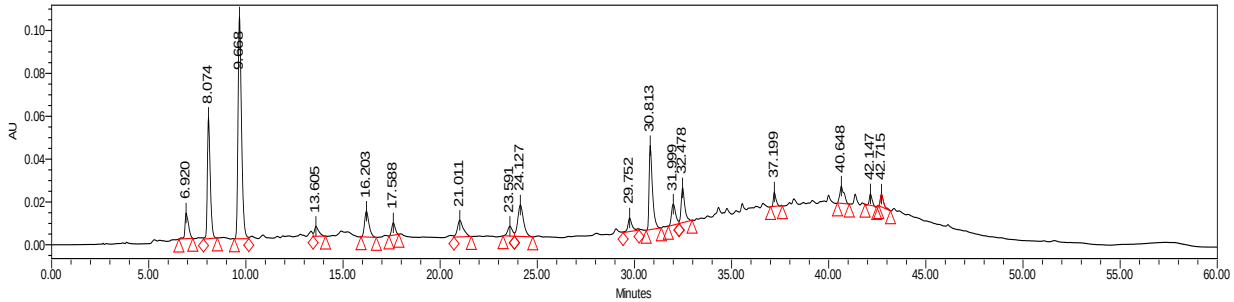


**Figure 142.** HPLC@280 nm, charred layer 3, reflux rep 2, run 1

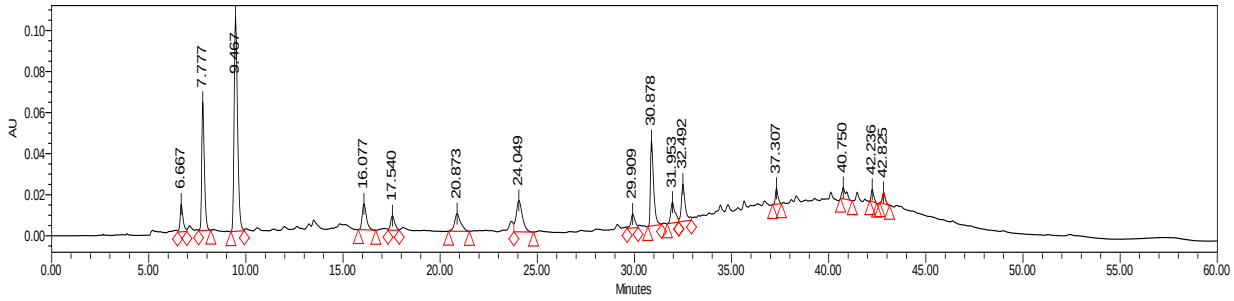


**Figure 143.** HPLC@280 nm, charred layer 3, reflux rep 2, run 2

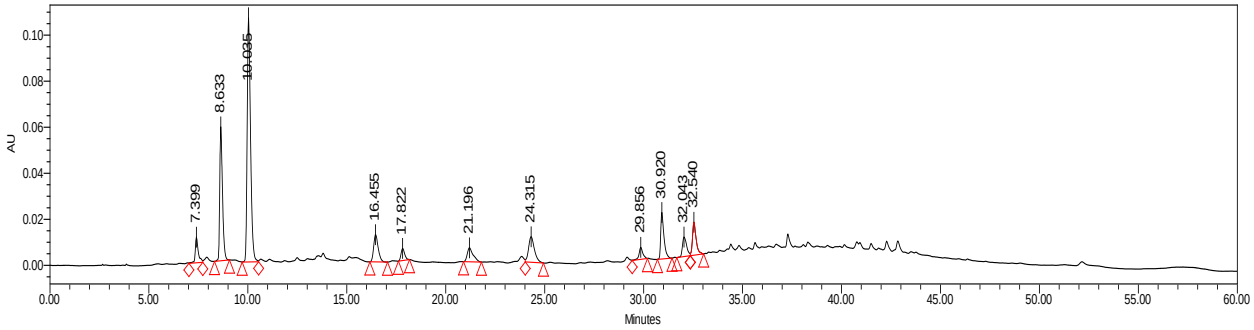


**Figure 144.** HPLC@280 nm, charred layer 3, control rep 1, run 1

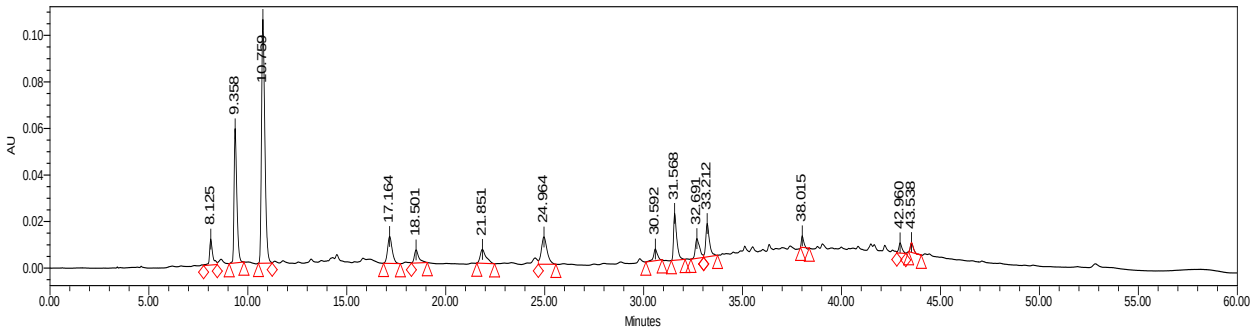


**Figure 145.** HPLC@280 nm, charred layer 3, control rep 1, run 2

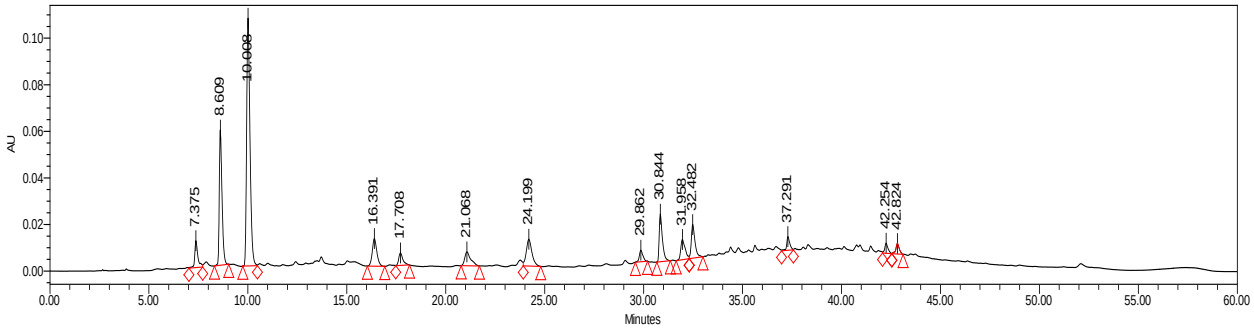


**Figure 146.** HPLC@280 nm, charred layer 3, control rep 2, run 1

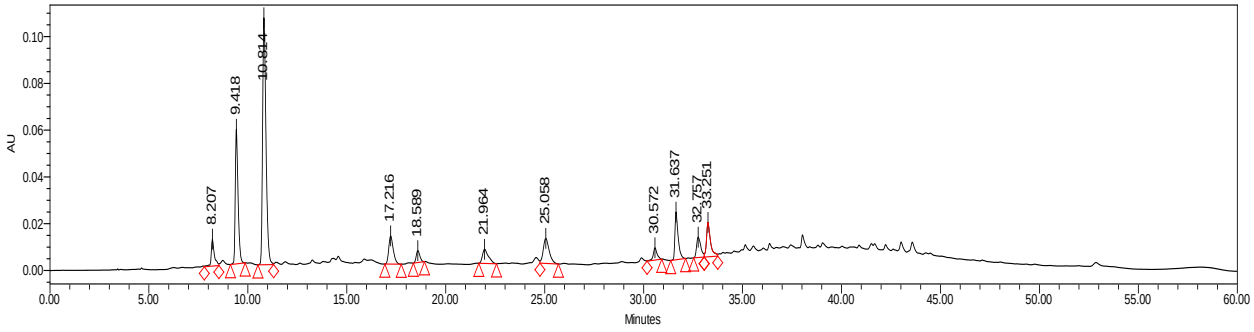


**Figure 147.** HPLC@280 nm, charred layer 3, control rep 2, run 2

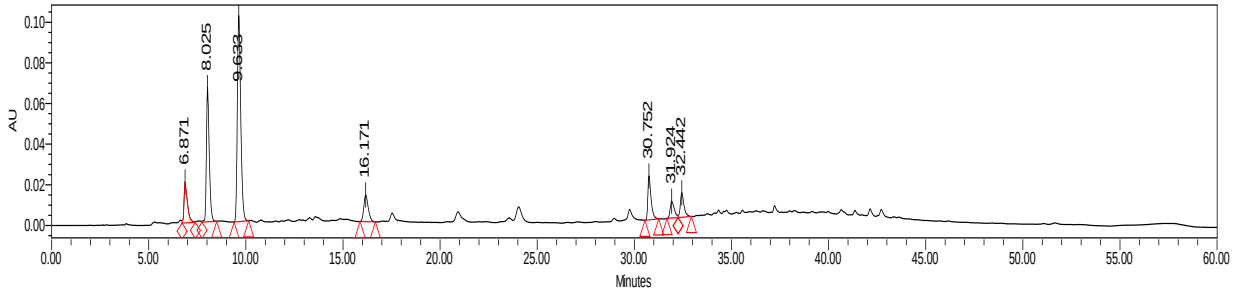


**Figure 148.** HPLC @280 nm, charred layer 4, sonicated rep 1, run 1

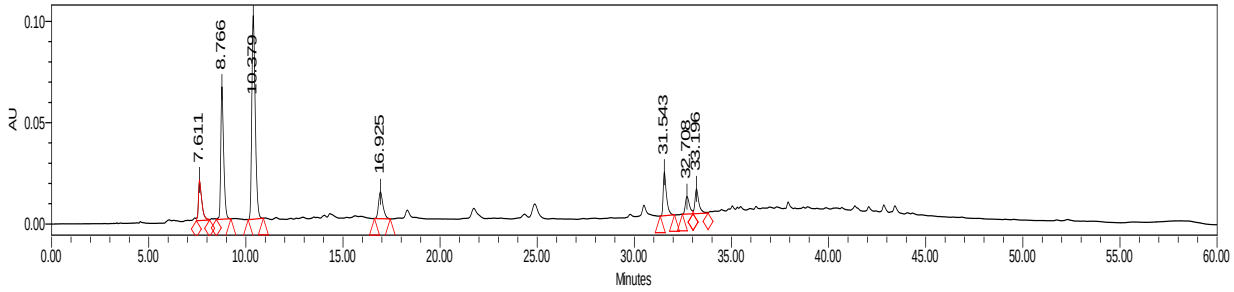


**Figure 149.** HPLC@280 nm, charred layer 4, sonicated rep 1, run 2

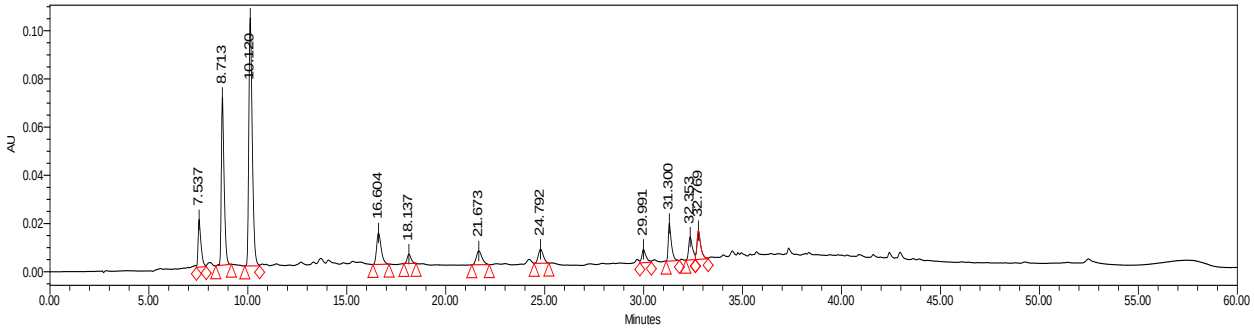


**Figure 150.** HPLC@280 nm, charred layer 4, sonicated rep 2, run 1

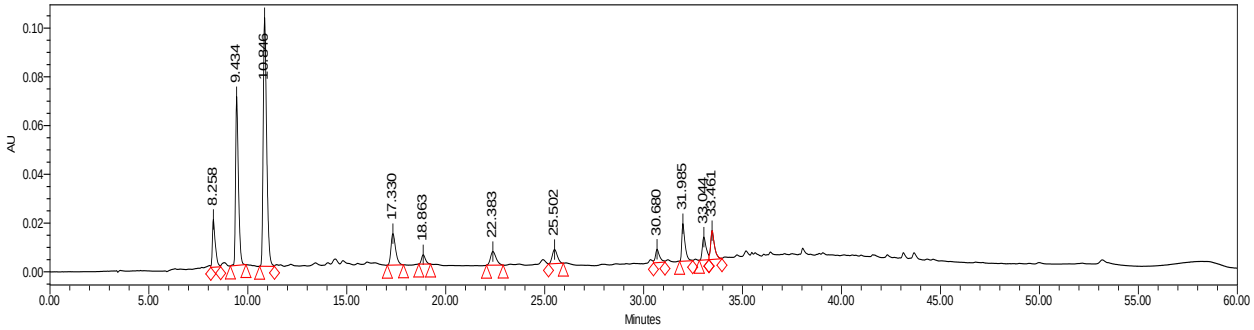


**Figure 151.** HPLC@280 nm, charred layer 4, sonicated rep 2, run 2

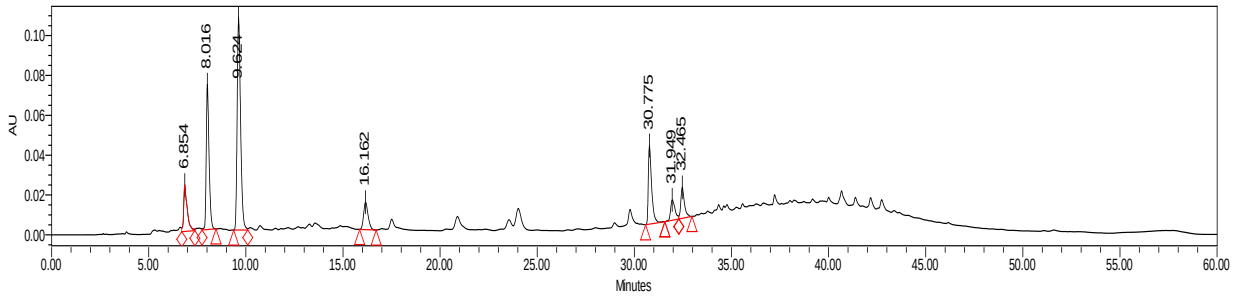


**Figure 152.** HPLC @280 nm, charred layer 4, reflux rep 1, run 1

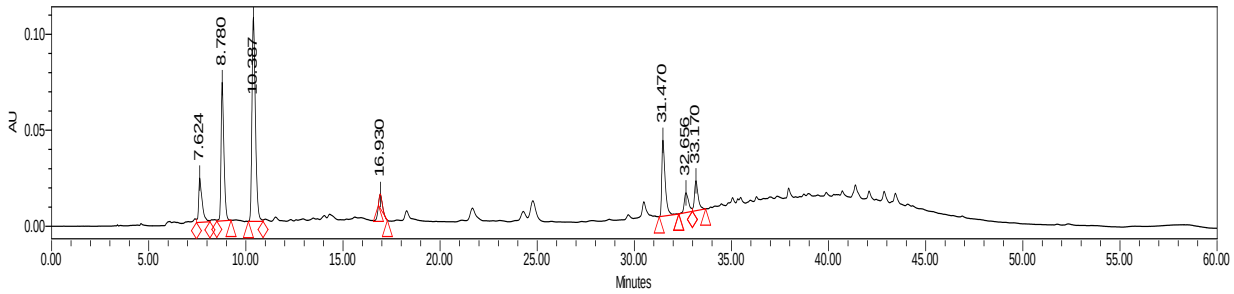


**Figure 153.** HPLC @280 nm, charred layer 4, reflux rep 1, run 2

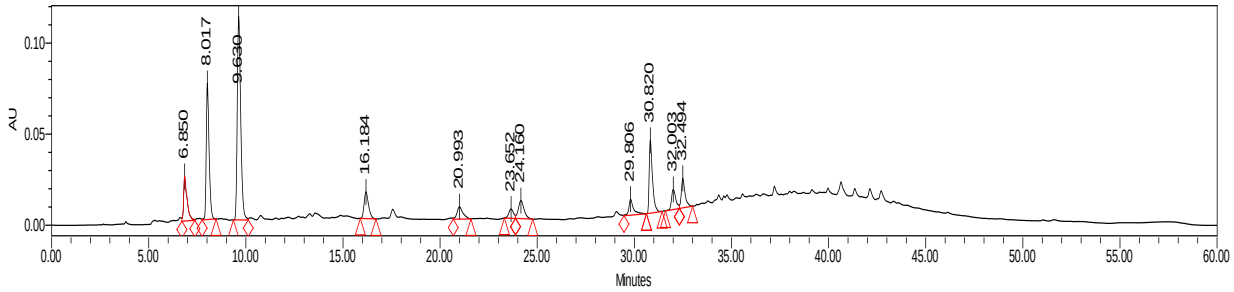


**Figure 154.** HPLC @280 nm, charred layer 4, reflux rep 2, run 1

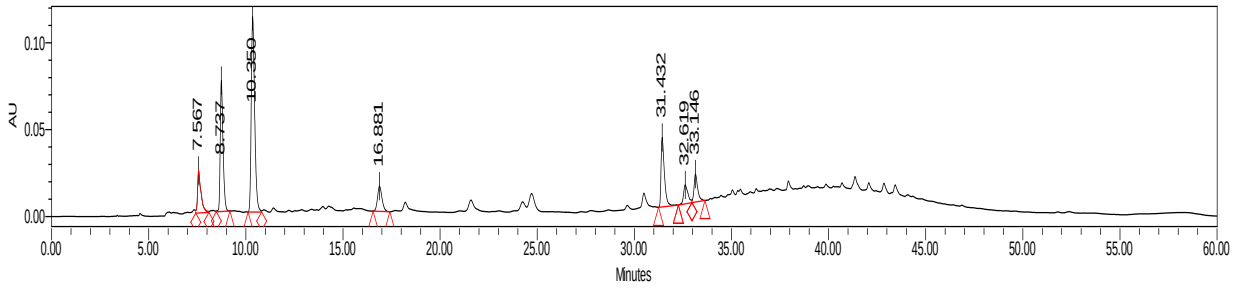


**Figure 155.** HPLC @280 nm, charred layer 4, reflux rep 2, run 2

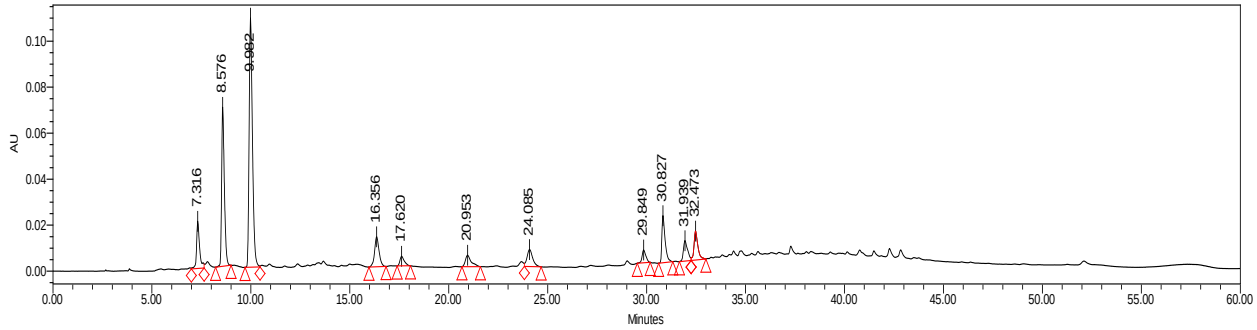


**Figure 156.** HPLC @280 nm, charred layer 4, control rep 1, run 1

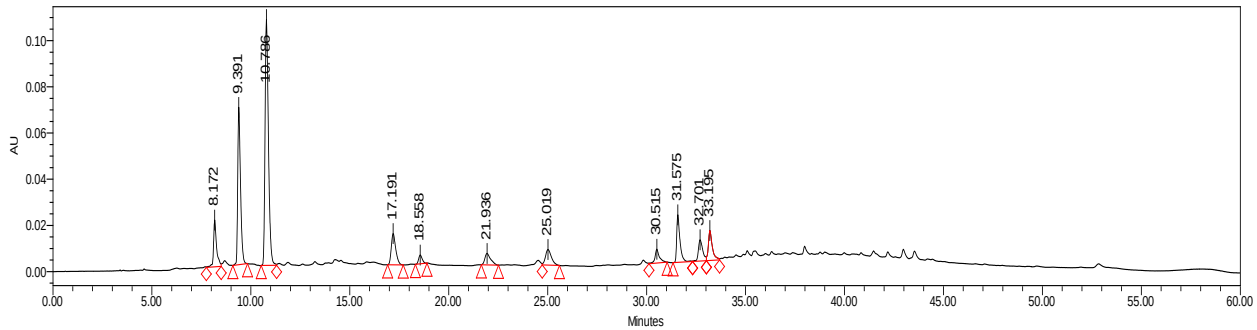


**Figure 157.** HPLC@280 nm, charred layer 4, control rep 1, run 2

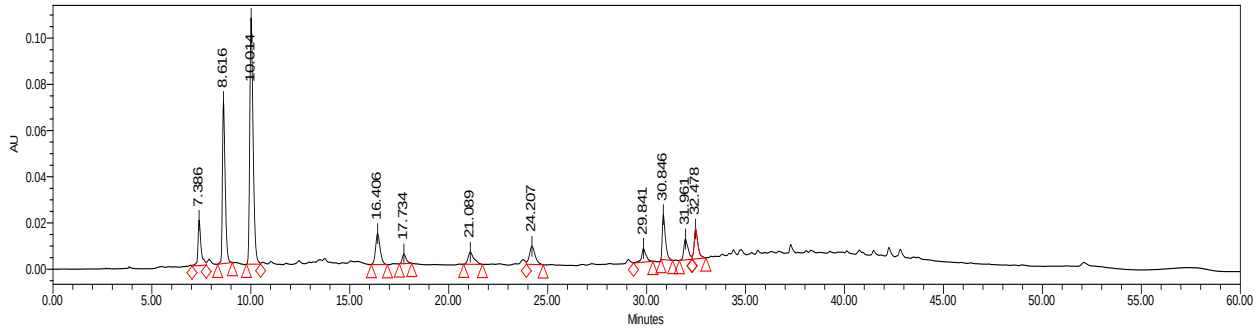


**Figure 158.** HPLC@280 nm, charred layer 4, control rep 2, run 1

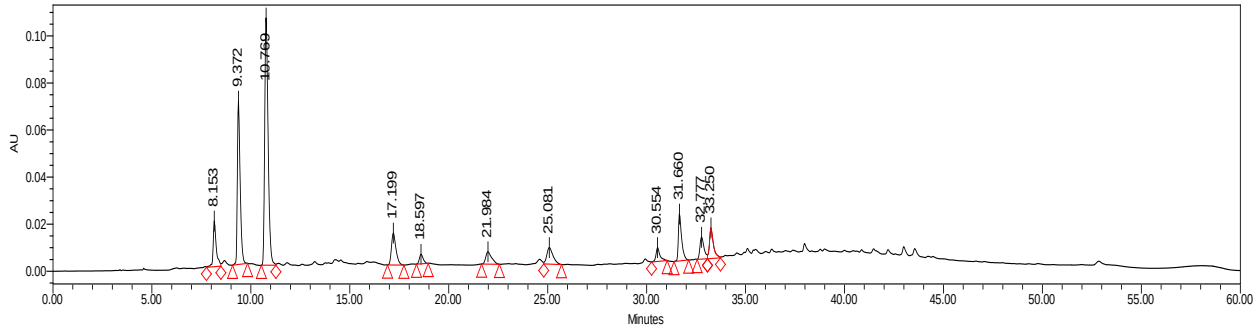


**Figure 159.** HPLC @280 nm, charred layer 4, control rep 2, run 2

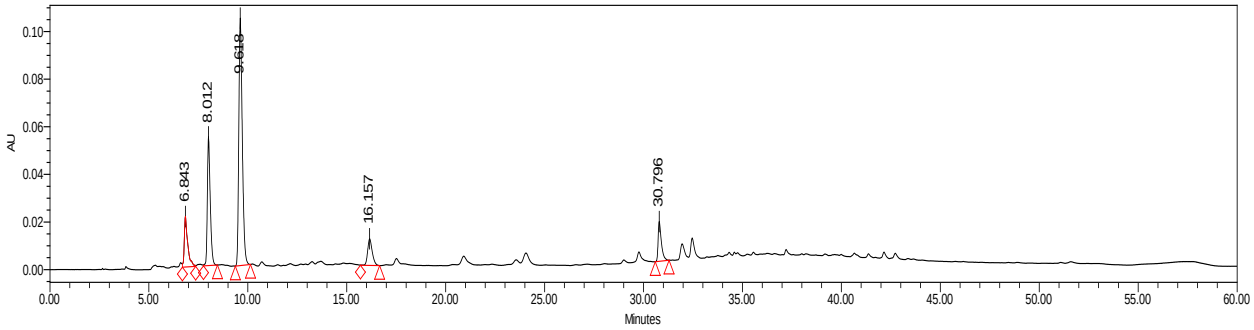


**Figure160.** HPLC @280 nm, charred layer 5, sonicated rep 1, run 1

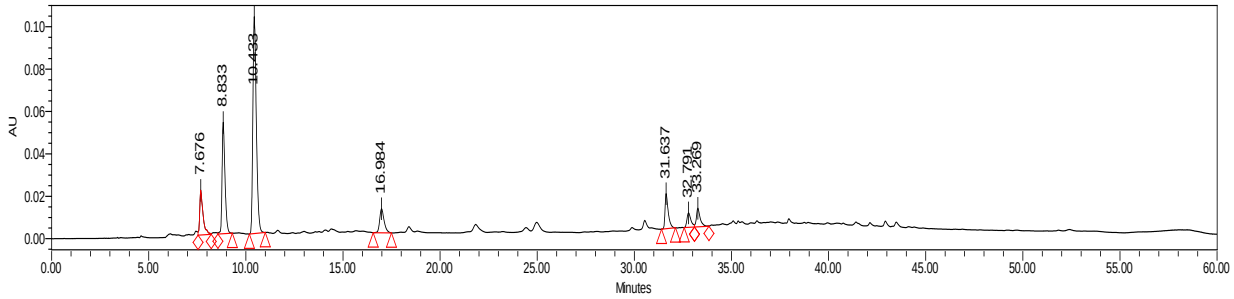


**Figure 161.** HPLC @280, charred layer 5, sonicated rep 1, run 2

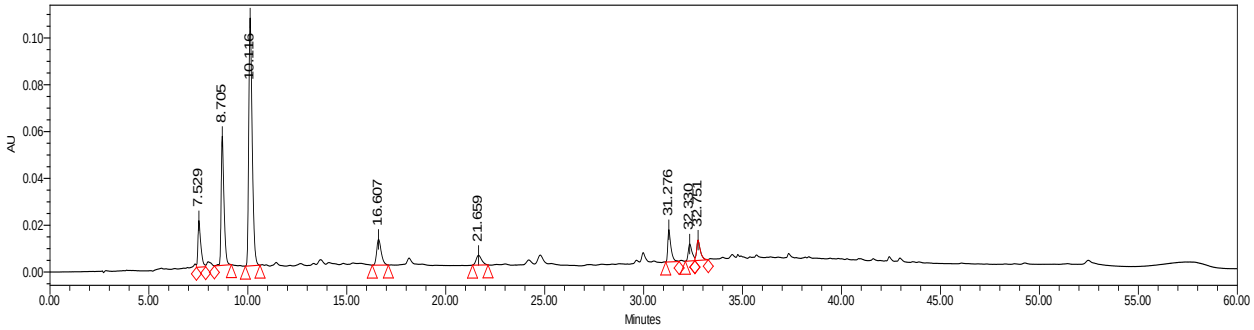


**Figure 162.** HPLC@280 nm, charred layer 5, sonicated rep 2, run 1

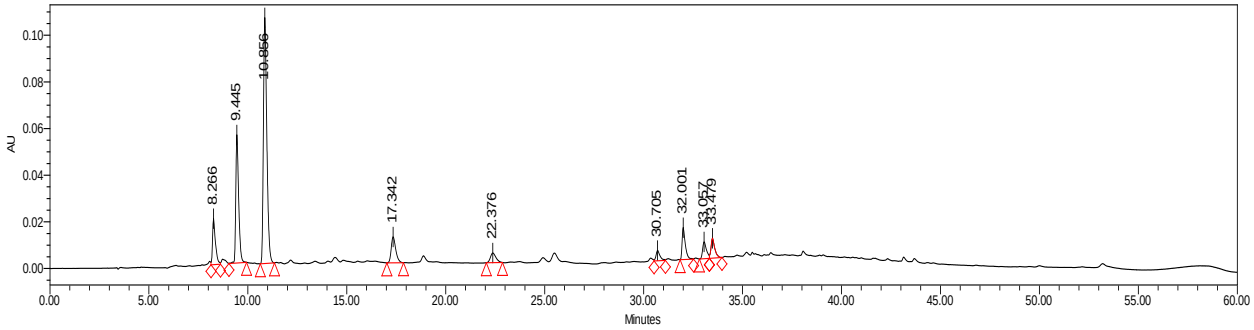


**Figure 163.** HPLC@280 nm, charred layer 5, sonicated rep 2, run 2

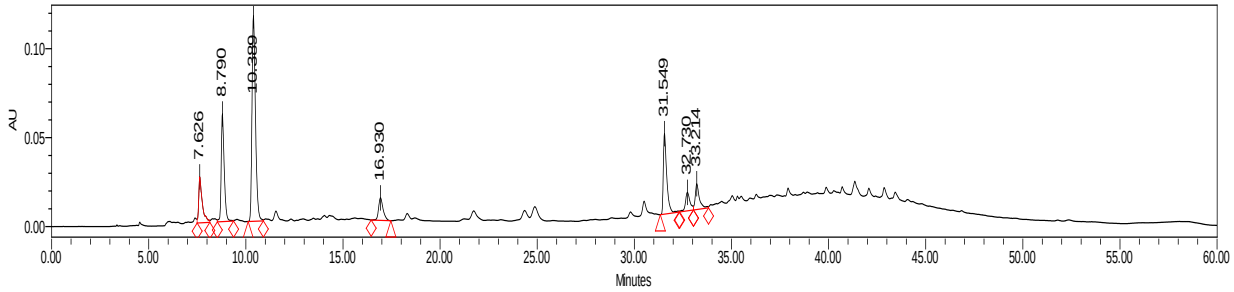


**Figure 164.** HPLC @280 nm, charred layer 5, reflux rep 1, run 1

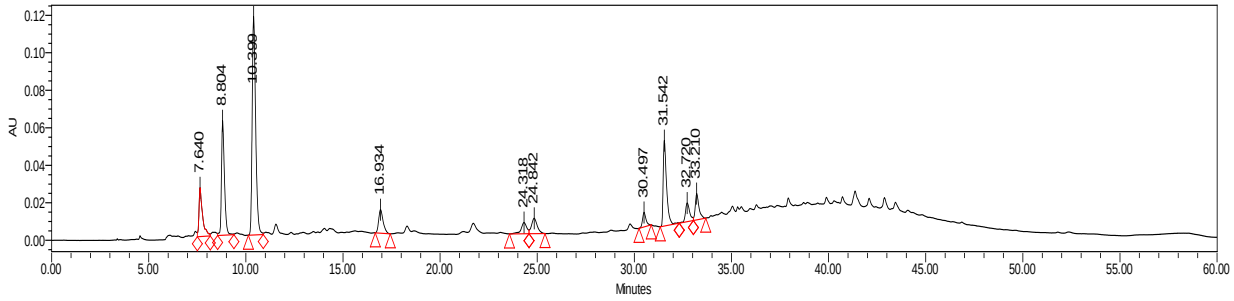


**Figure 165.** HPLC@280 nm, charred layer 5, reflux rep 1, run 2

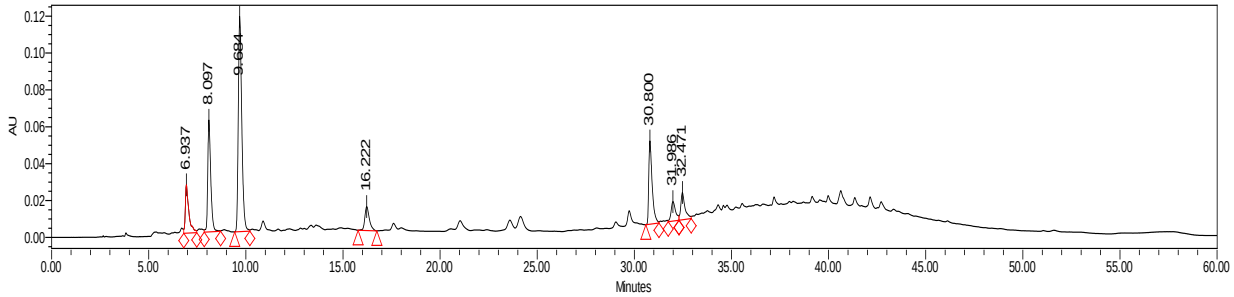


**Figure 166.** HPLC @280 nm, charred layer 5, reflux rep 2, run 1

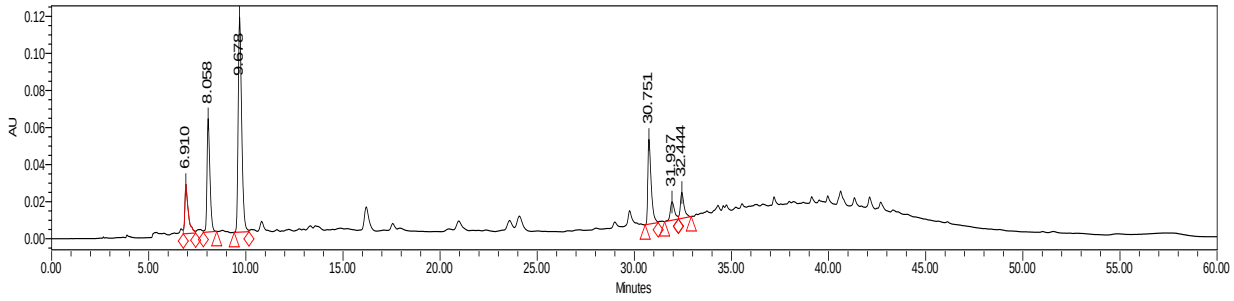


**Figure 167.** HPLC @280 nm, charred layer 5, reflux rep 2, run 2

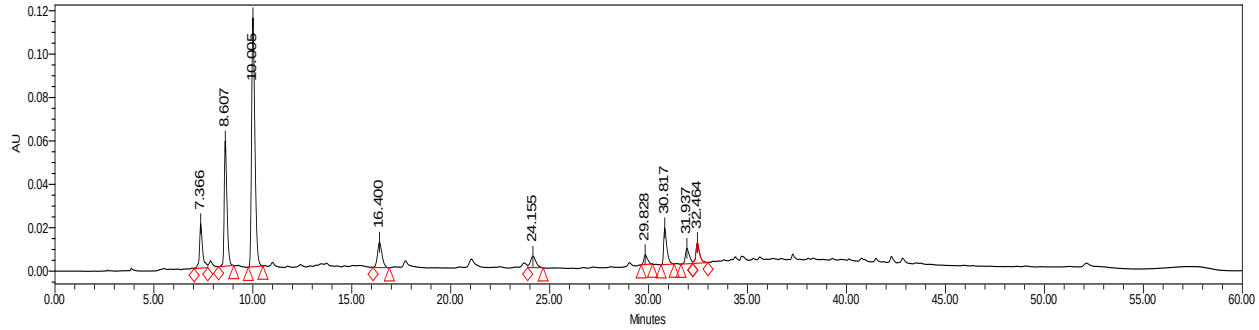


**Figure 168.** HPLC@280 nm, charred layer 5, control rep 1, run 1

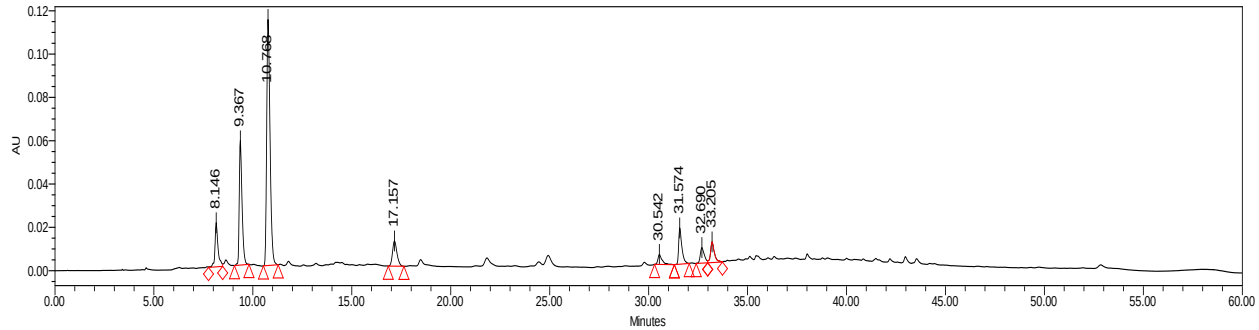


**Figure 169.** HPLC@280 nm, charred layer 5, control rep 1, run 2

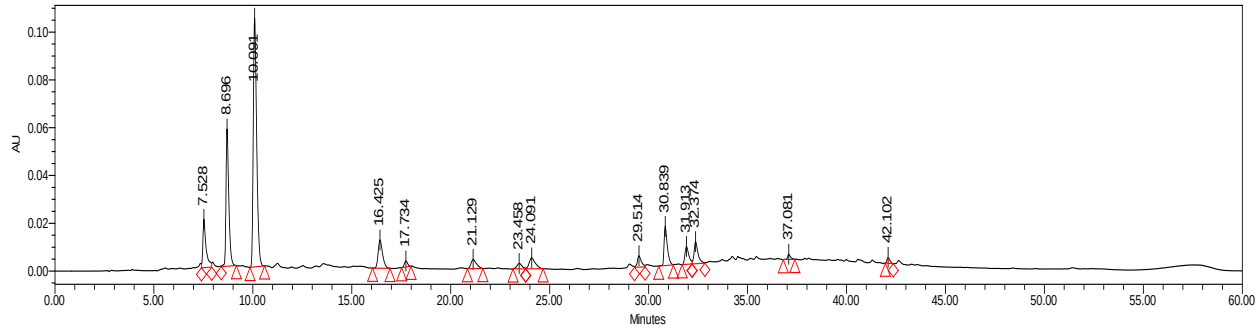


**Figure 170.** HPLC@280 nm, charred layer 5, control rep 2, run 1

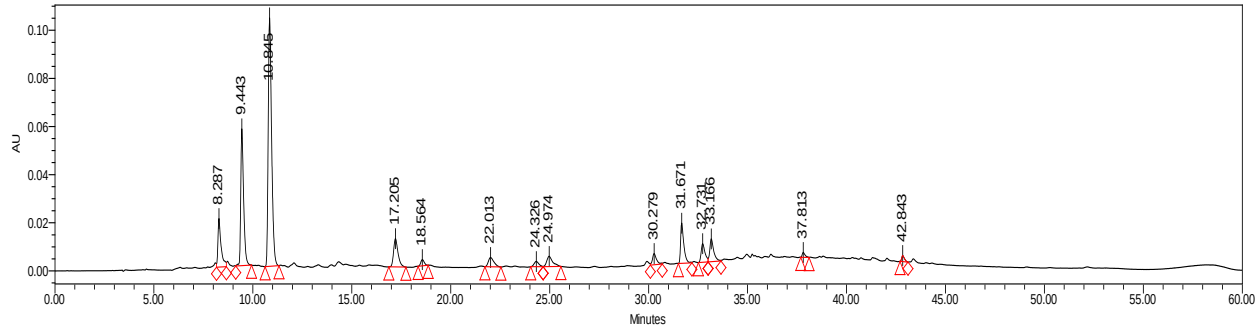


**Figure 171.** HPLC@280 nm, charred layer 5, control rep 2, run 2

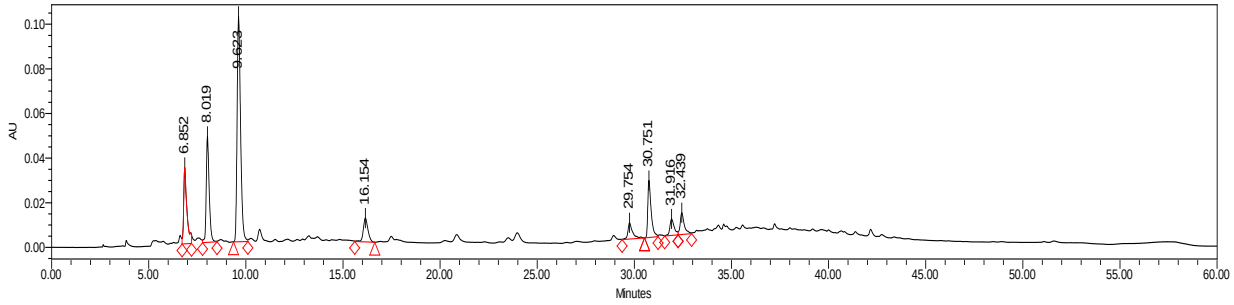


**Figure 172.** HPLC @280 nm, charred layer 6, sonicated rep 1, run 1

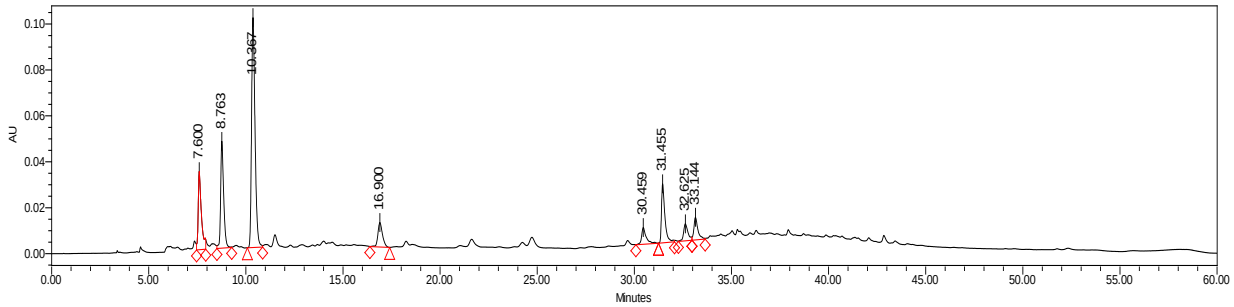


**Figure 173.** HPLC @280 nm, charred layer 6, sonicated rep 1, run 2

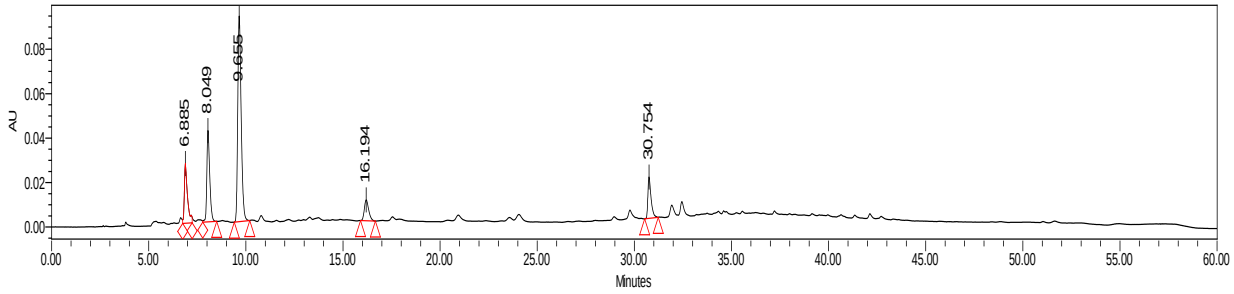


**Figure 174.** HPLC@280 nm, charred layer 6, sonicated rep 2, run 1

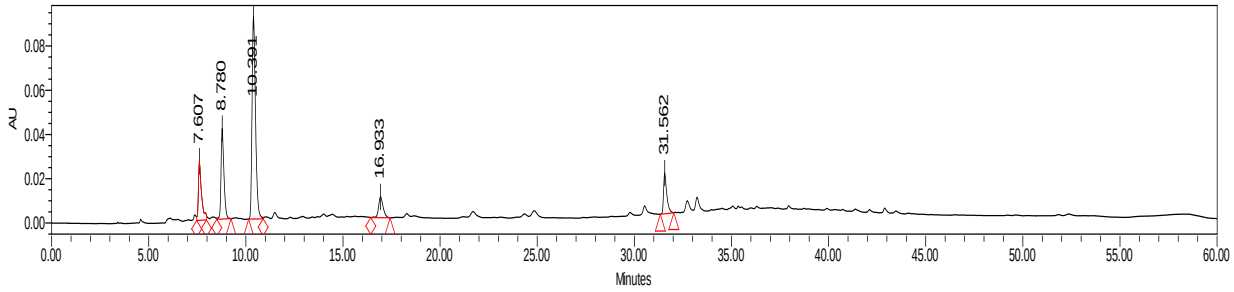


**Figure 175.** HPLC @280 nm, charred layer 6, sonicated rep 2, run 2

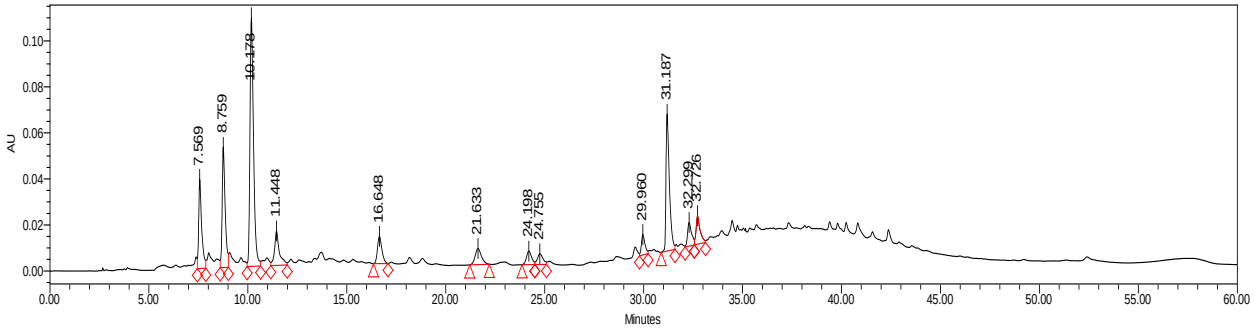


**Figure 176.** HPLC@280 nm, charred layer 6, reflux rep 1, run 1

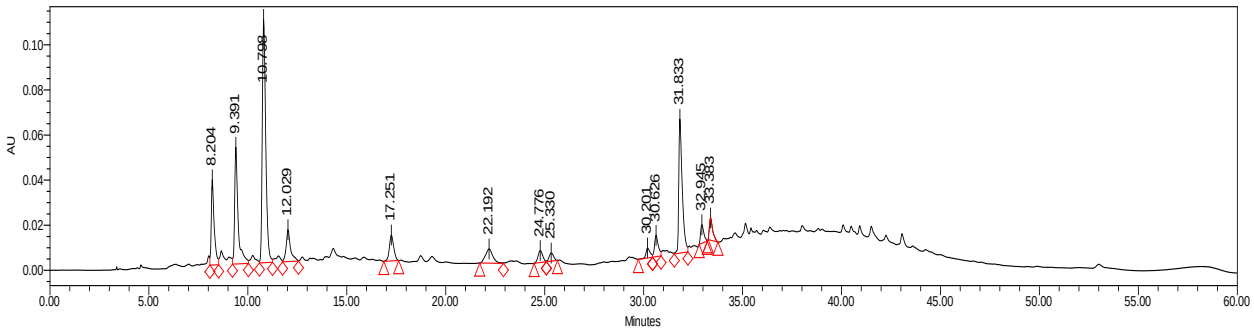


**Figure 177.** HPLC @280 nm, charred layer 6, reflux rep 1, run 2

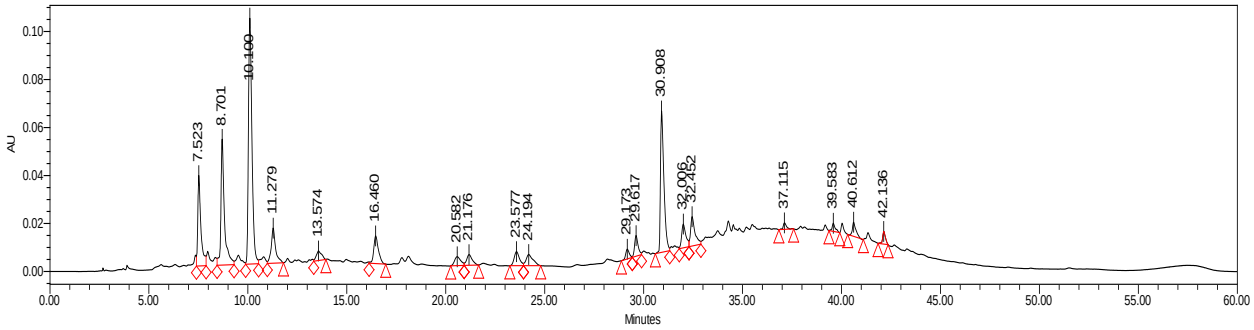


**Figure 178.** HPLC@280 nm, charred layer 6, reflux rep 2, run 1

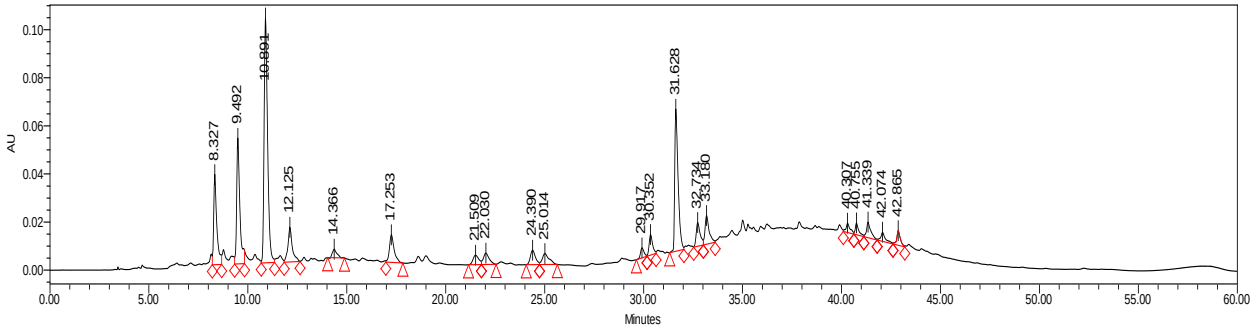


**Figure 179.** HPLC@280 nm, charred layer 6, reflux rep 2, run 2

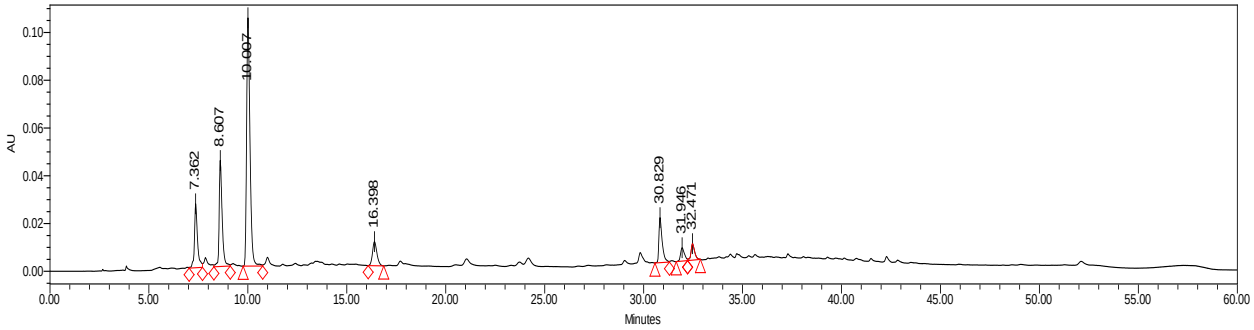


**Figure 180.** HPLC@280 nm, charred layer 6, control rep 1, run 1

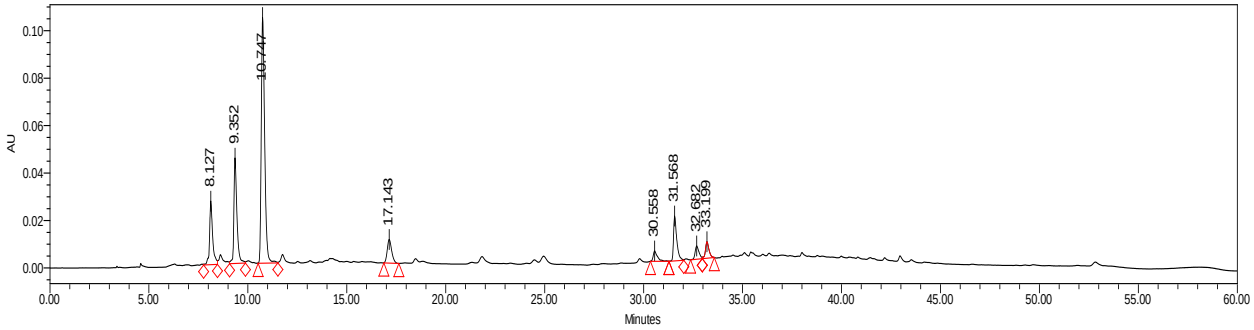


**Figure 181.** HPLC @280 nm, charred layer 6, control rep 1, run 2

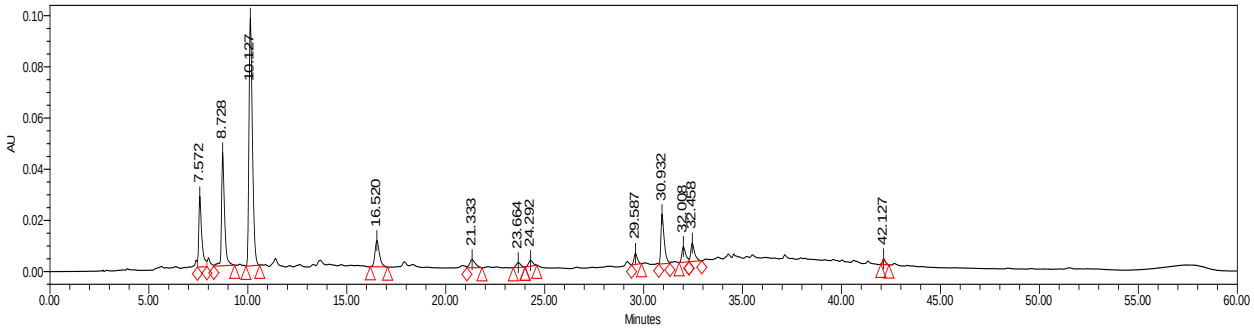


**Figure 182.** HPLC@280 nm, charred layer 6, control rep 2, run 1

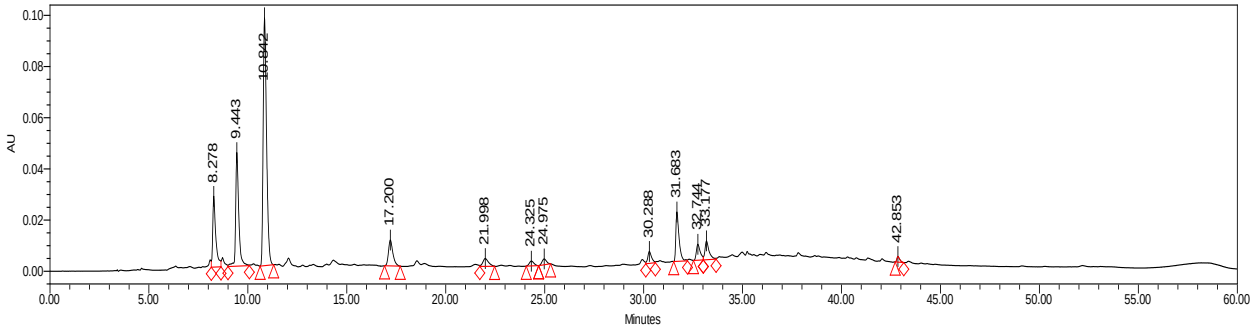


**Figure 183.** HPLC@280 nm, charred layer 6, control rep 2, run 2

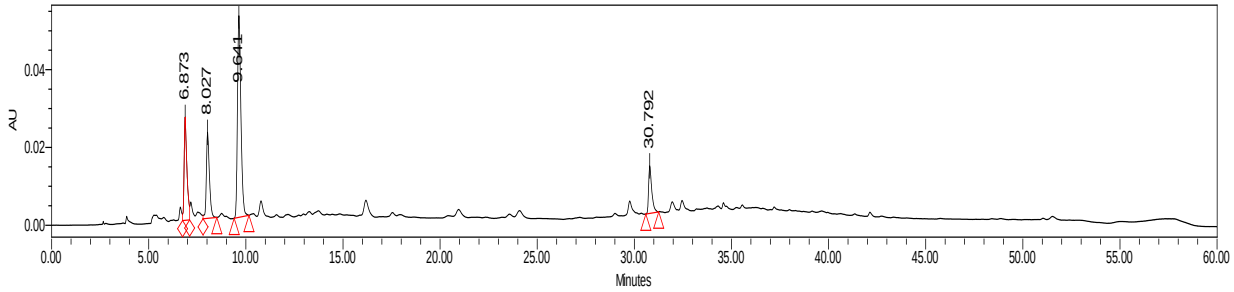


**Figure 184.** HPLC@280 nm, charred layer 7, sonicated rep 1, run 1

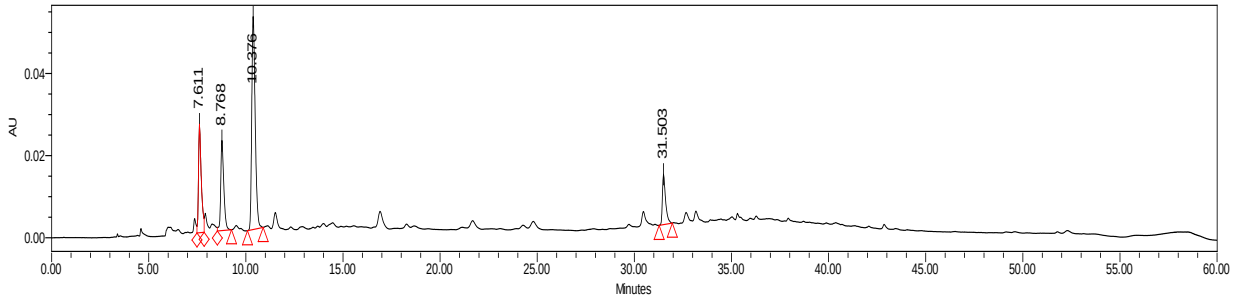


**Figure 185.** HPLC@280 nm, charred layer 7, sonicated rep 1, run 2

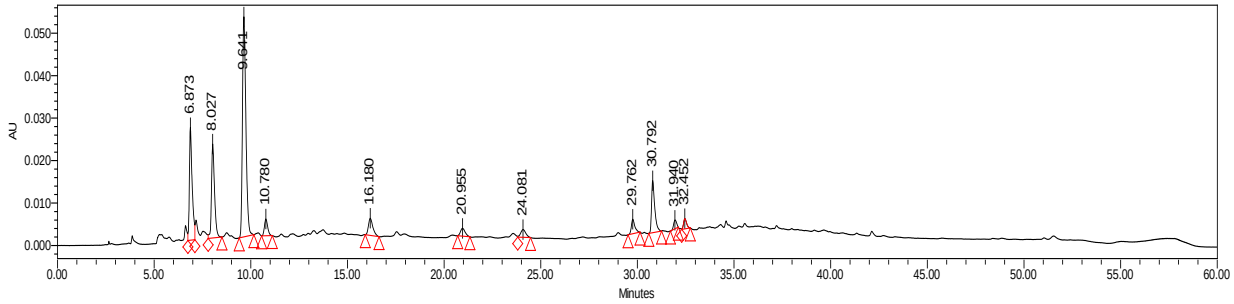


**Figure 186.** HPLC@280 nm, charred layer 7, sonicated rep 2, run 1

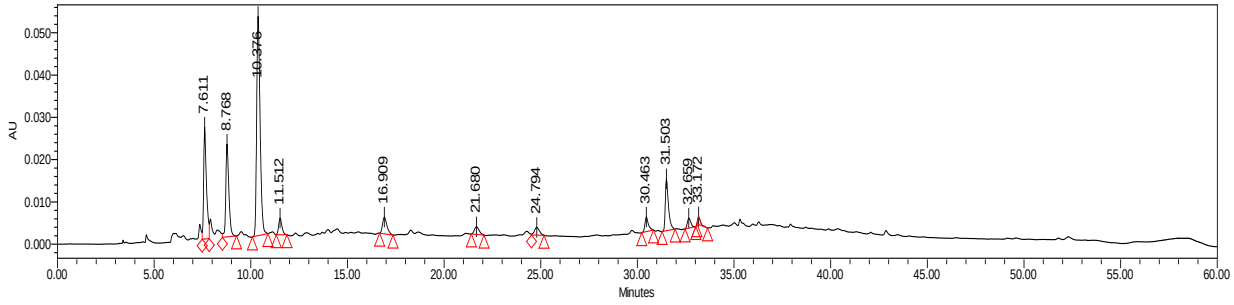


**Figure 187.** HPLC@280 nm, charred layer 7, sonicated rep 2, run 2

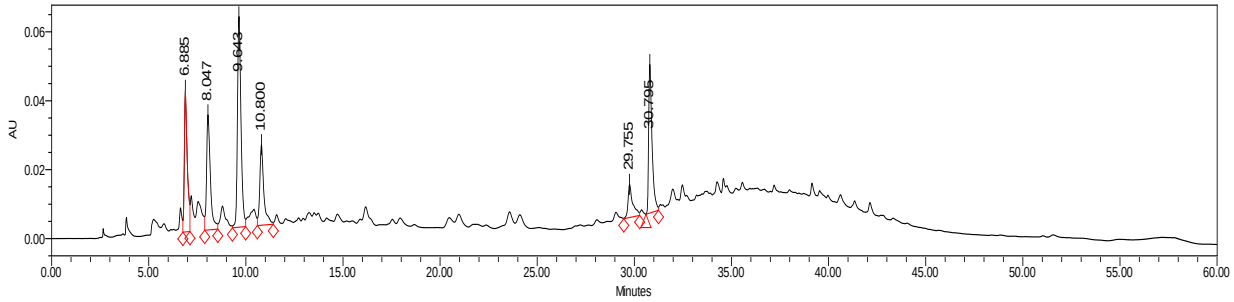


**Figure 188.** HPLC@280 nm, charred layer 7, reflux rep 1, run 1

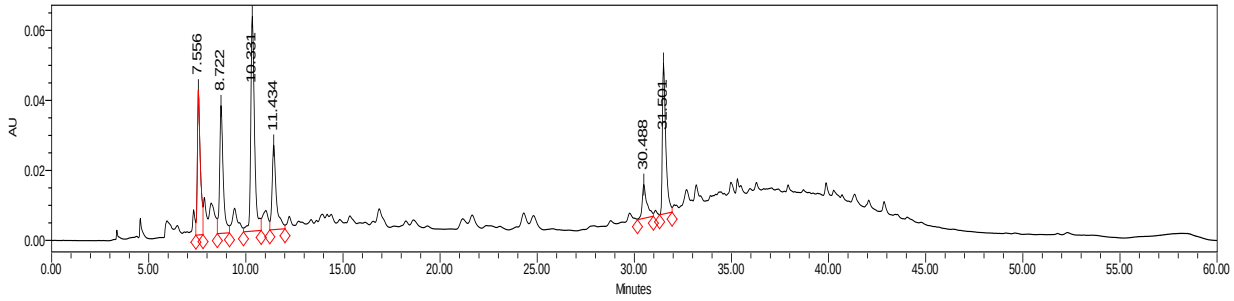


**Figure 189.** HPLC@280 nm, charred layer 7, reflux rep 1, run 2

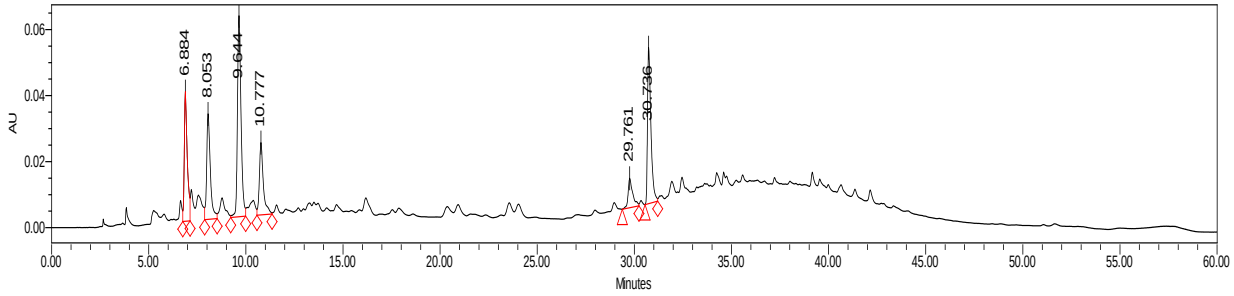


**Figure 190.** HPLC@280 nm, charred layer 7, reflux rep 2, run 1

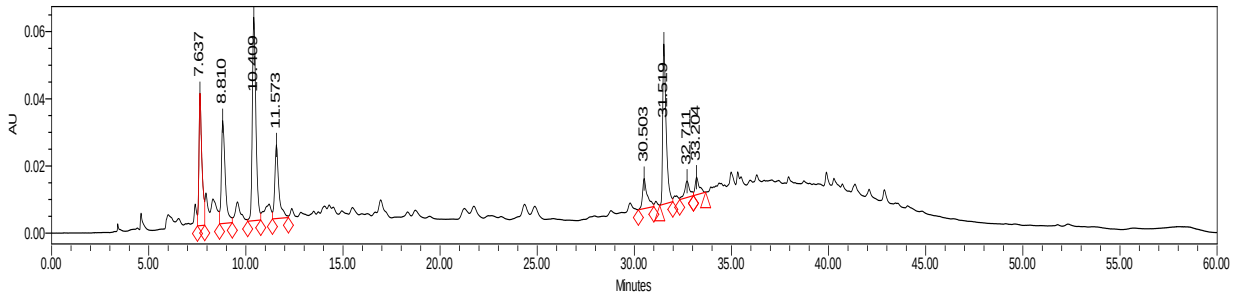


**Figure 191.** HPLC @ 280 nm, charred layer 7, reflux rep 2, run 2 time 0

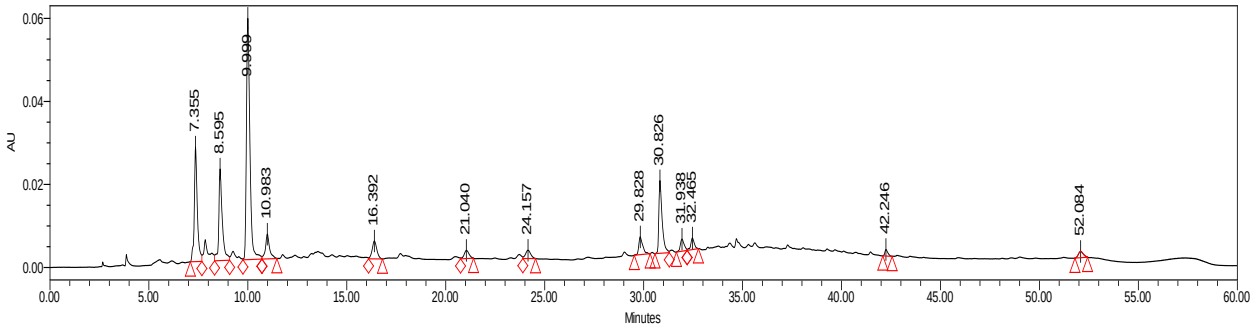


**Figure 192.** HPLC@280 nm, charred layer 7, control rep 1, run 1

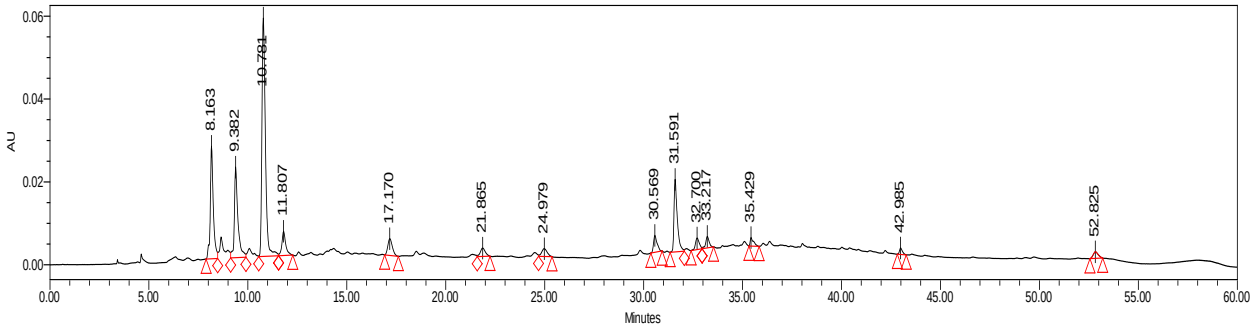


**Figure 193.** HPLC@280 nm, charred layer 7, control rep 1, run 2

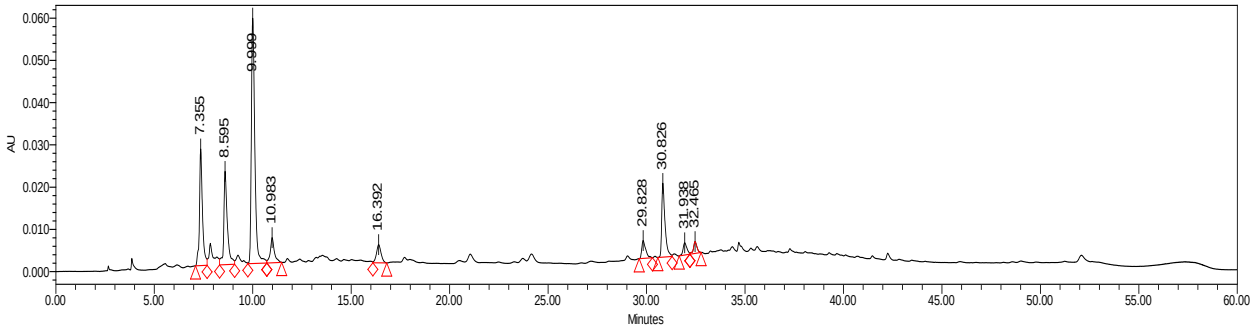


**Figure 194.** HPLC@280 nm, charred layer 7, control rep 2, run 1

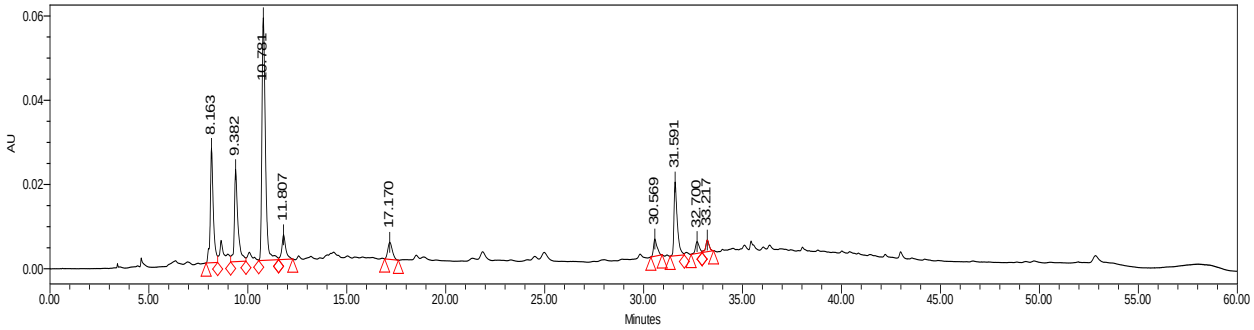


**Figure 195.** HPLC@280 nm, charred layer 7, control rep 2, run 2

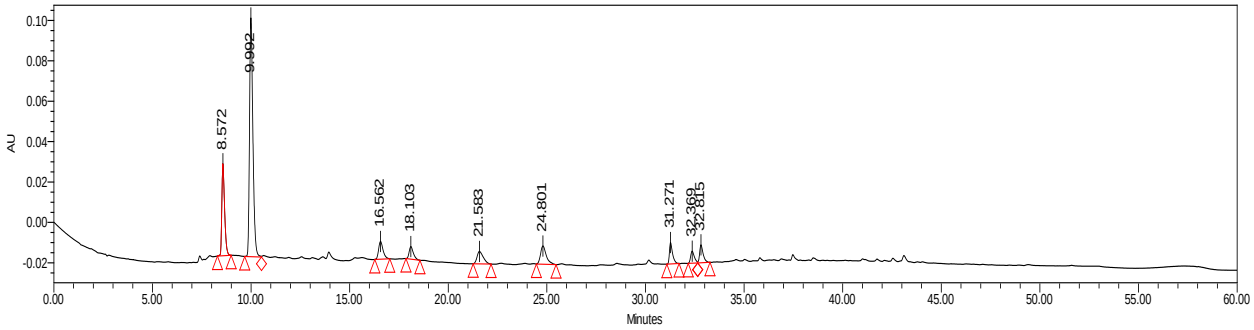


**Figure 196.** HPLC@280 nm, toasted layer 1, sonicated rep 1, run 1

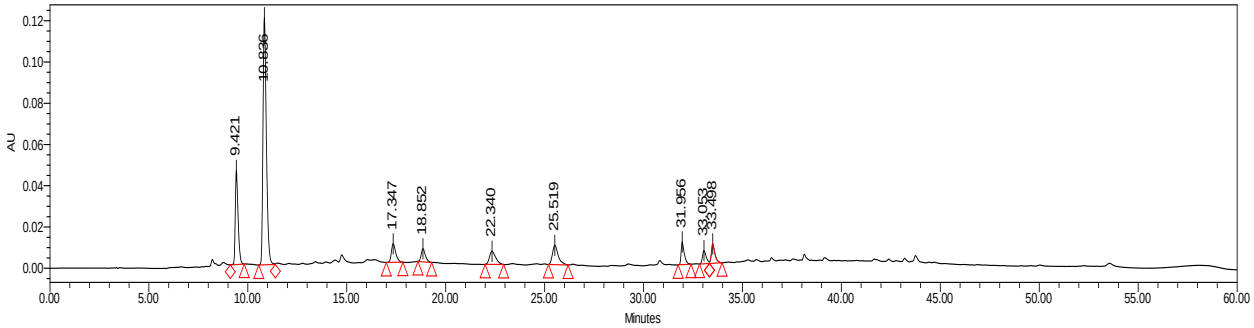


**Figure 197.** HPLC@280 nm, toasted layer 1, sonicated rep 1, run 2

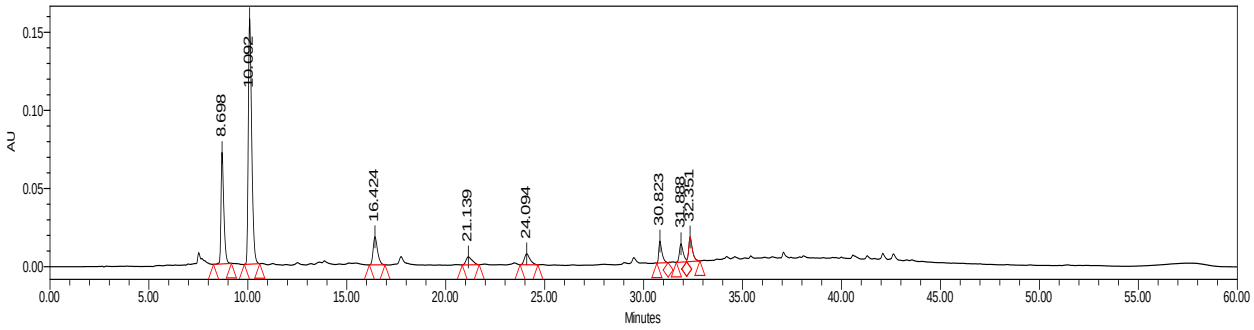


**Figure 198.** HPLC @280 nm, toasted layer 1, sonicated rep 2, run 1

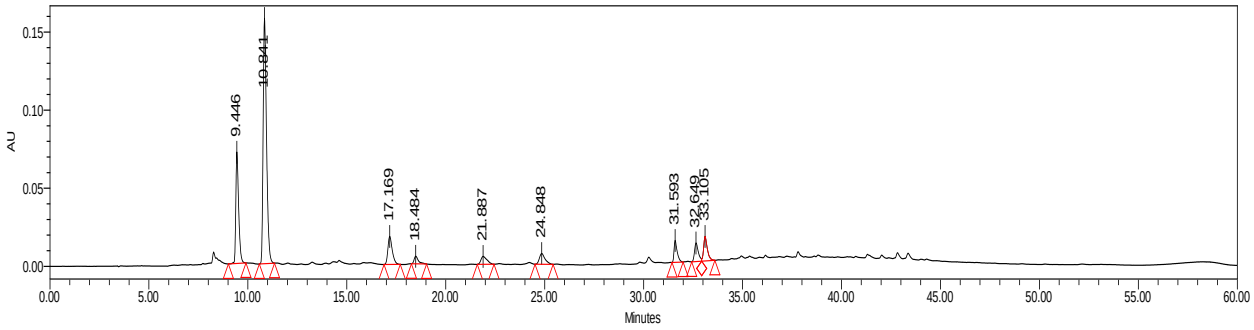


**Figure 199.** HPLC@280 nm, toasted layer 1, sonicated rep 2, run 1

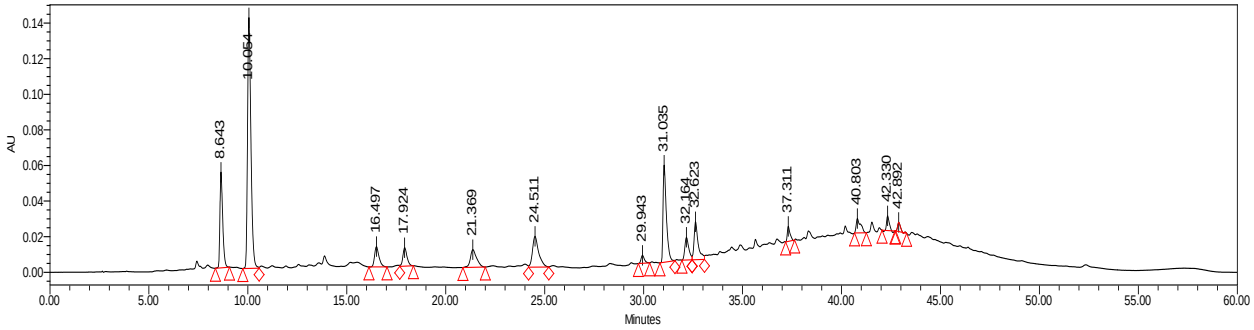


**Figure 200.** HPLC@280 nm, toasted layer 1, reflux rep 1, run 1

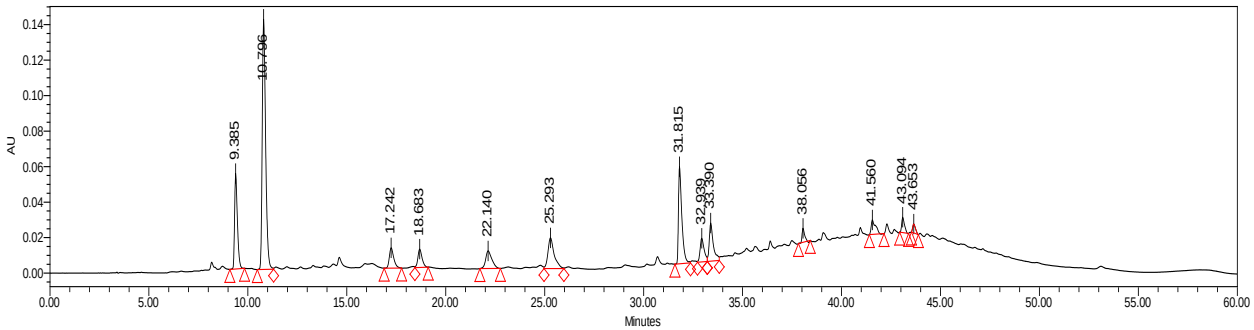


**Figure 201.** HPLC@280 nm, toasted layer 1, reflux rep 1, run 2

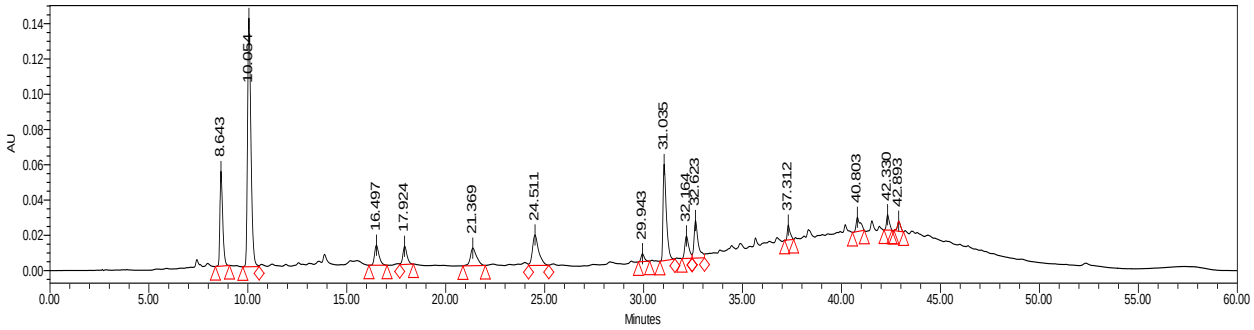


**Figure 202.** HPLC@280 nm, toasted layer 1, reflux rep 2, run 1

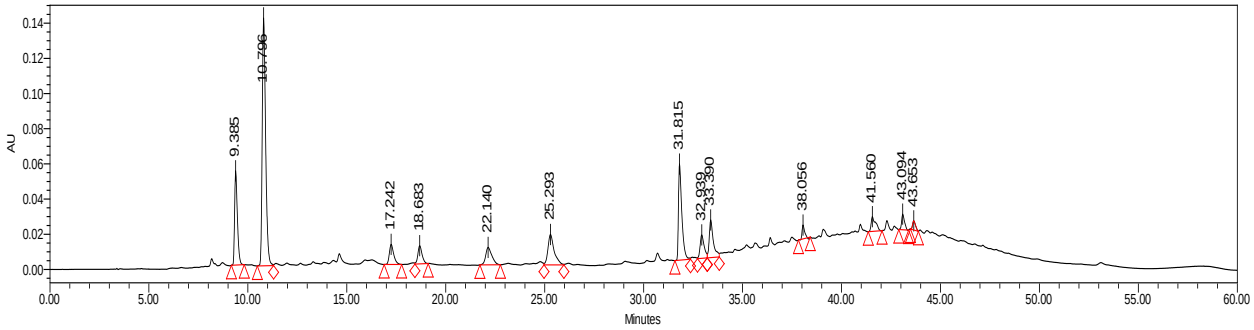


**Figure 203.** HPLC@280 nm, toasted layer 1, reflux rep 2, run 2

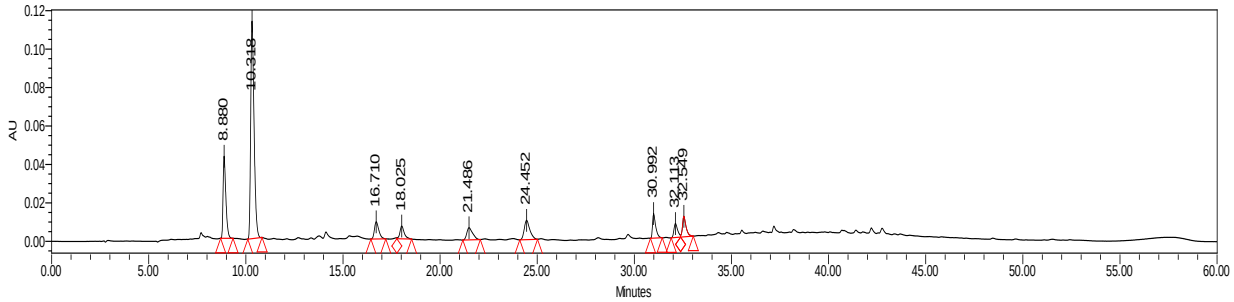


**Figure 204.** HPLC @280 nm, toasted layer 1, control rep 1, run 1, time 0

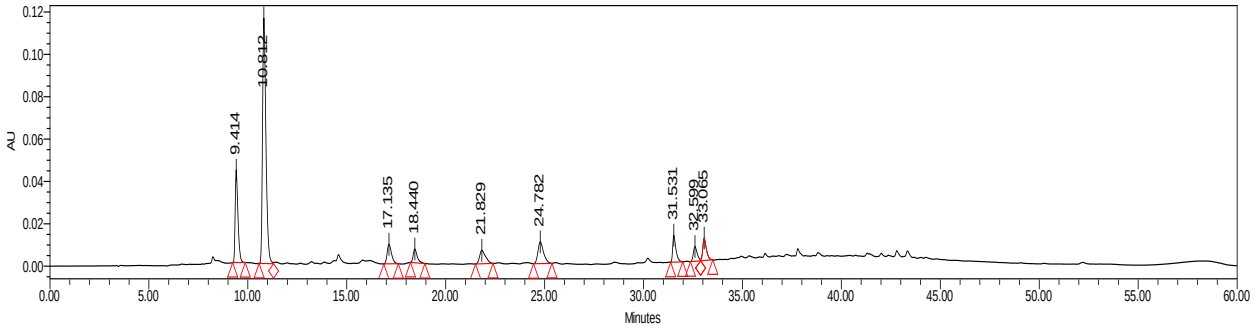


**Figure 205.** HPLC @280 nm, toasted layer 1, control rep 1, run 2, time 0

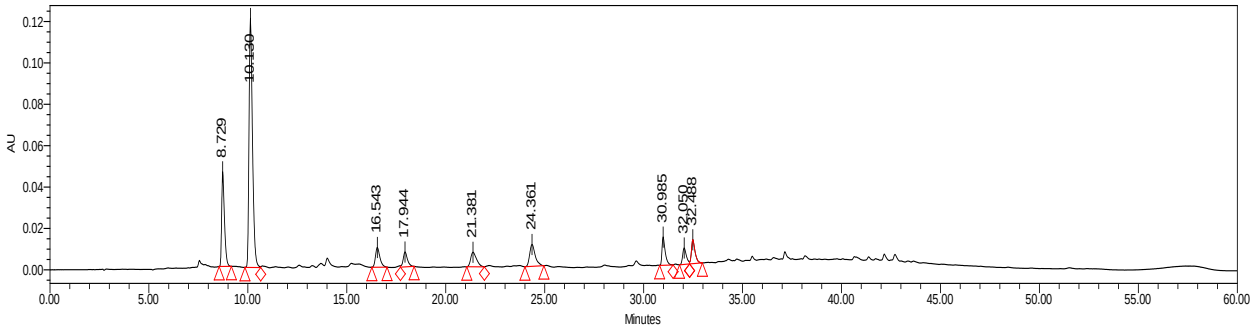


**Figure 206.** HPLC@280 nm, toasted layer 1, control rep 2, run 1

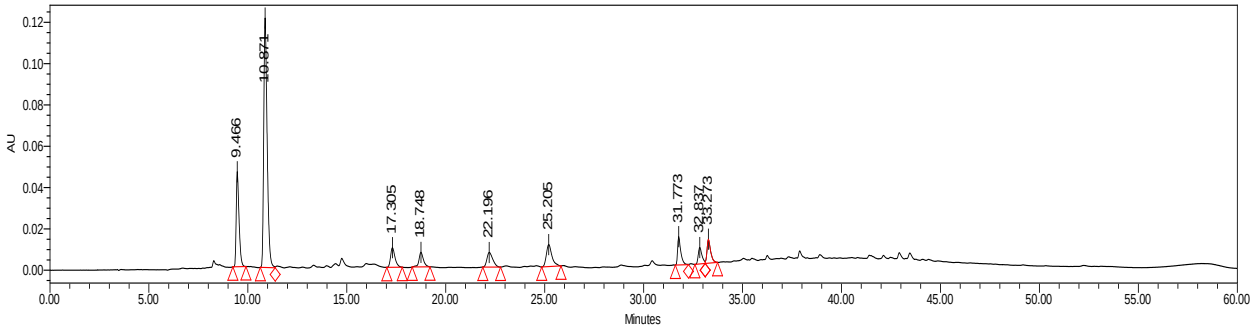


**Figure 207.** HPLC@280 nm, toasted layer 1, control rep 1, run

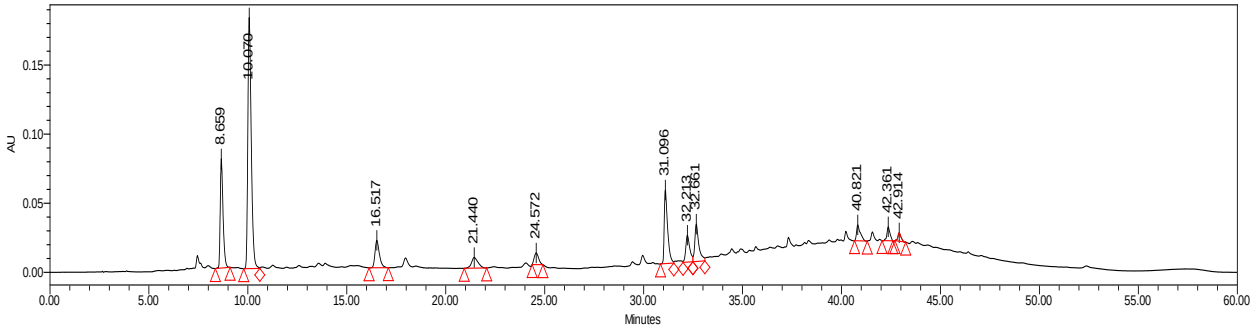


**Figure 208.** HPLC@280 nm, toasted layer 2, reflux rep 1, run 1

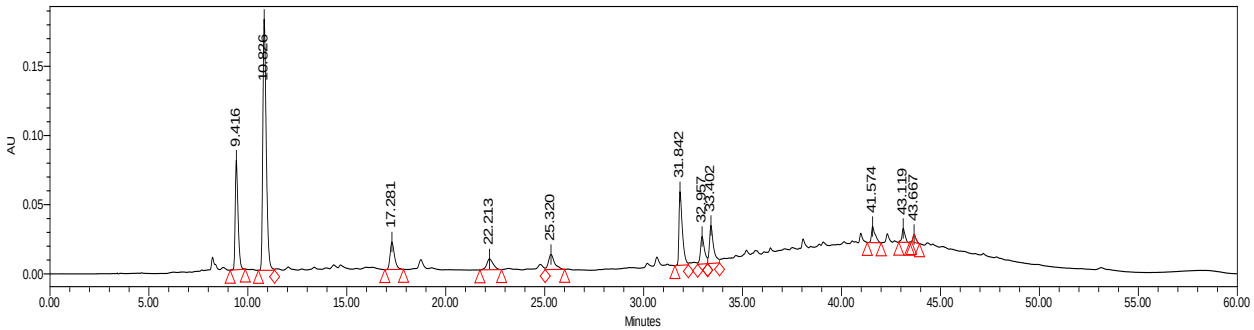


**Figure 209.** HPLC@280 nm, toasted layer 2, reflux rep 1, run 2

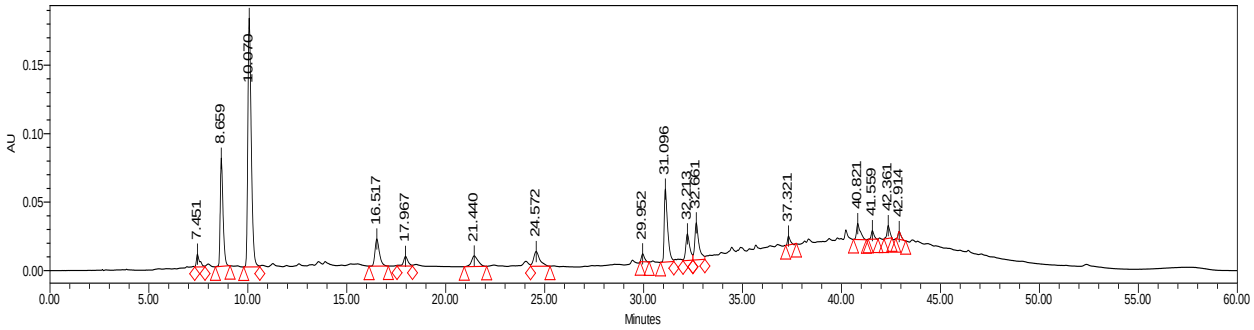


**Figure 210.** HPLC@280 nm, toasted layer 2, reflux rep 2, run 1

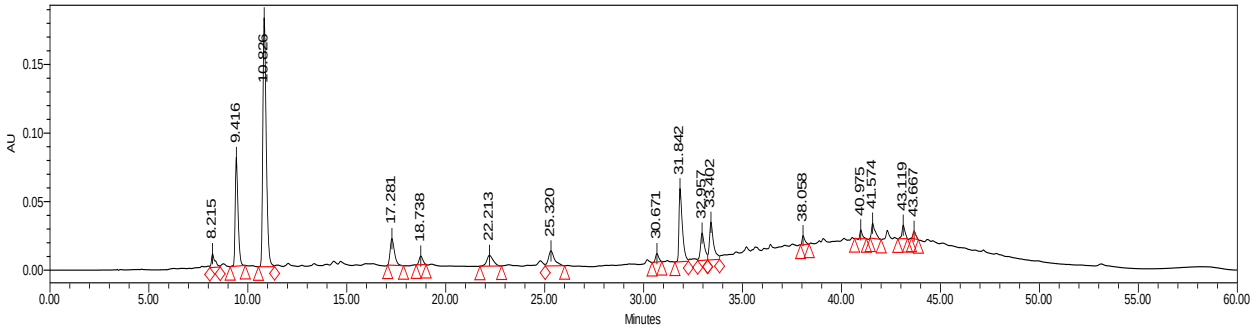


**Figure 211.** HPLC@280 nm, toasted layer 2, reflux rep 2, run 2

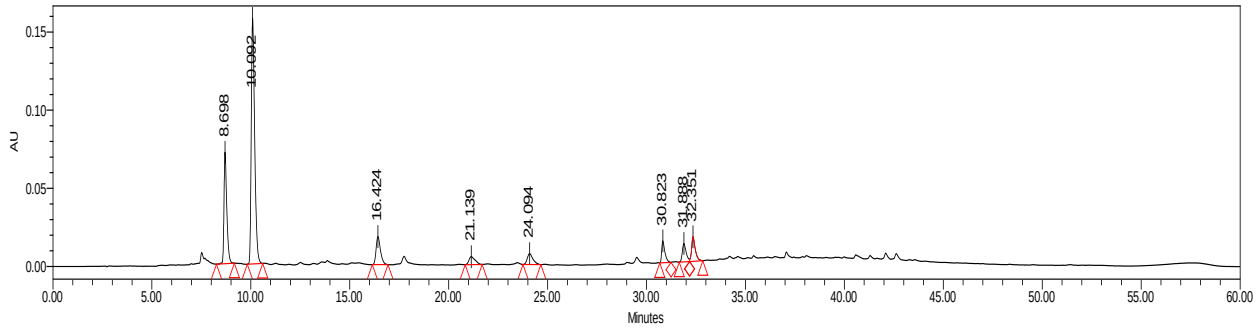


**Figure 212.** HPLC @280nm, toasted layer 2, control rep 1, run 1

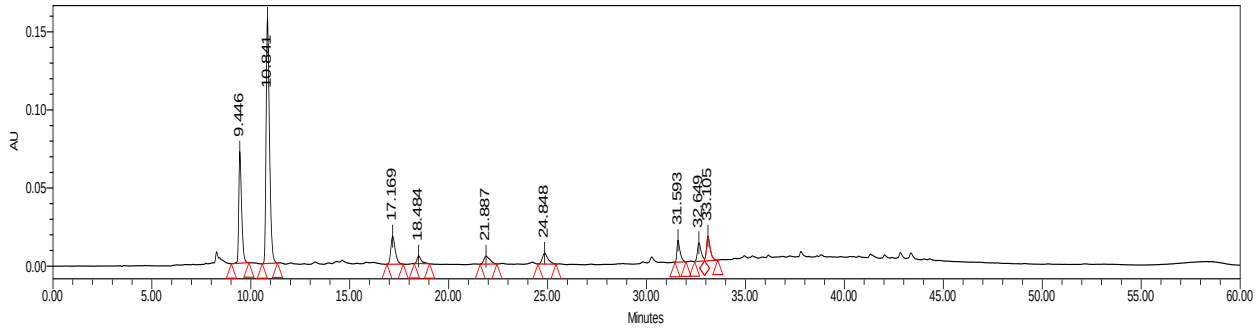


**Figure 213.** HPLC@280 nm, toasted layer 2, control rep 1, run 2

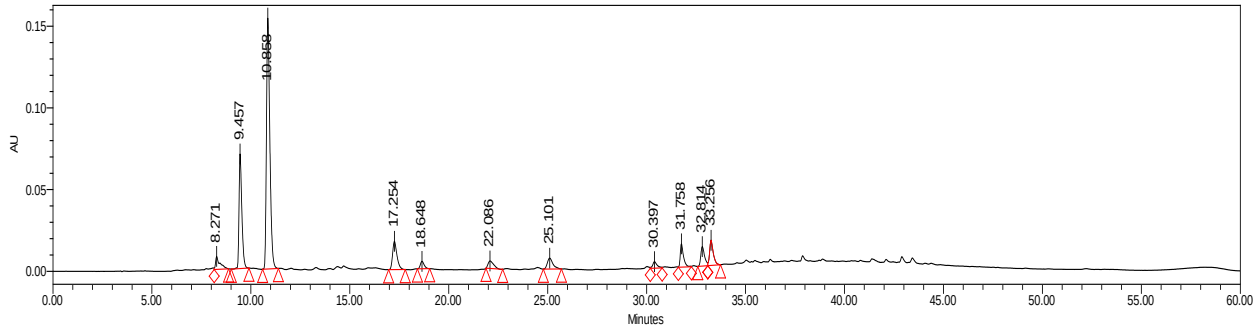


**Figure 214.** HPLC@280 nm, toasted layer 2, control rep 2, run 1

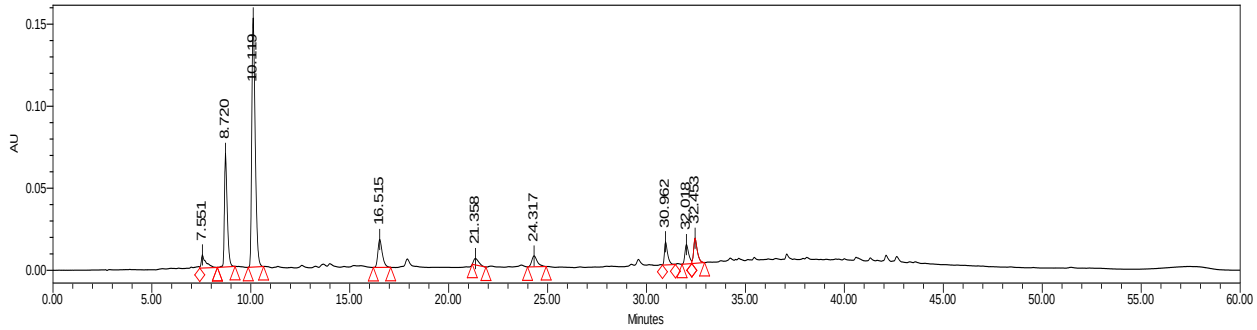


**Figure 215.** HPLC@280 nm, toasted layer 2, control rep 2, run 2

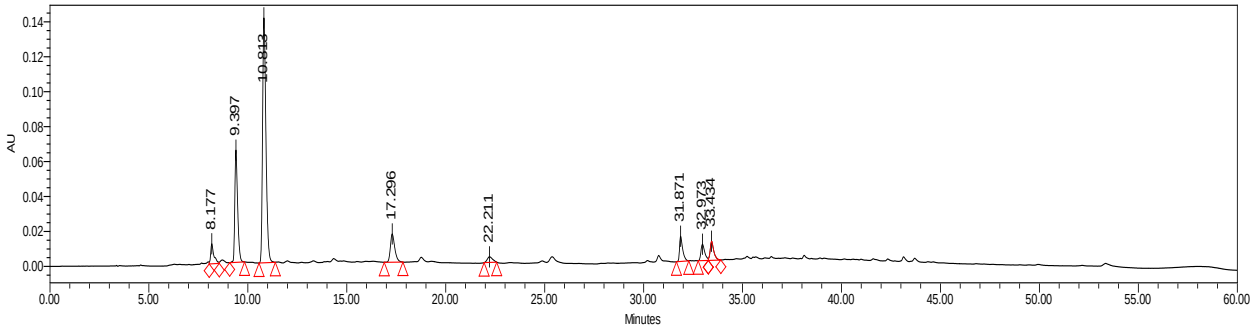


**Figure 216.** HPLC@280 nm, toasted layer 3, sonicated rep 1, run 1

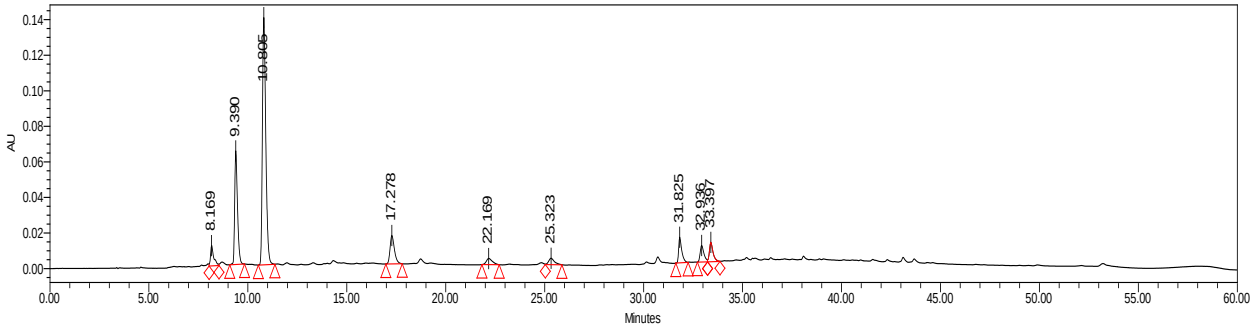


**Figure 217.** HPLC@280 nm, toasted layer 3, sonicated rep 1, run 2

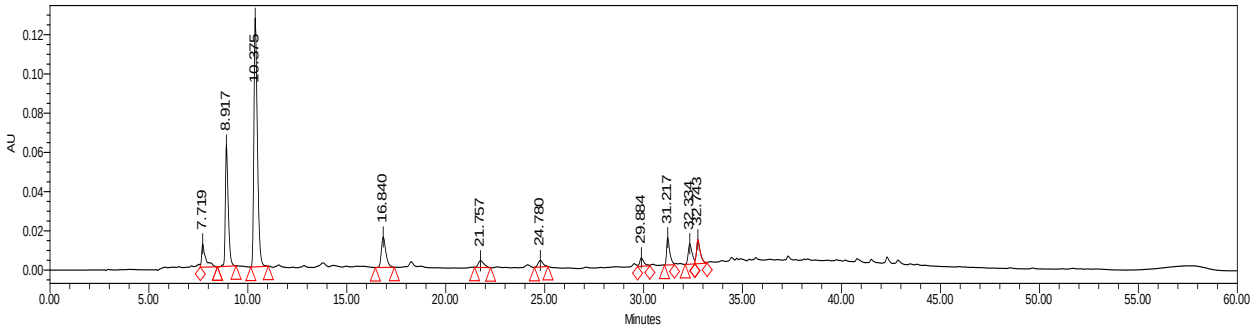


**Figure 218.** HPLC@280 nm, toasted layer 3, sonicated rep 2, run 1

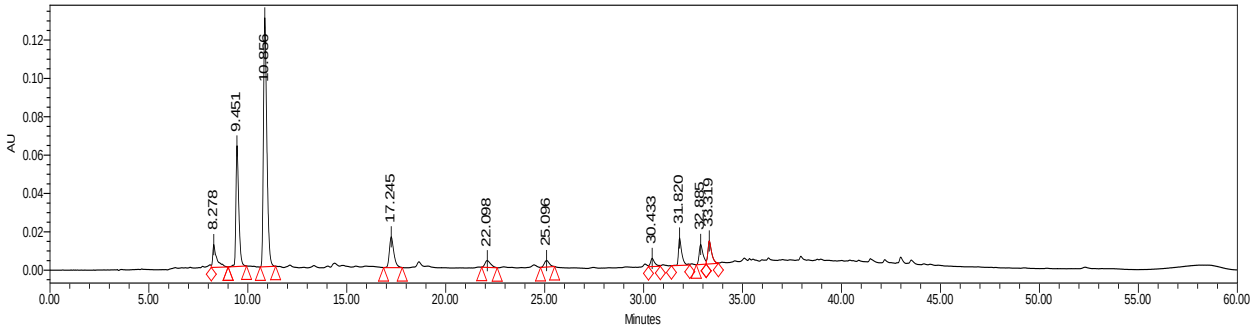


**Figure 219.** HPLC@280 nm, toasted layer 3, sonicated rep 2, run 2

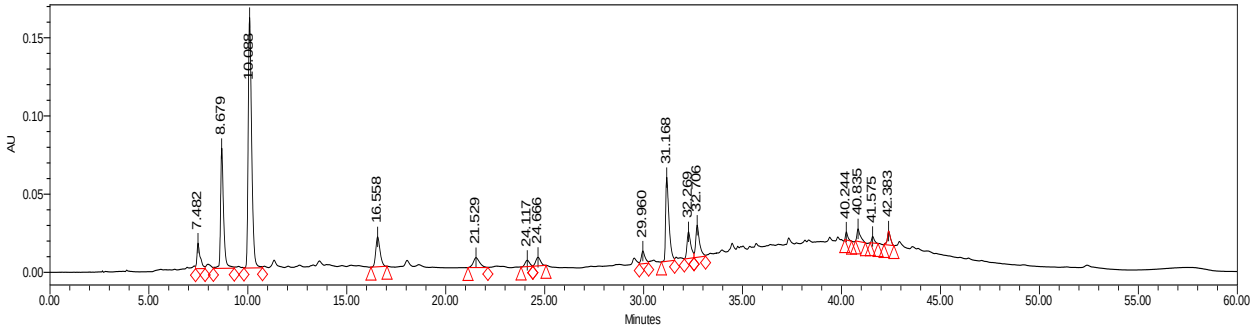


**Figure 220.** HPLC@280 nm, toasted layer 3, reflux rep 1, run 1

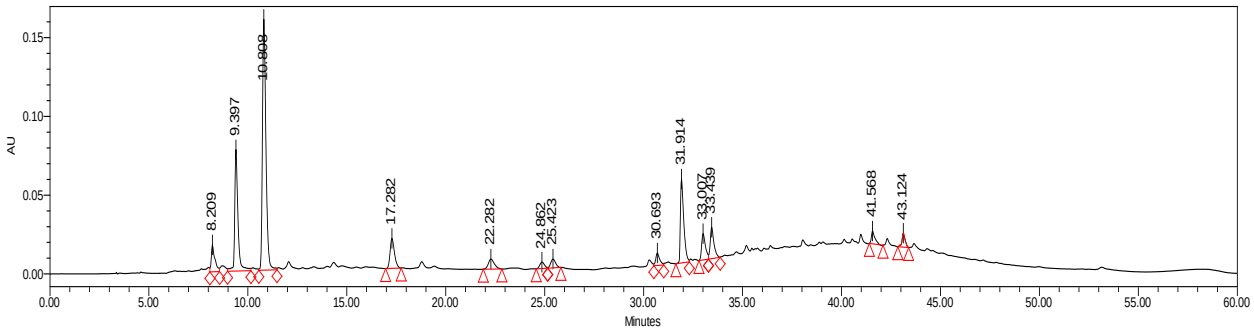


**Figure 221.** HPLC@280 nm, toasted layer 3, reflux rep 1, run 2

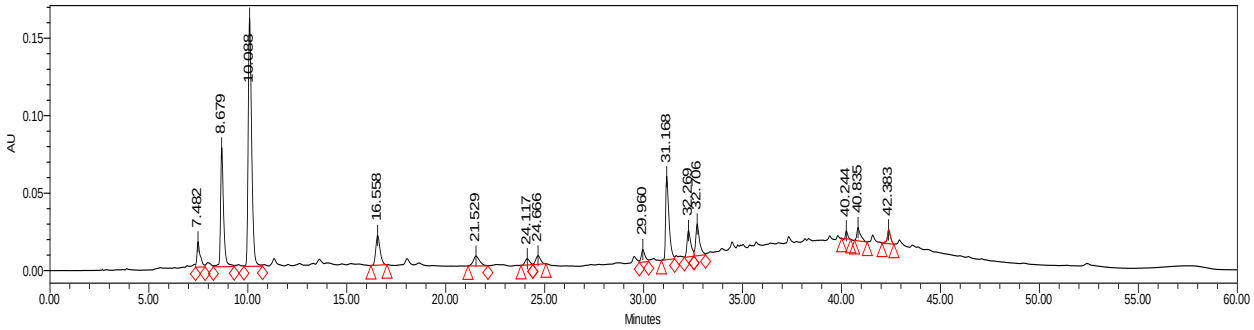


**Figure 222.** HPLC@280 nm, toasted layer 3, reflux rep 2, run 1

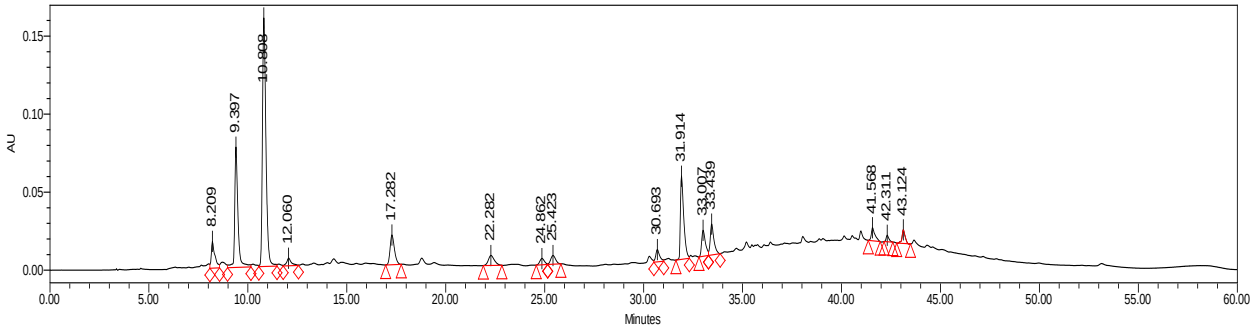


**Figure 223.** HPLC@280 nm, toasted layer 3, reflux rep 2, run 2

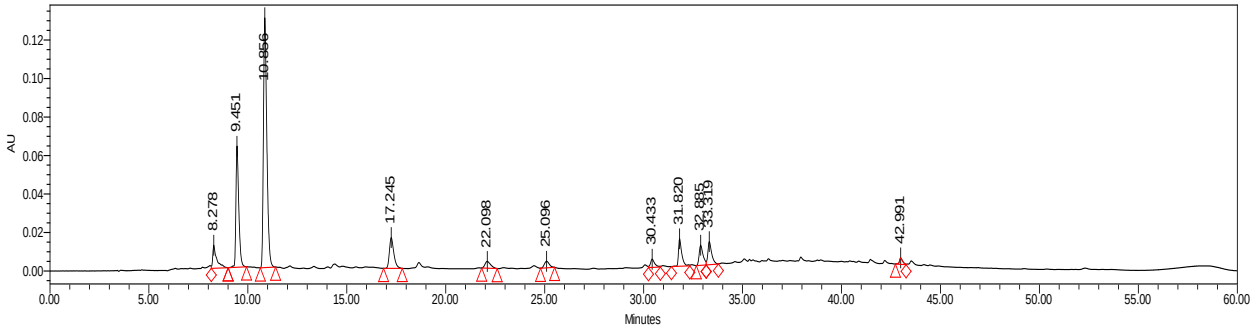


**Figure 224.** HPLC@280, toasted layer 3, control rep 1, run 1

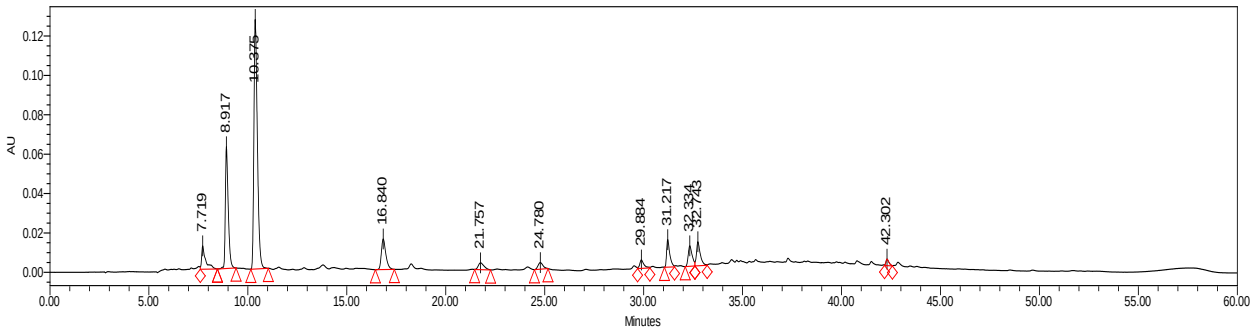


**Figure 225.** HPLC@280 nm, toasted layer 3, control rep 1, run 2

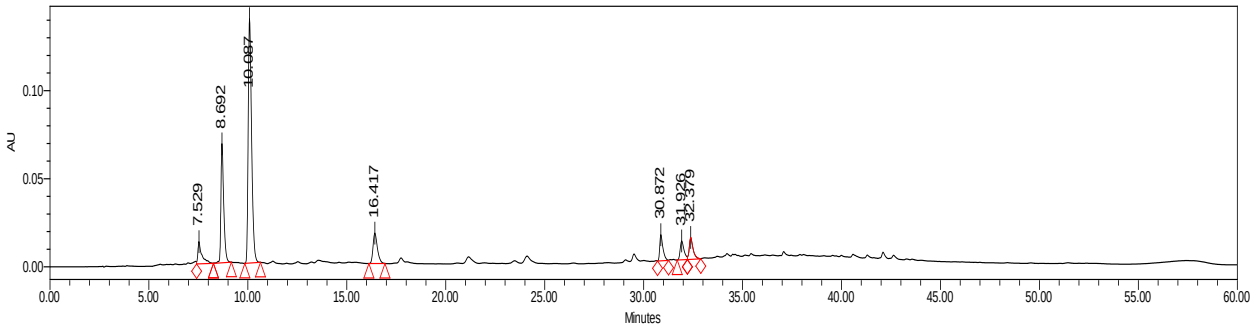


**Figure 226.** HPLC @280 nm, toasted layer 3, control rep 2, run 1

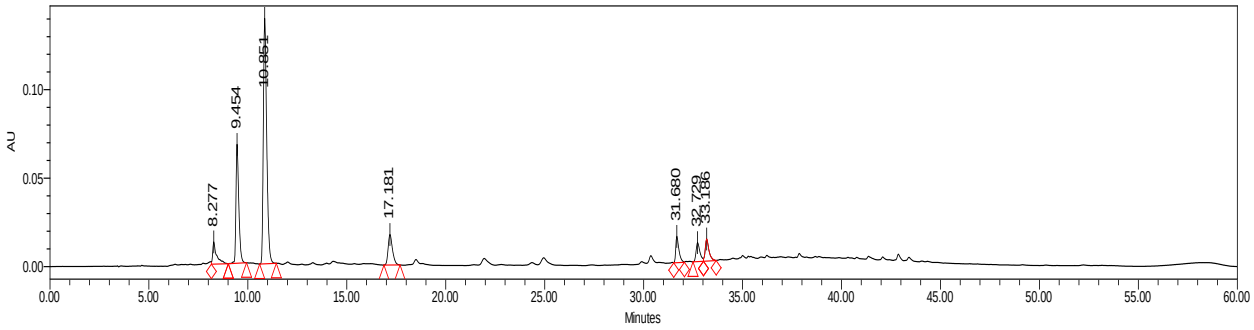


**Figure 227.** HPLC @280 nm, toasted layer 3m control rep 2, run 2

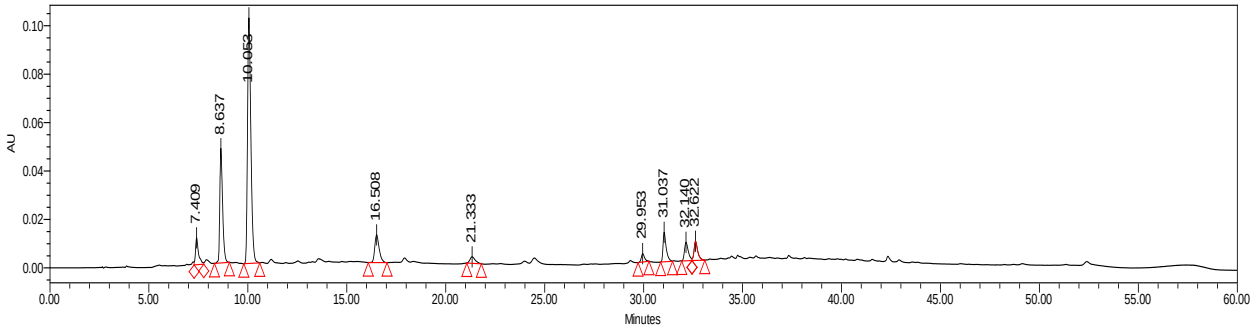


**Figure 228.** HPLC@280 nm, toasted layer 4, sonicated rep 1, run 1

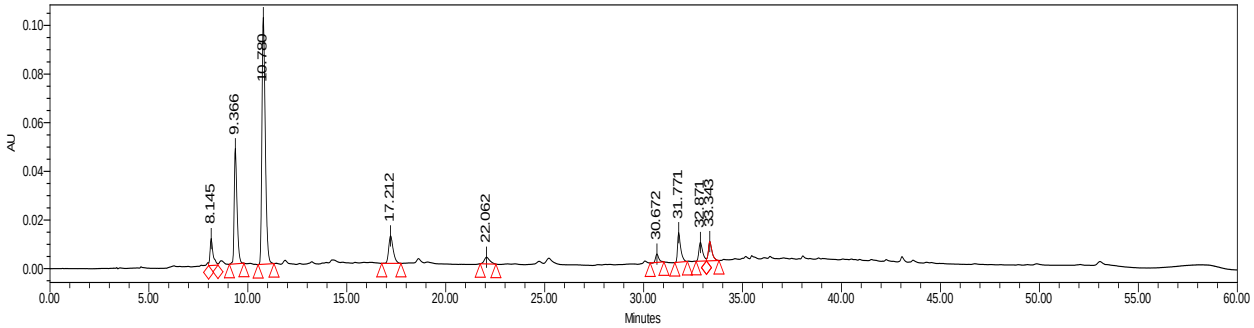


**Figure 229.** HPLC@280 nm, toasted layer 4, sonicated rep 1, run 2

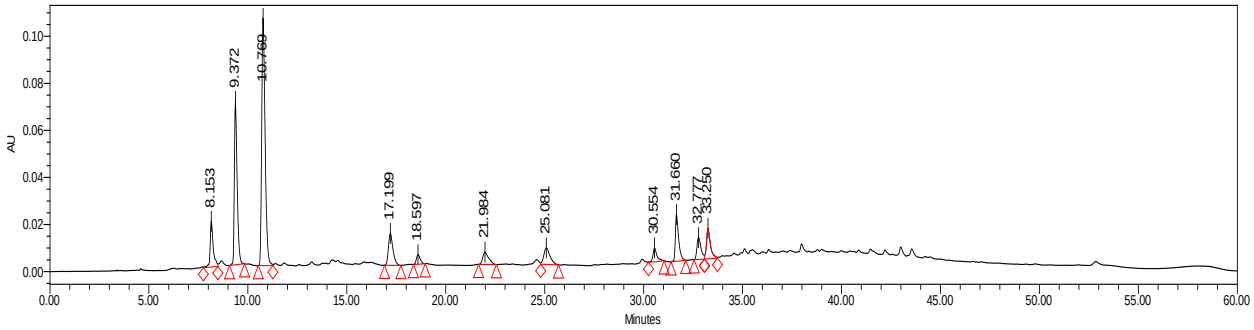


**Figure 230.** HPLC@280 nm, toasted layer 4, sonicated rep 2, run 1

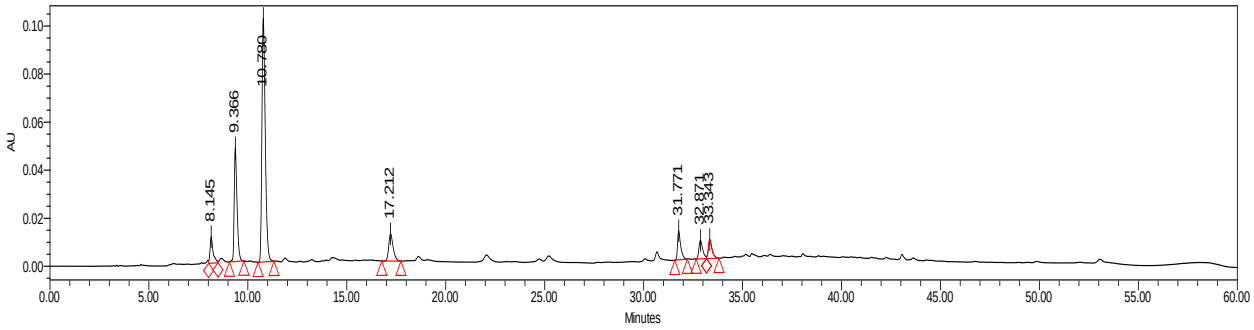


**Figure 231.** HPLC@280 nm, toasted layer 4, sonicated rep 2, run 2

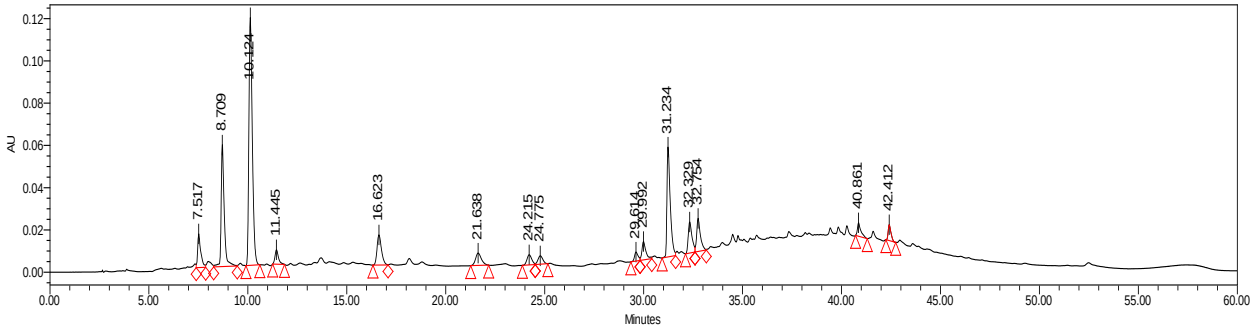


**Figure 232.** HPLC @280 nm, toasted layer 4, reflux rep 1, run 1

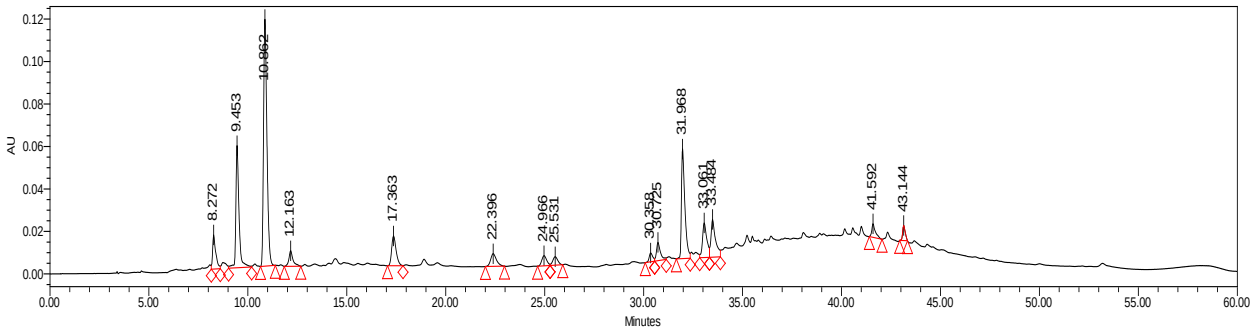


**Figure 233.** HPLC@280 nm, toasted layer 4, reflux rep 1, run 2

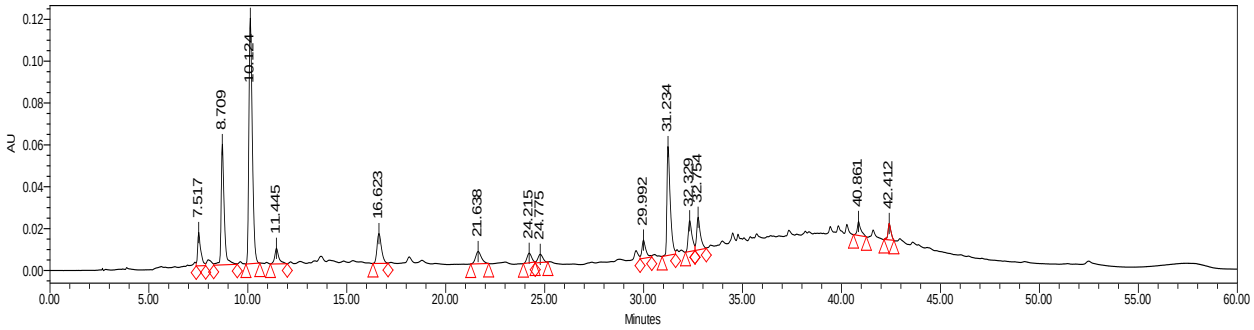


**Figure 234.** HPLC@280 nm, toasted layer 4, reflux rep 2, run 1

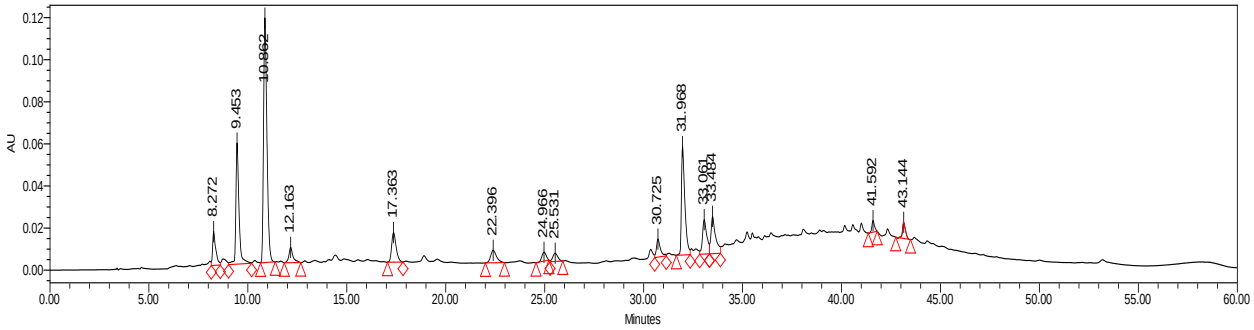


**Figure 235.** HPLC@280 nm, toasted layer 4, reflux rep 2, run 2

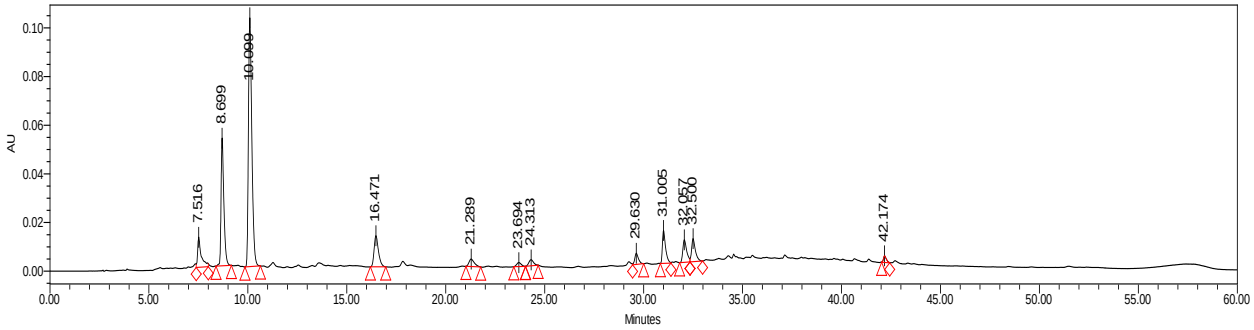


**Figure 236.** HPLC@280 nm, toasted layer 4, control rep 1, run 1

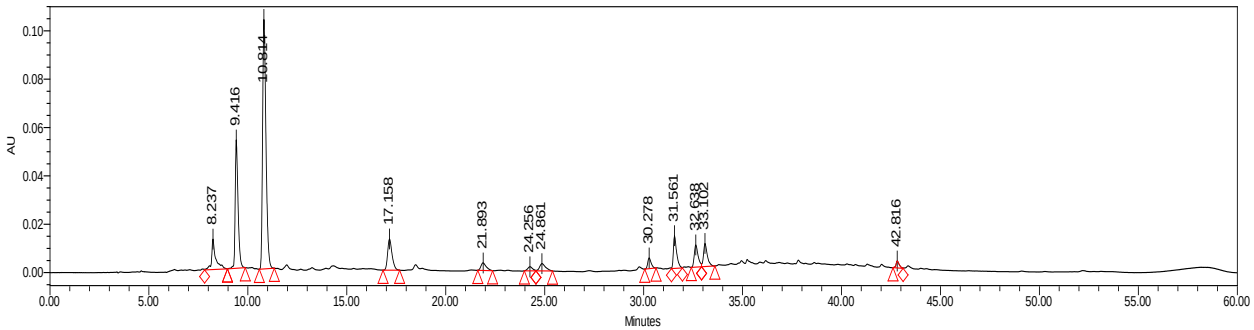


**Figure 237.** HPLC@280 nm, toasted layer 4, control rep 1, run 2

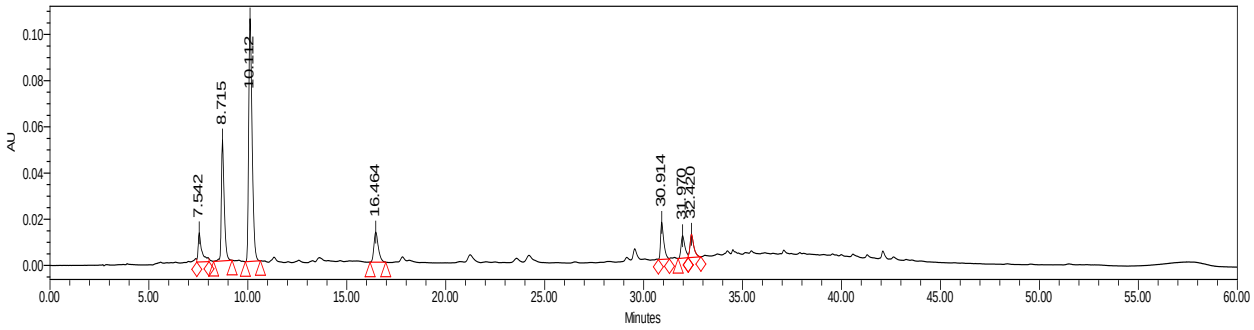


**Figure 238.** HPLC @280 nm, toasted layer 4, control rep 2, run 1

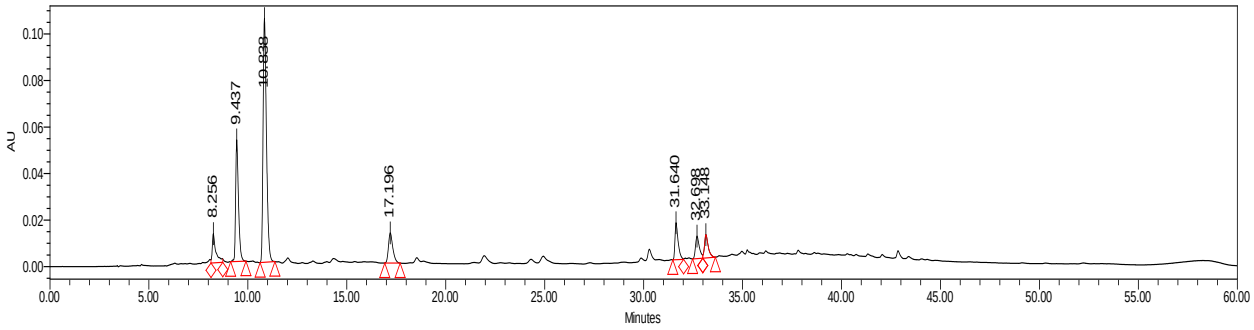


**Figure 239.** HPLC@280 nm, toasted layer 4, control rep 2, run 2

## HPLC Chromatograms After 3 months (280 nanometers)

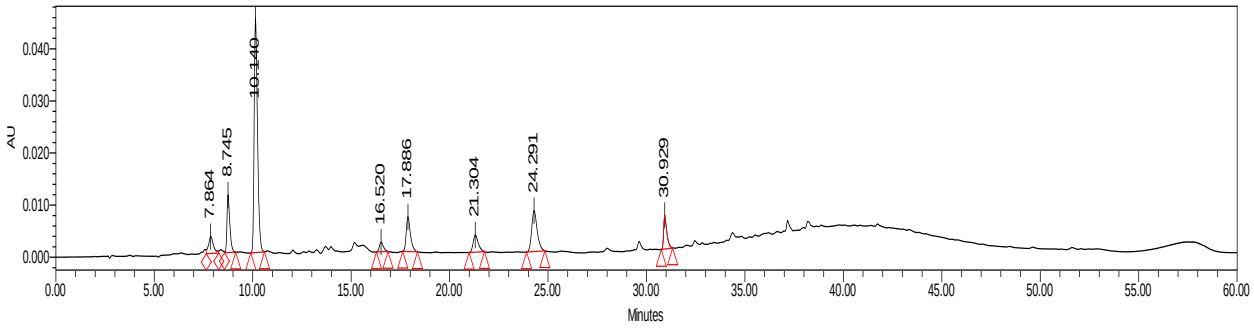


**Figure 240.** HPLC @280 nm, charred layer 1, sonicated rep 1, run 1

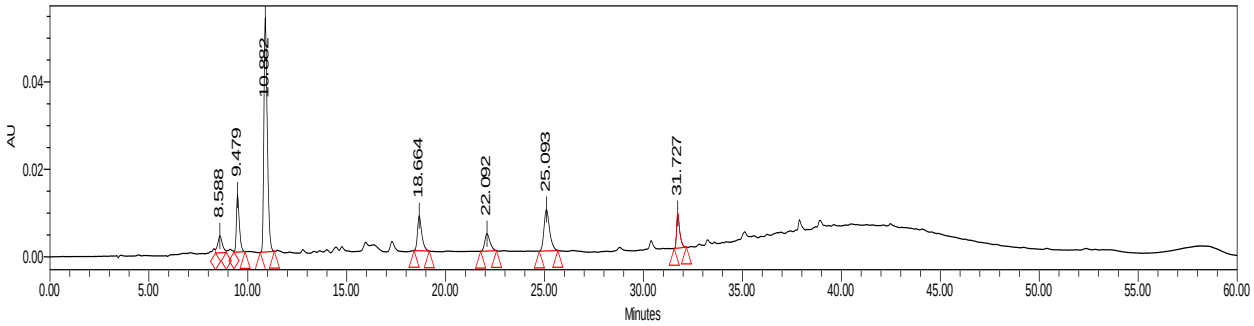


**Figure 241.** HPLC@280 nm, charred layer 1, sonicated rep 1, run 2

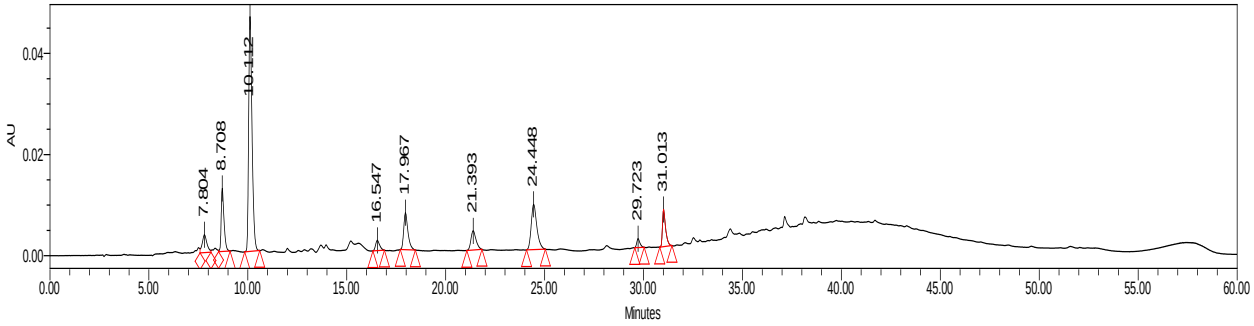


**Figure 242.** HPLC@280 nm, charred layer 1, sonicated rep 2, run 1

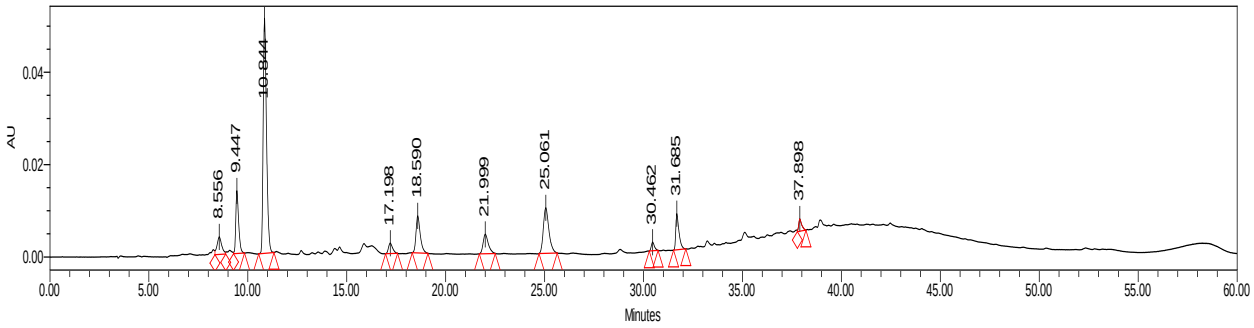


**Figure 243.** HPLC@280 nm, charred layer 1, sonicated rep 2, run 2

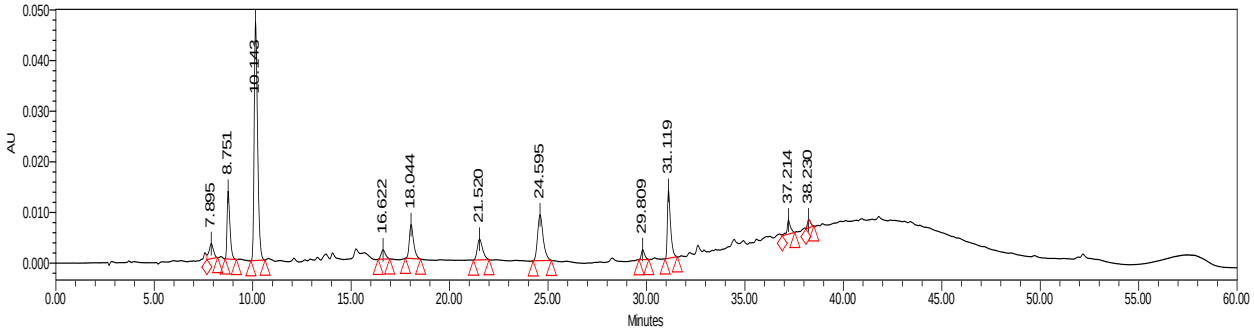


**Figure 244.** HPLC@280 nm, charred layer 1, reflux rep 1, run 1

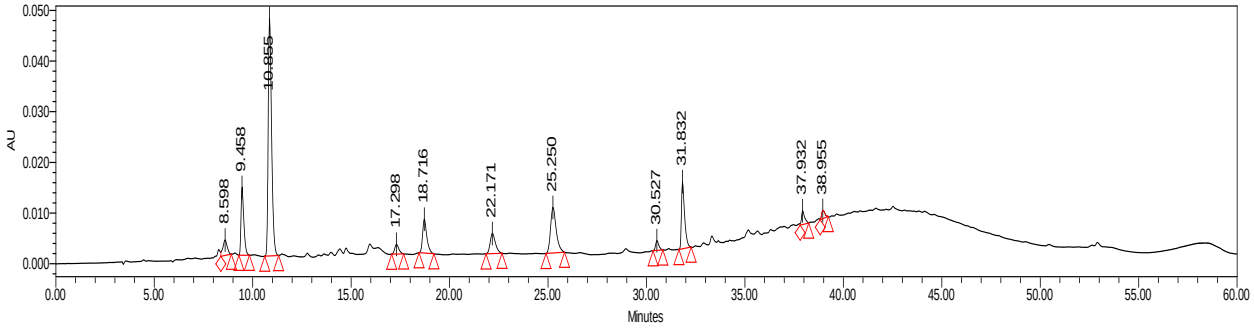


**Figure 245.** HPLC@280 nm, charred layer 1, reflux rep 1, run 2

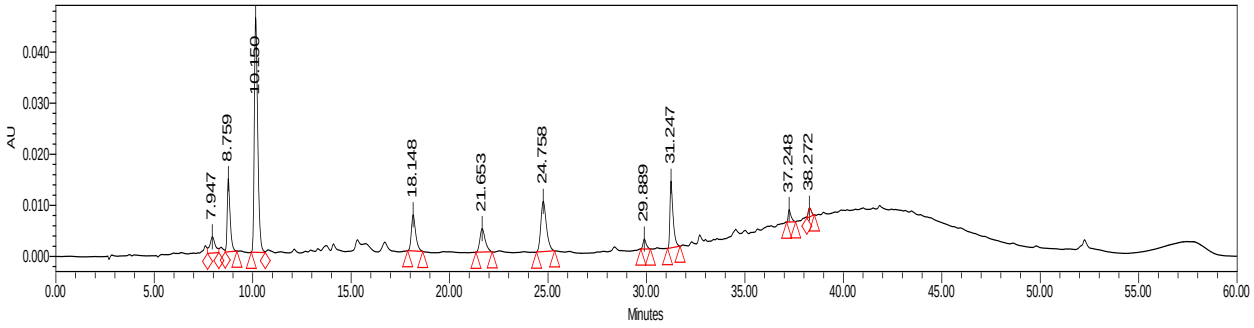


**Figure 246.** HPLC@280 nm, charred layer 1, reflux rep 2, run 1

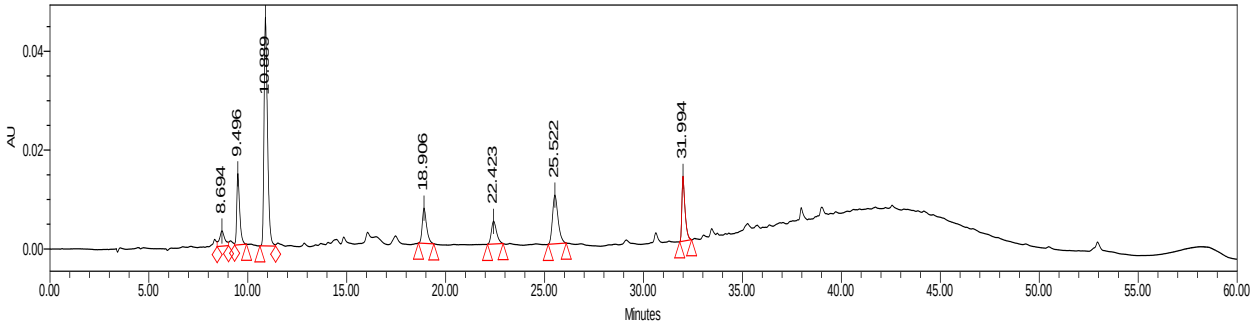


**Figure 247.** HPLC@280 nm, charred layer 1, reflux rep 2, run 2

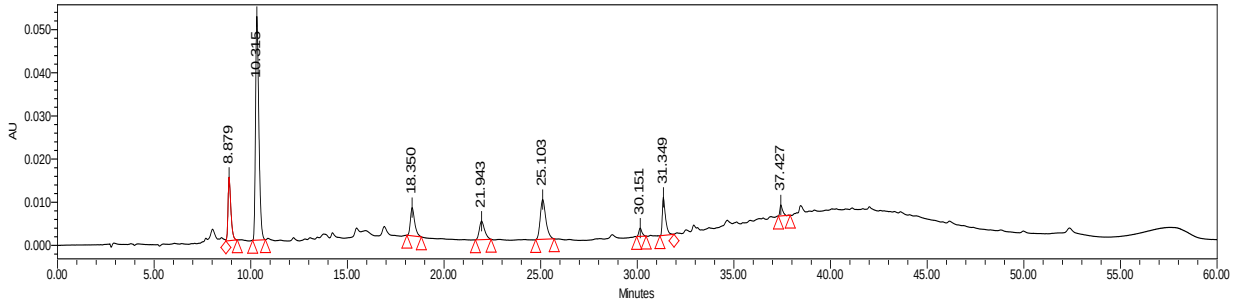


**Figure 248.** HPLC@280 nm, charred layer 1, control rep 1, run 1

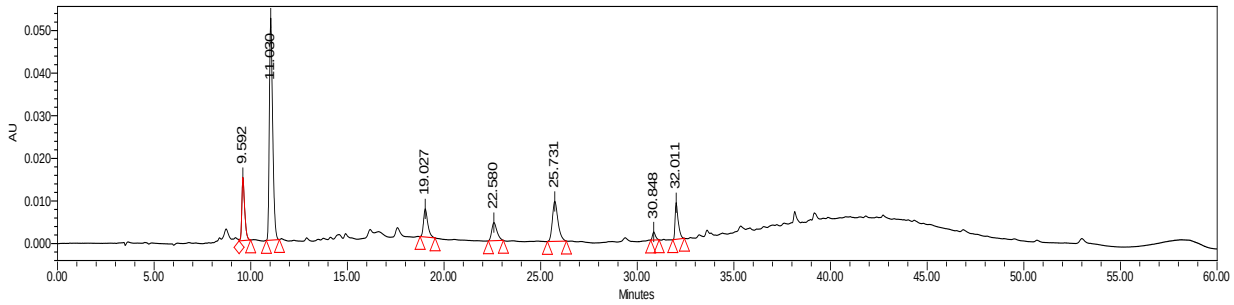


**Figure 249.** HPLC@280 nm, charred layer 1, control rep 1, run 2

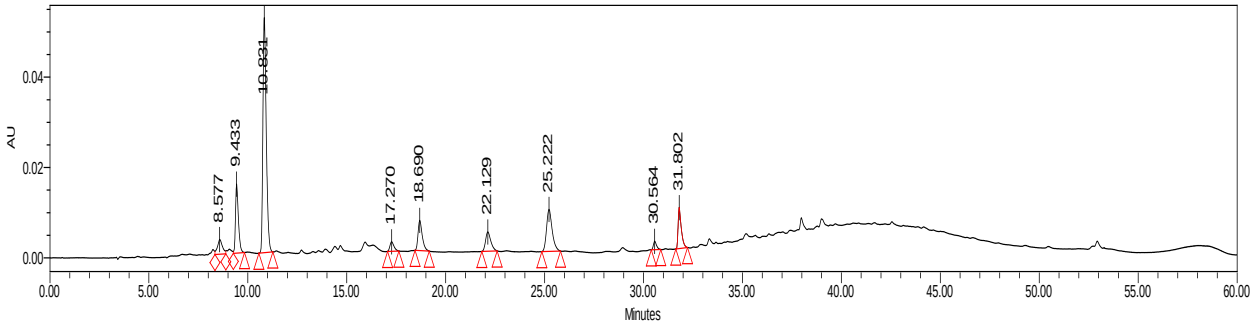


**Figure 250.** HPLC@280 nm, charred layer 1, control rep 2, run 1

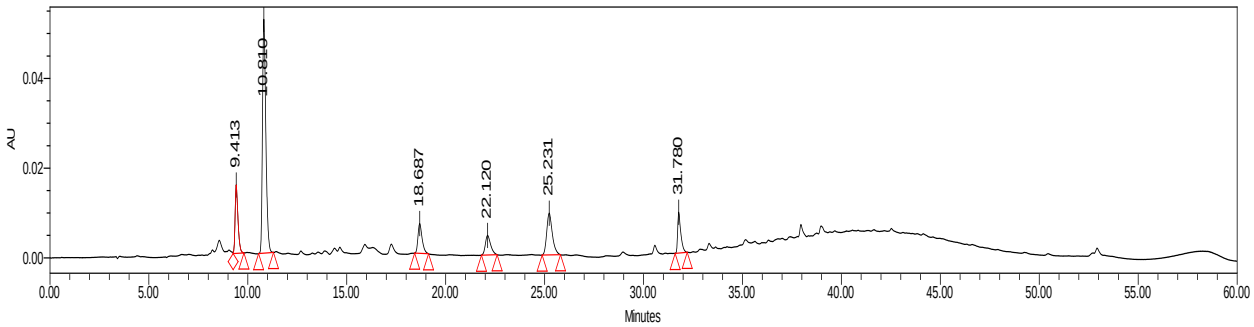


**Figure 251.** HPLC@280 nm, charred layer 1, control rep 2, run 2

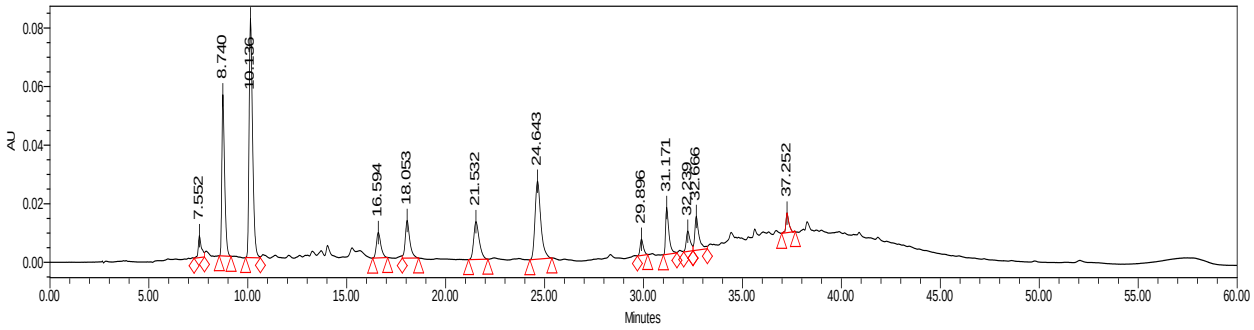


**Figure 252.** HPLC@280 nm, charred layer 2, sonicated rep 1, run 1

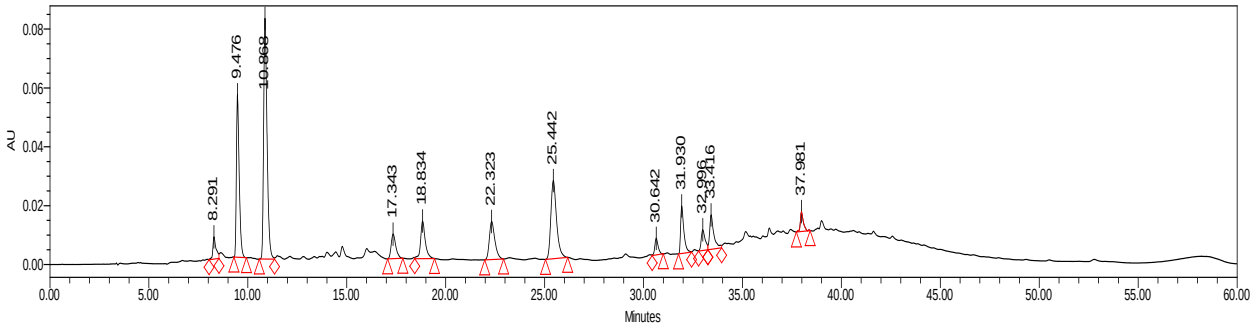


**Figure 253.** HPLC@280 nm, charred layer 2, sonicated rep 1, run 2

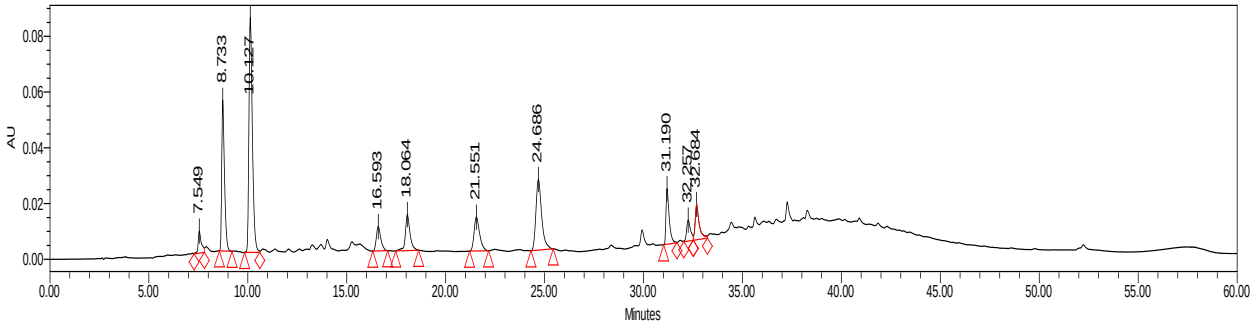


**Figure 254.** HPLC@280 nm, charred layer 2, sonicated rep 2, run 1

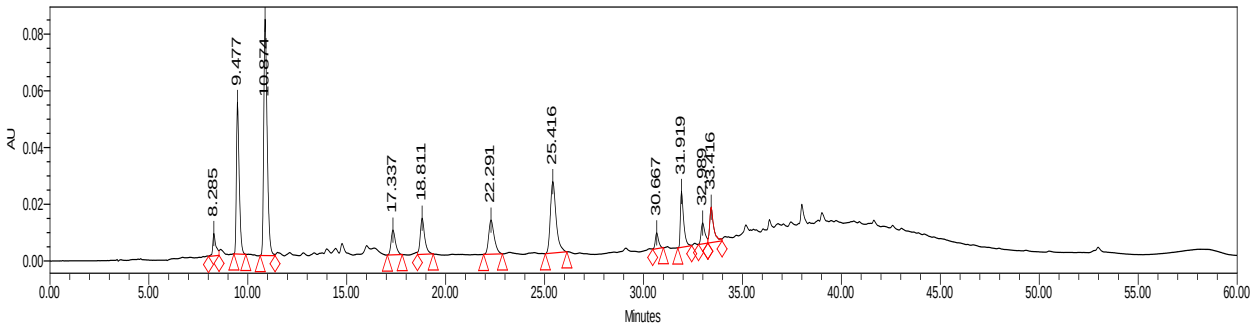


**Figure 255.** HPLC@280 nm, charred layer 2, sonicated rep 2, run 2

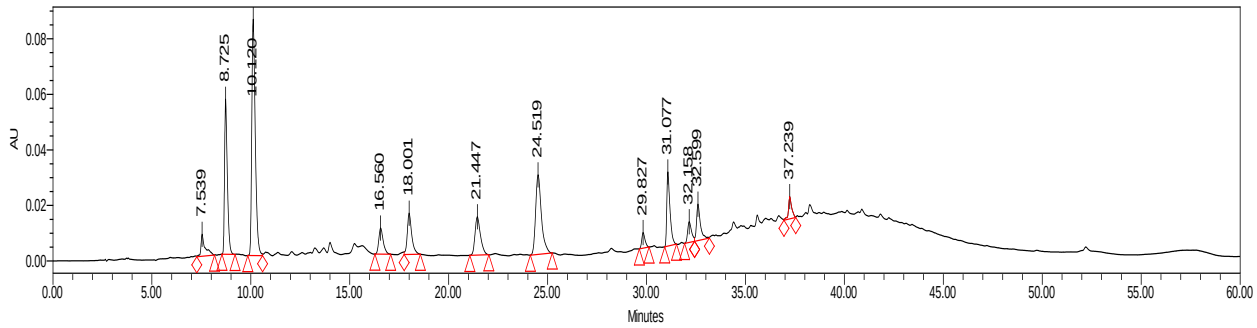


**Figure 256.** HPLC@280 nm, charred layer 2, reflux rep 1, run 1

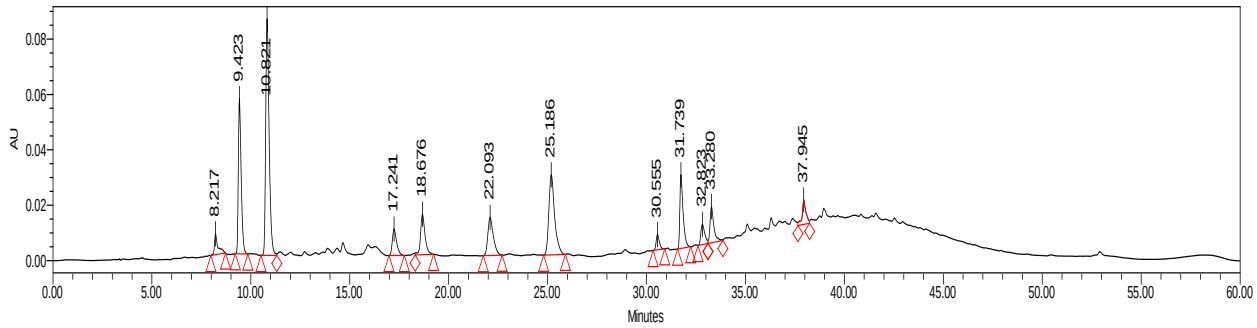


**Figure 257.** HPLC@280 nm, charred layer 2, reflux rep 1, run 2

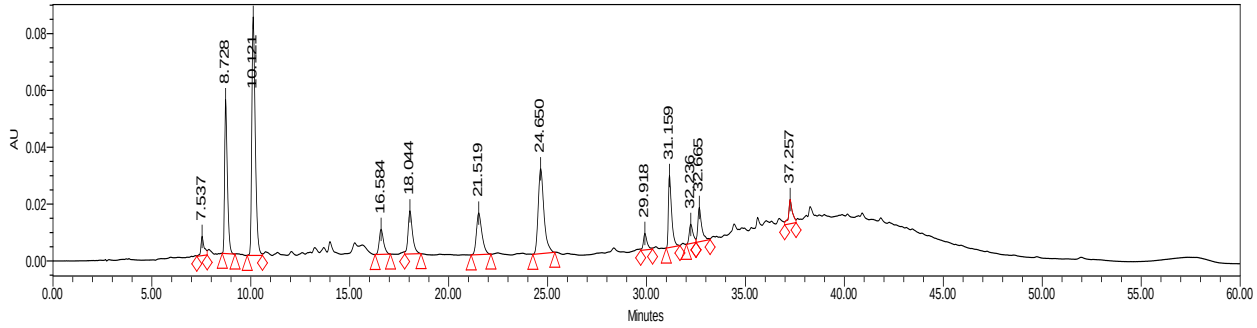


**Figure 258.** HPLC@280 nm, charred layer 2, reflux rep 2, run 1

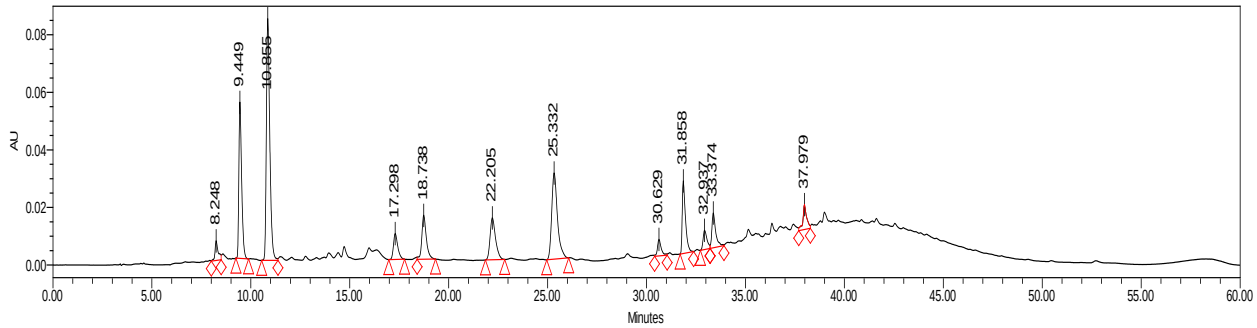


**Figure 259.** HPLC@280 nm, charred layer 2, reflux rep 2, run 2

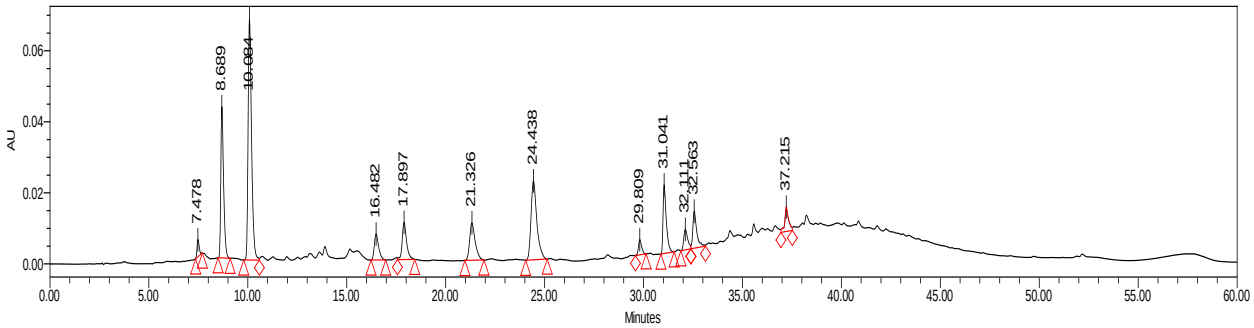


**Figure 260.** HPLC@280 nm, charred layer 2, control rep 1, run 1

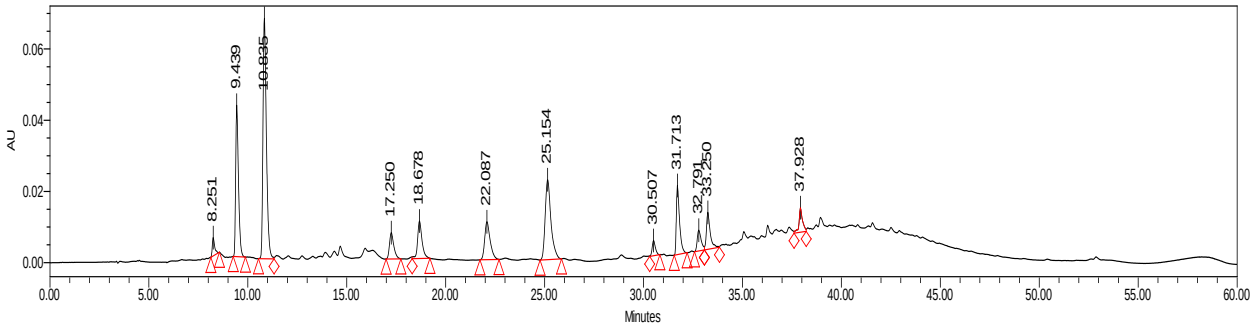


**Figure 261.** HPLC@280 nm, charred layer 2, control rep 1, run 2

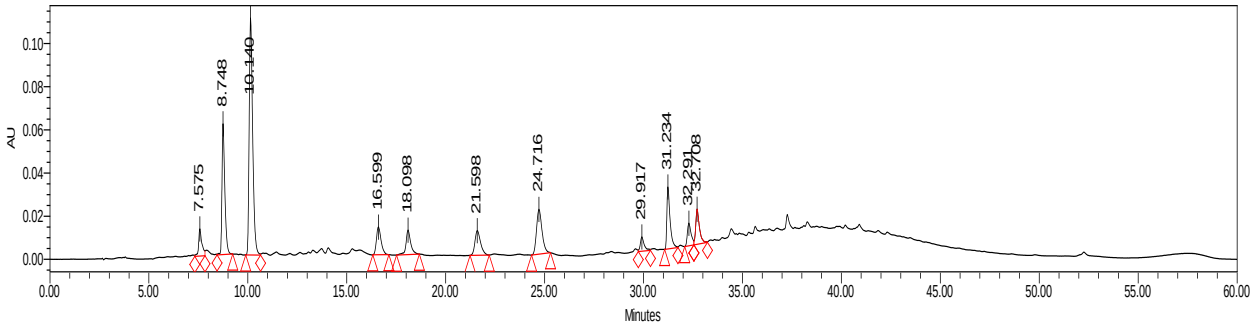


**Figure 262.** HPLC@280 nm, charred layer 3, sonicated rep 1, run 1

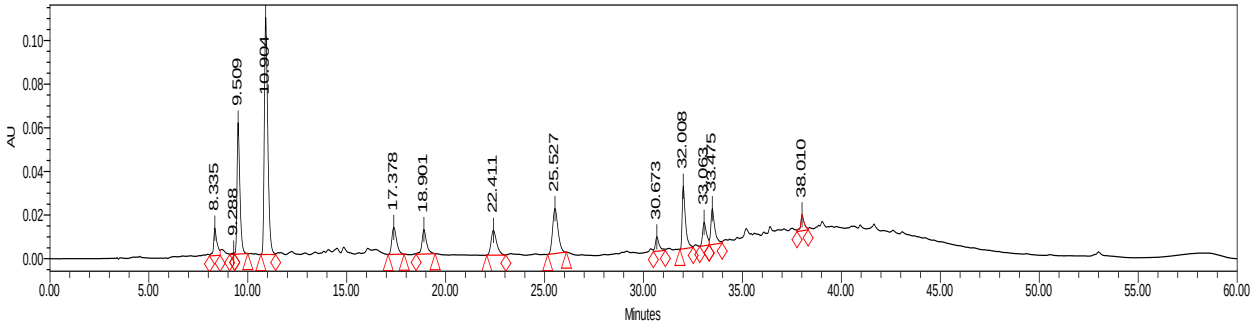


**Figure 263.** HPLC@280 nm, charred layer 3, sonicated rep 1, run 2

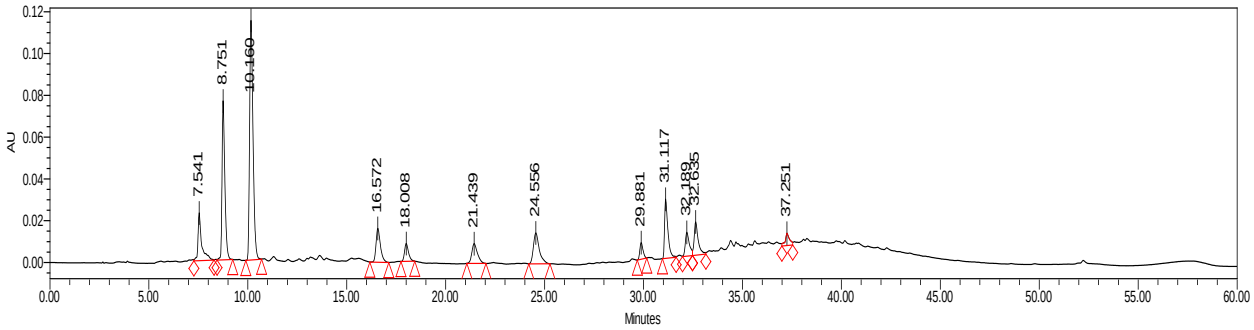


**Figure 264.** HPLC@280 nm, charred layer 3, sonicated rep 2, run 1

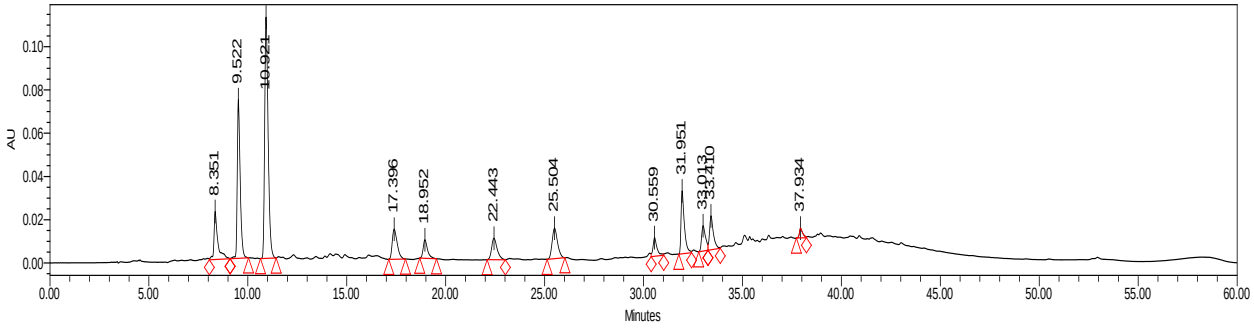


**Figure 265.** HPLC@280 nm, charred layer 3, sonicated rep 2, run 2

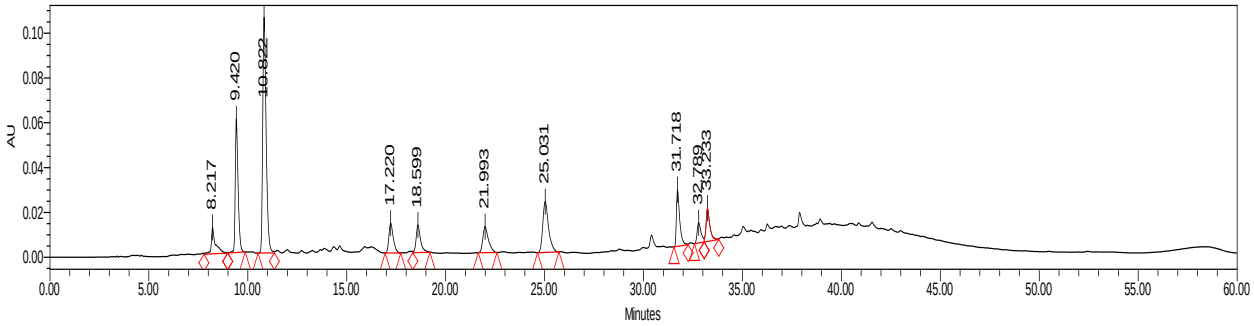


**Figure 266.** HPLC@280 nm, charred layer 3, reflux rep 1, run 1

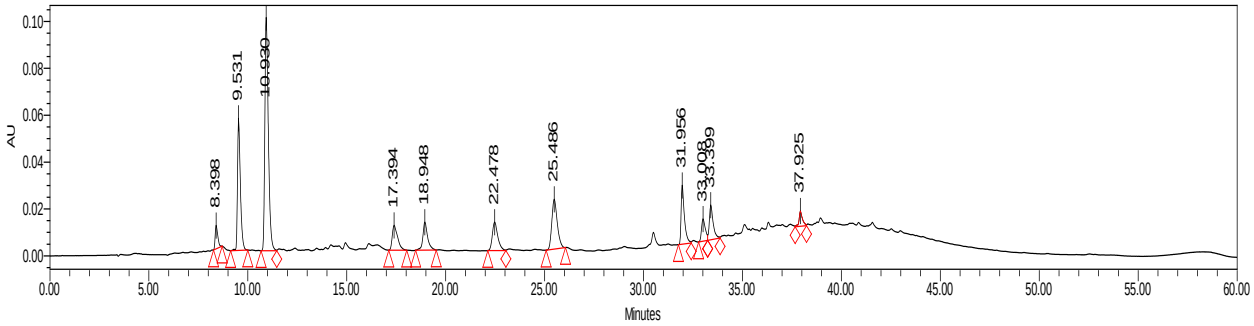


**Figure 267.** HPLC@280 nm, charred layer 3, reflux rep 1, run 2

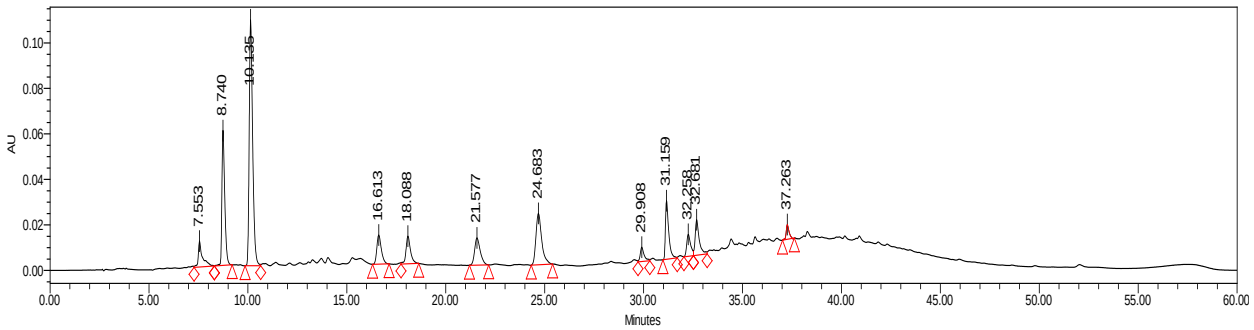


**Figure 268.** HPLC@280 nm, charred layer 3, reflux rep 2, run 1

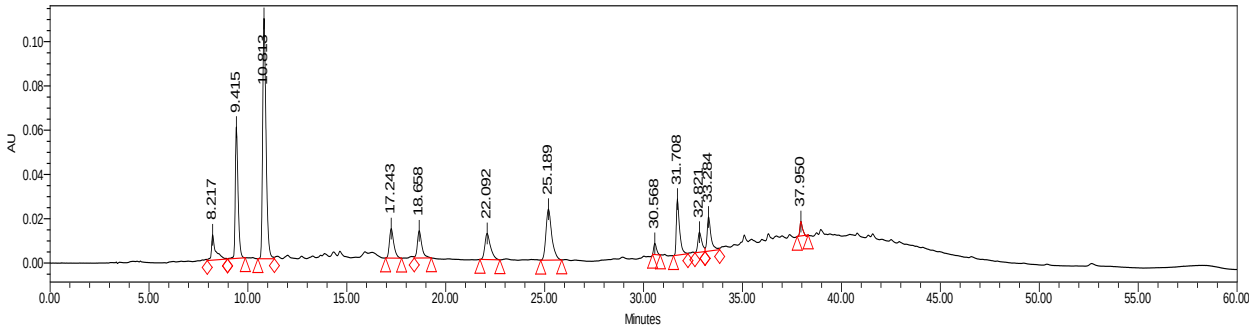


**Figure 269.** HPLC@280 nm, charred layer 3, reflux rep 2, run 2

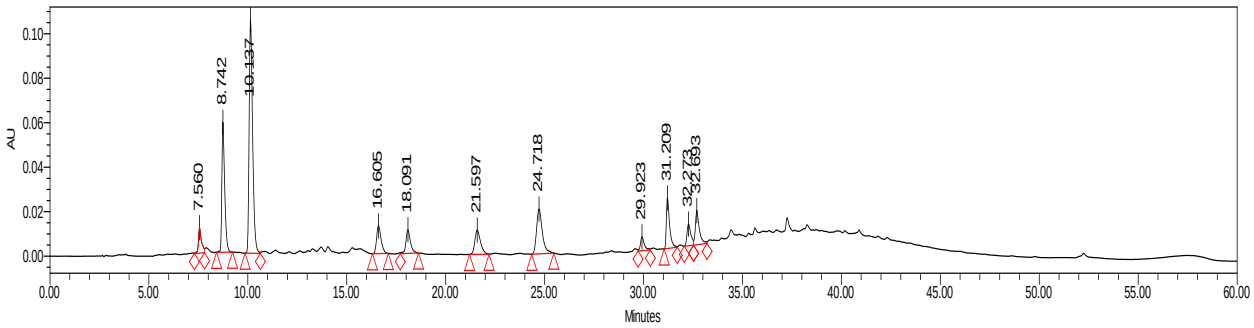


**Figure 270.** HPLC@280 nm, charred layer 3, control rep 1, run 1

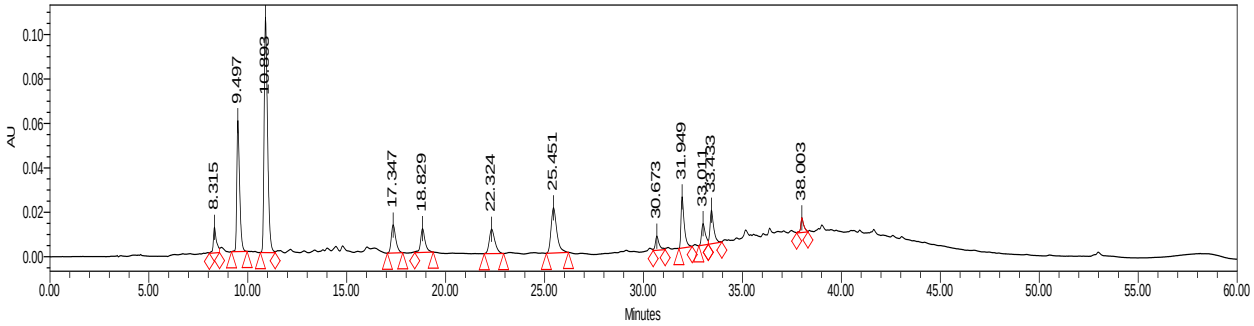


**Figure 271.** HPLC@280 nm, charred layer 3, control rep 1, run 2

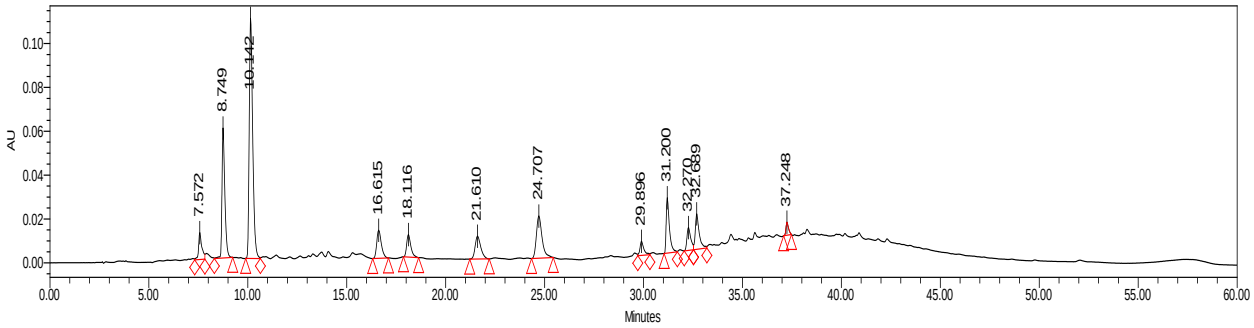


**Figure 272.** HPLC@280 nm, charred layer 3, control rep 2, run 1

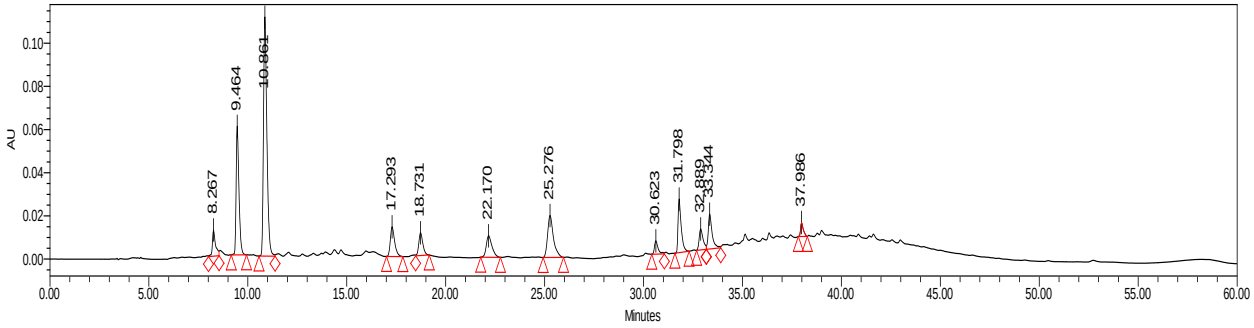


**Figure 273.** HPLC@280 nm, charred layer 3, control rep 2, run 2

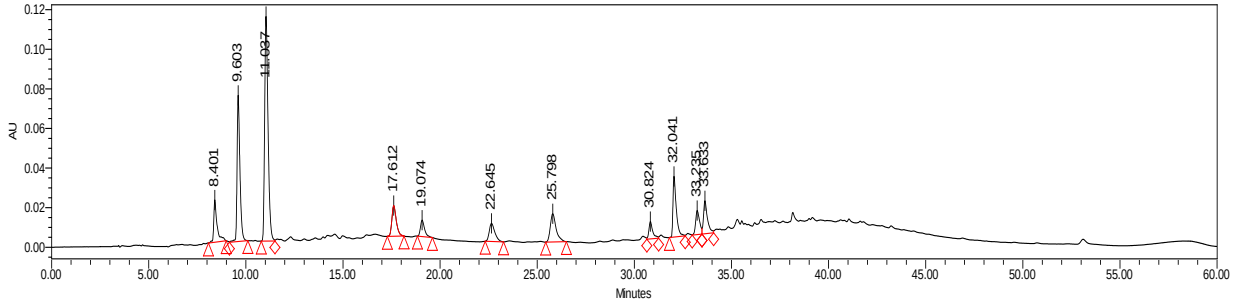


**Figure 274.** HPLC@280 nm, charred layer 4, sonicated rep 1, run 1

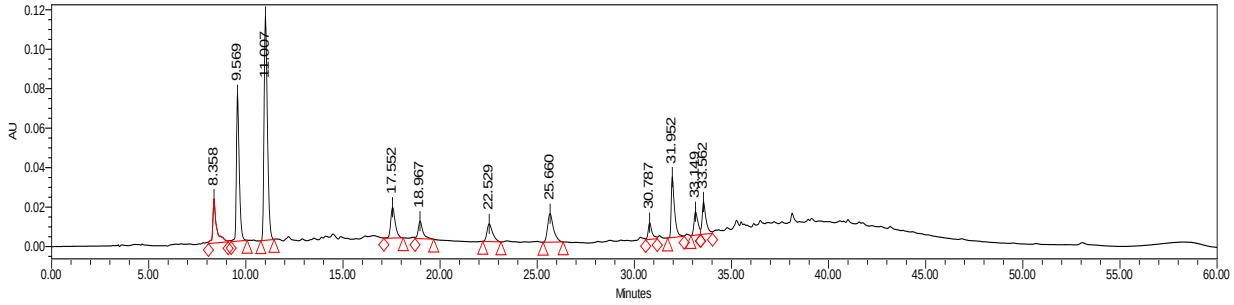


**Figure 275.** HPLC@280 nm, charred layer 4, sonicated rep 1, run 2

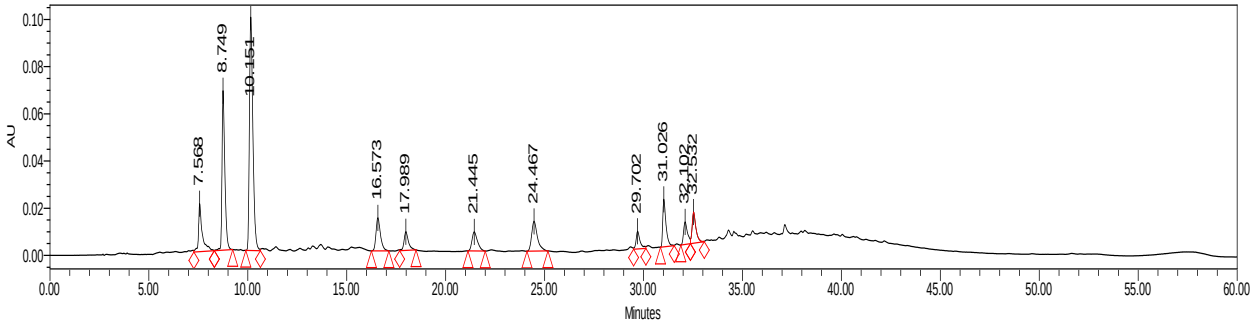


**Figure 276.** HPLC@280 nm, charred layer 4, sonicated rep 2, run 1

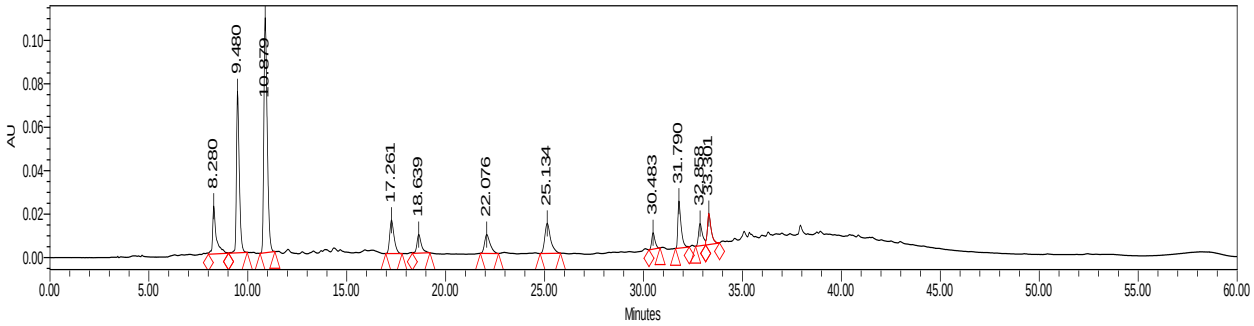


**Figure 277.** HPLC@280 nm, charred layer 4, sonicated rep 2, run 2

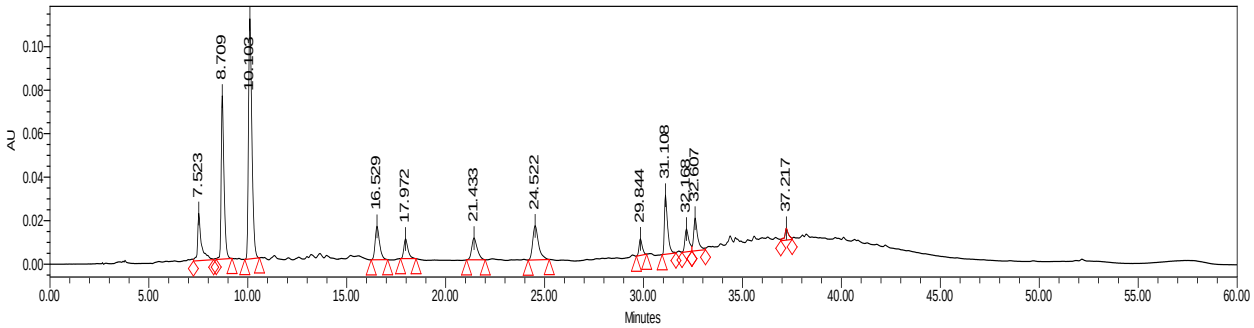


**Figure 278.** HPLC@280 nm, charred layer 4, reflux rep 1, run 1

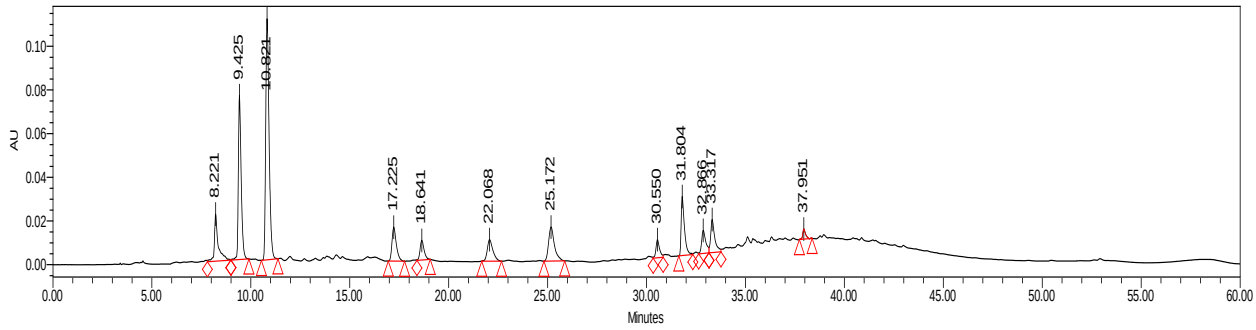


**Figure 279.** HPLC@280 nm, charred layer 4, reflux rep 1, run 2

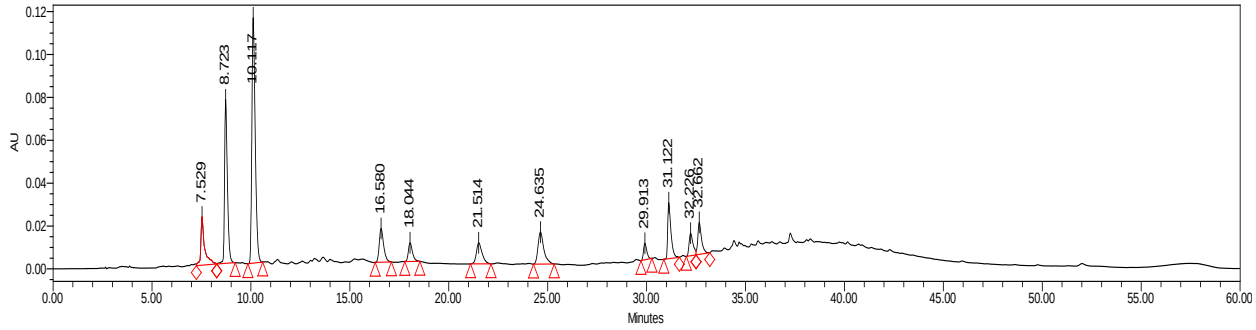


**Figure 280.** HPLC@280 nm, charred layer 4, reflux rep 2, run 1

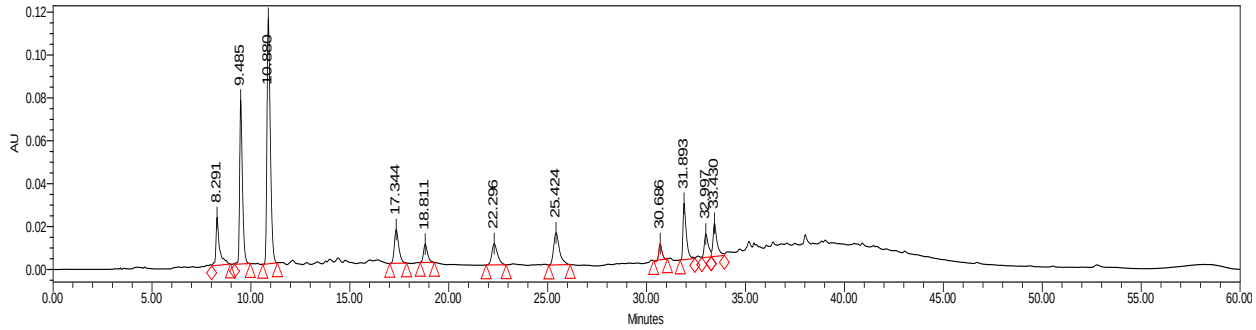


**Figure 281.** HPLC@280 nm, charred layer 4, reflux rep 2, run 2

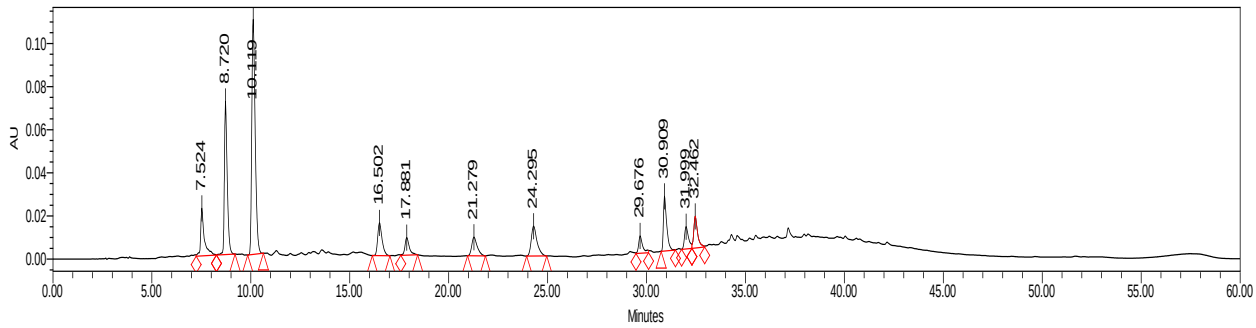


**Figure 282.** HPLC@280 nm, charred layer 4, control rep 1, run 1

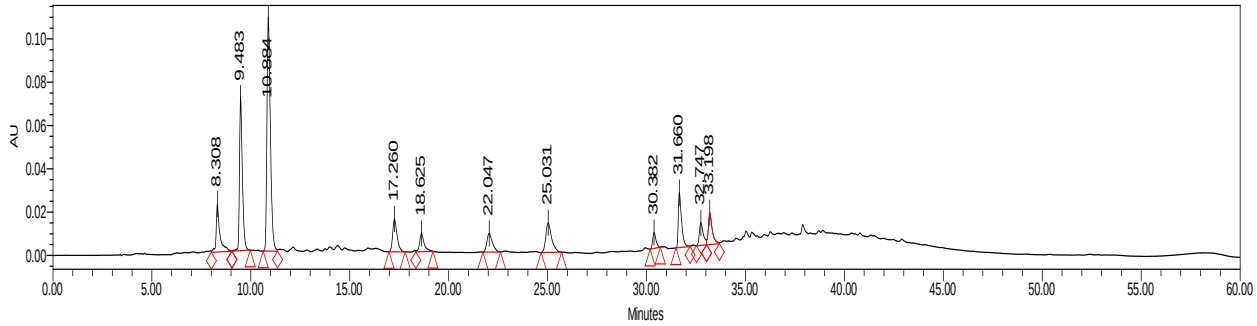


**Figure 283.** HPLC@280 nm, charred layer 4, control rep 1, run 2

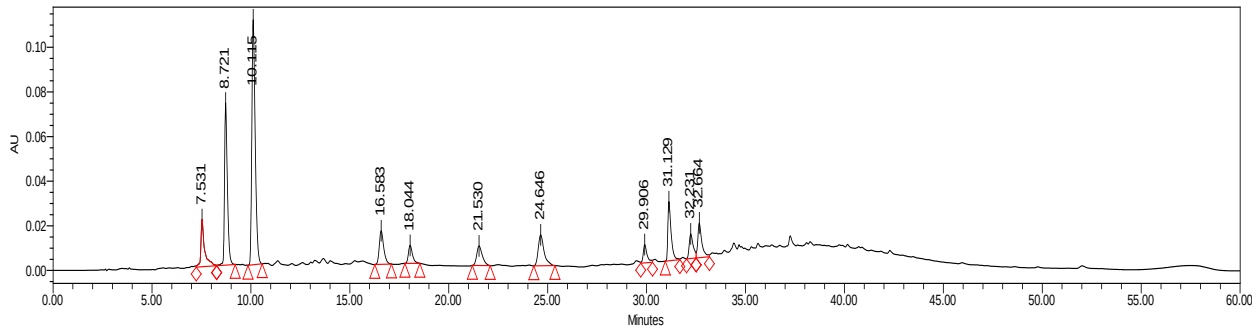


**Figure 284.** HPLC@280 nm, charred layer 4, control rep 2, run 1

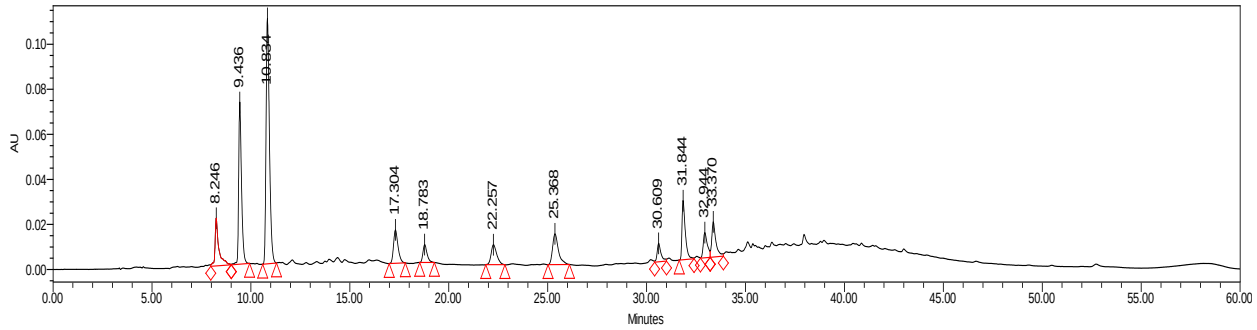


**Figure 285.** HPLC@280 nm, charred layer 4, control rep 2, run 2

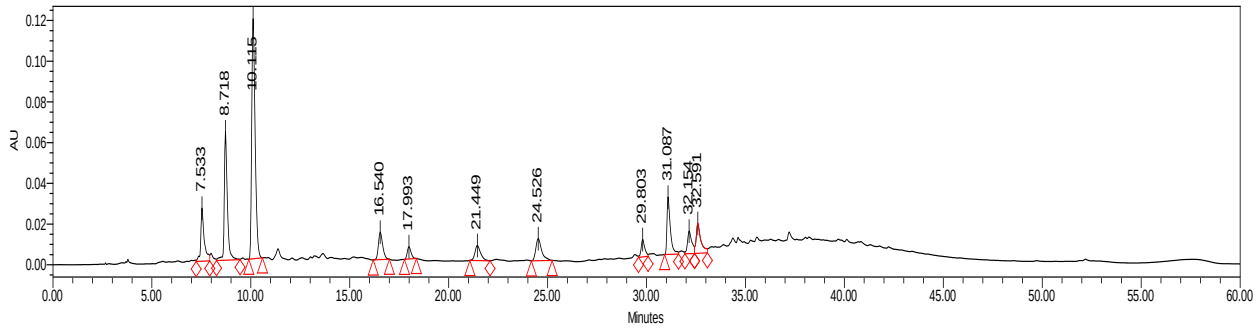


**Figure 286.** HPLC@280 nm, charred layer 5, sonicated rep 1, run 1

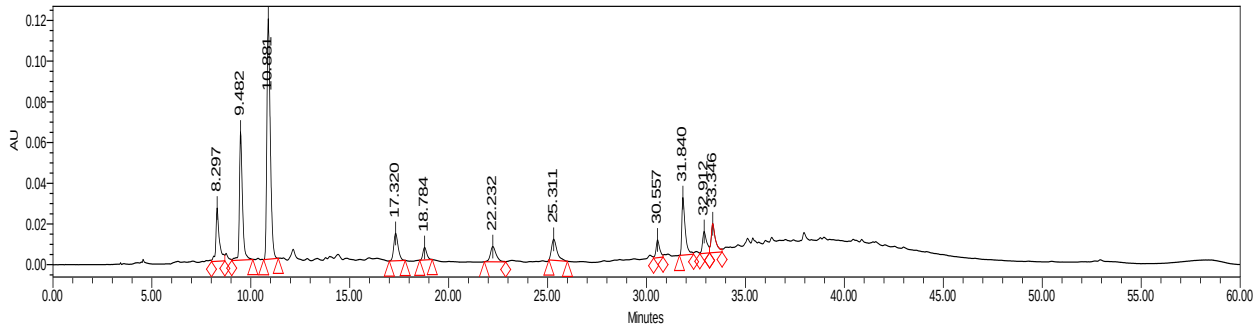


**Figure 287.** HPLC@280 nm, charred layer 5, sonicated rep 1, run 2

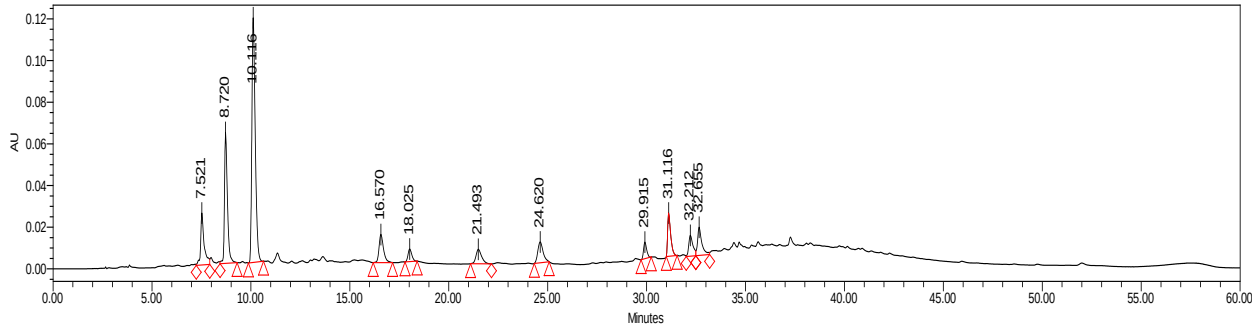


**Figure 288.** HPLC@280 nm, charred layer 5, sonicated rep 2, run 1

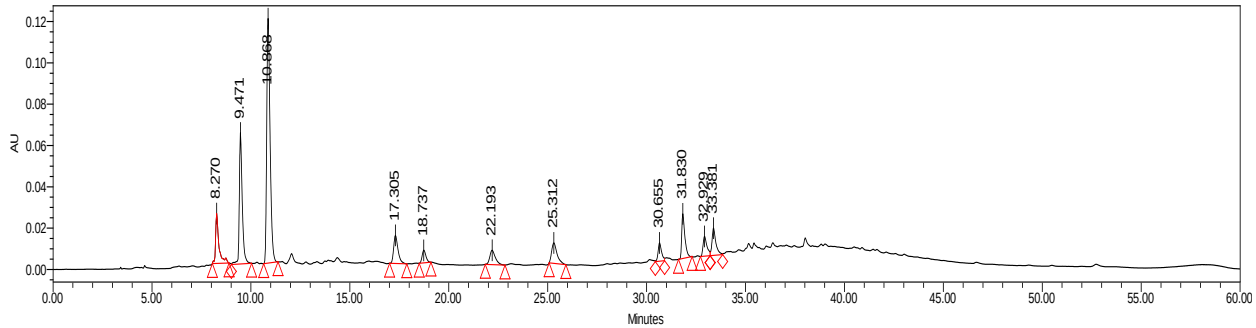


**Figure 289.** HPLC@280 nm, charred layer 5, sonicated rep 2, run 2

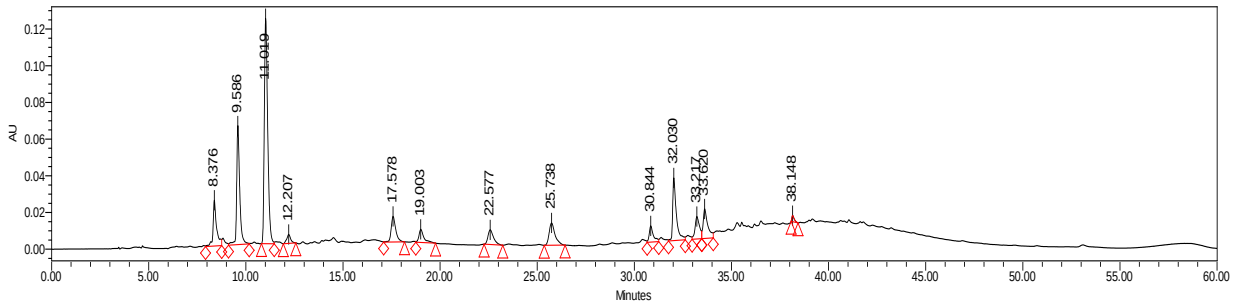


**Figure 290.** HPLC@280 nm, charred layer 5, reflux rep 1, run 1

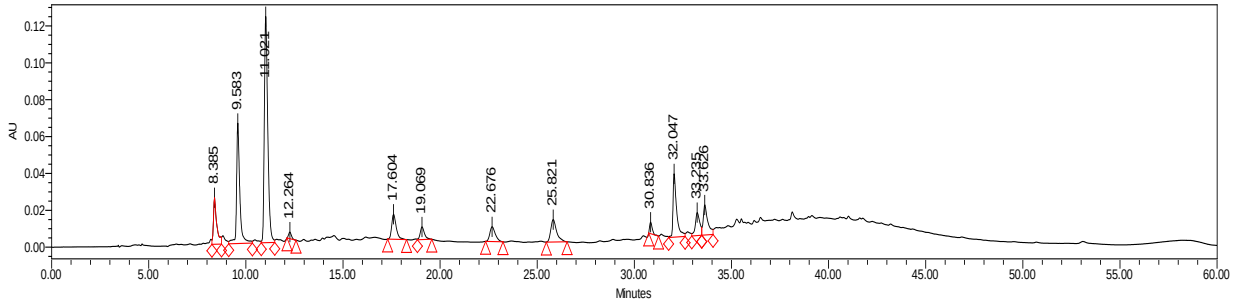


**Figure 291.** HPLC@280 nm, charred layer 5, reflux rep 1, run 2

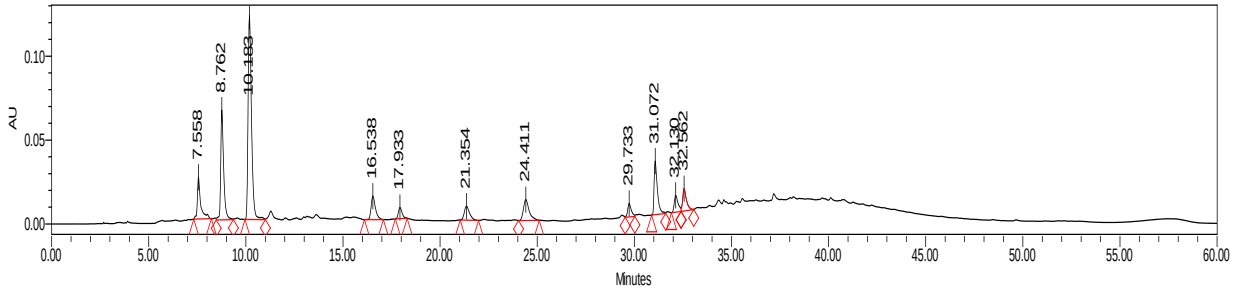


**Figure 292.** HPLC@280 nm, charred layer 5, reflux rep 2, run 1

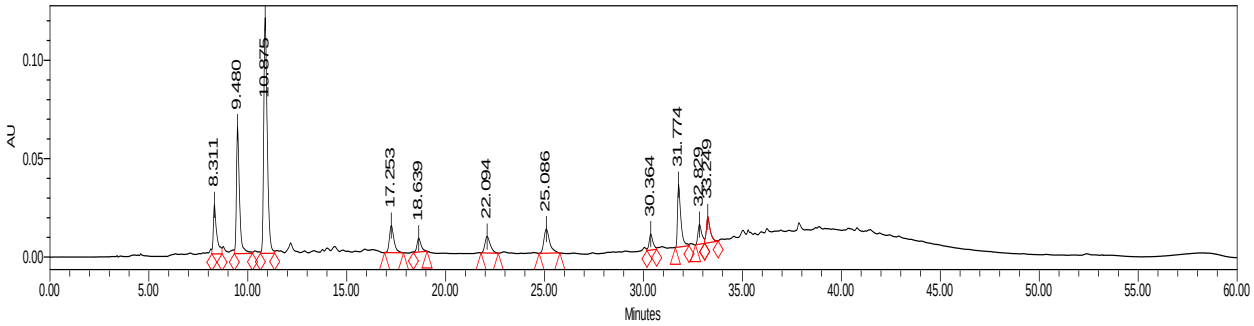


**Figure 293.** HPLC@280 nm, charred layer 5, reflux rep 2, run 2

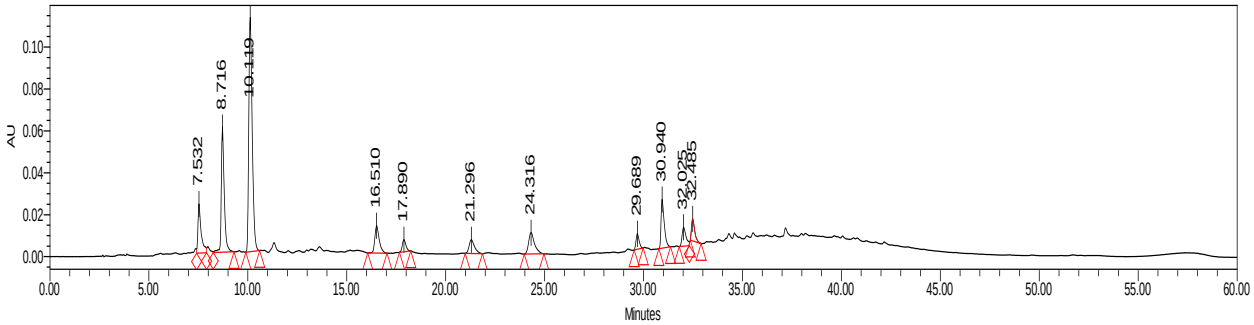


**Figure 294.** HPLC@280 nm, charred layer 5, control rep 1, run 1

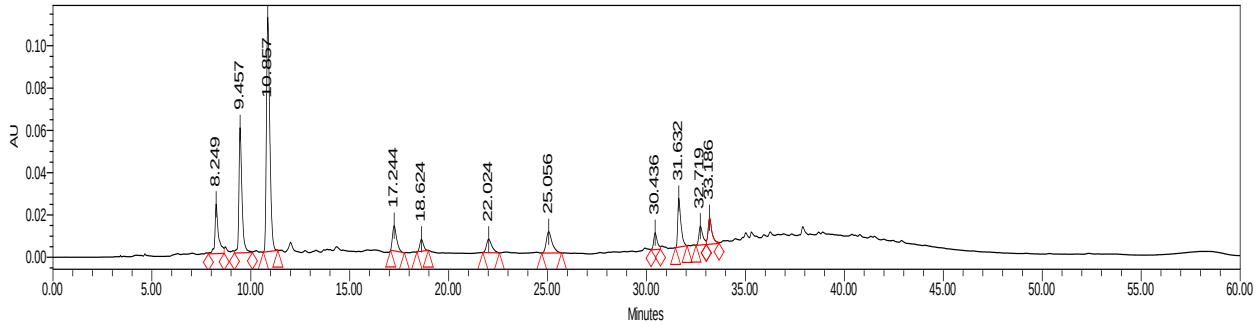


**Figure 295.** HPLC@280 nm, charred layer 5, control rep 1, run 2

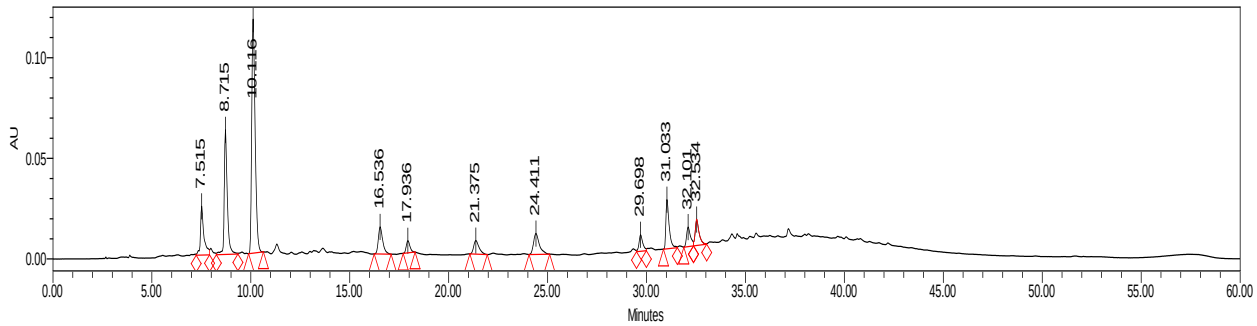


**Figure 296.** HPLC@280 nm, charred layer 5, control rep 2, run 1

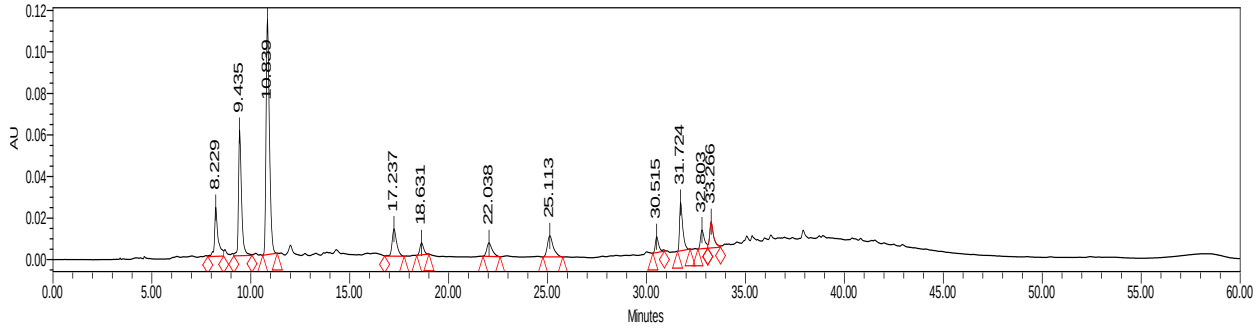


**Figure 297.** HPLC@280 nm, charred layer 5, control rep 2, run 2

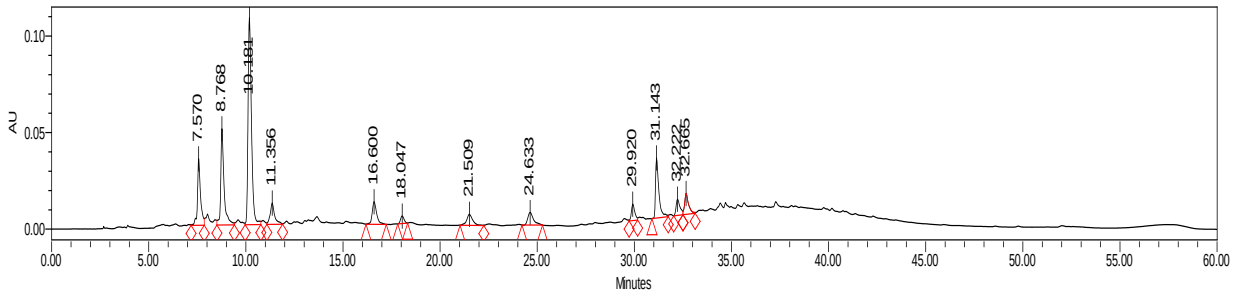


**Figure 298.** HPLC@280 nm, charred layer 6, sonicated rep 1, run 1

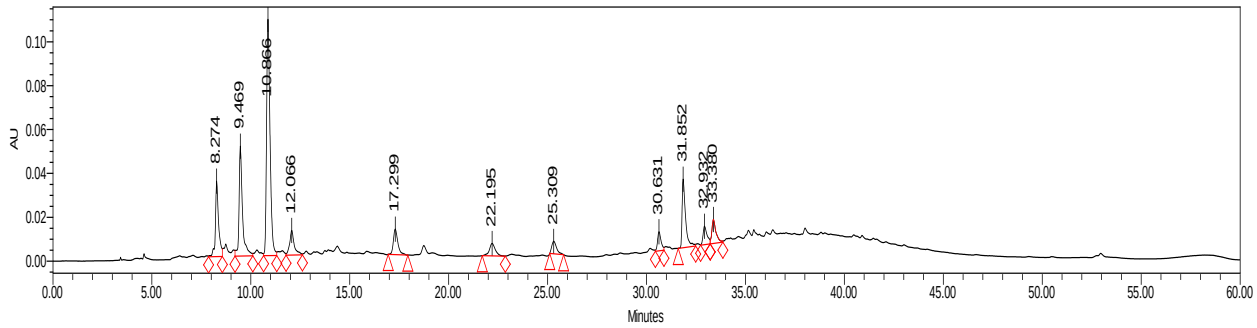


**Figure 299.** HPLC@280 nm, charred layer 6, sonicated rep 1, run 2

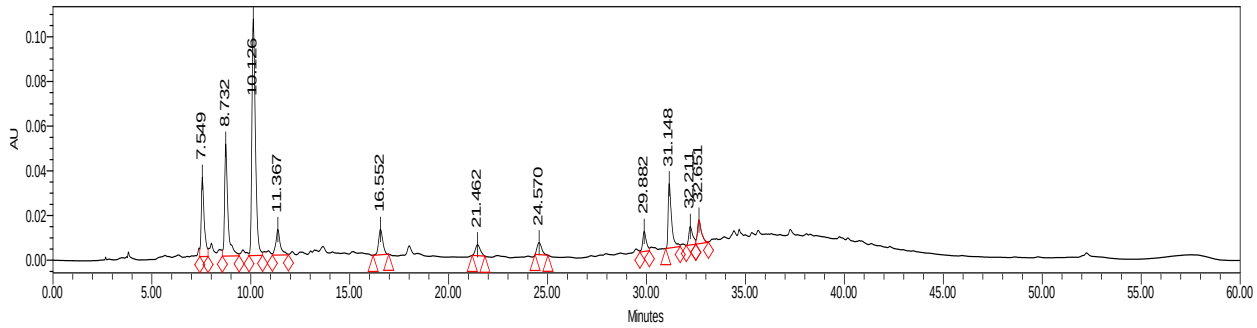


**Figure 300.** HPLC@280 nm, charred layer 6, sonicated rep 2, run 1

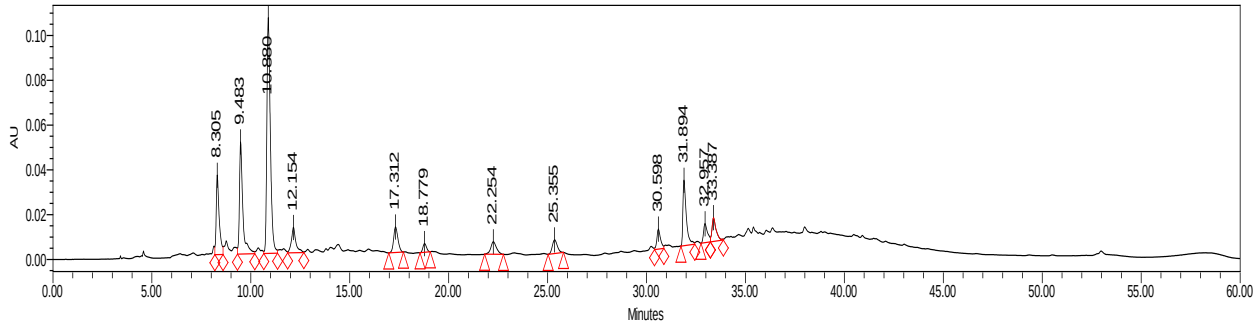


**Figure 301.** HPLC@280 nm, charred layer 6, sonciated rep 2, run 2

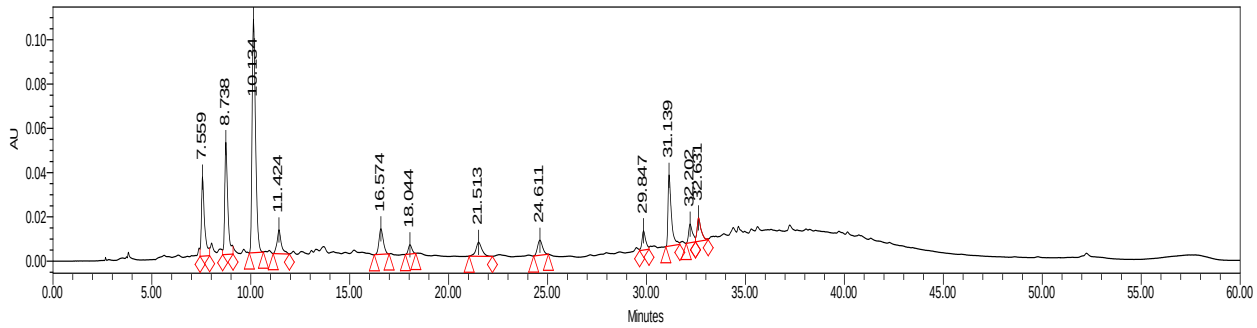


**Figure 302.** HPLC@280 nm, charred layer 6, reflux rep 1, run 1

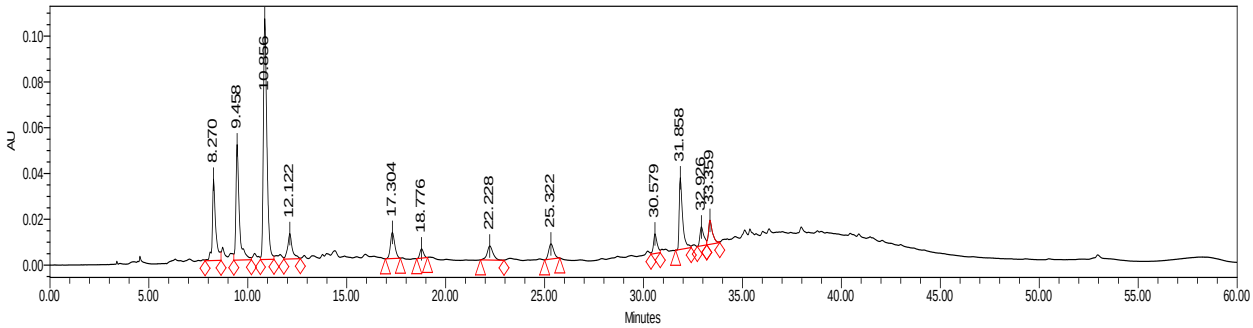


**Figure 303.** HPLC@280 nm, charred layer 6, reflux rep 1, run 2

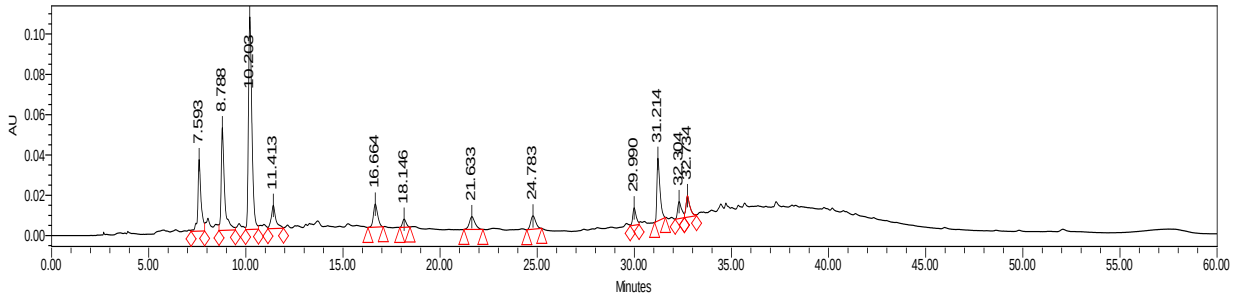


**Figure 304.** HPLC@280 nm, charred layer 6, reflux rep 2, run 1

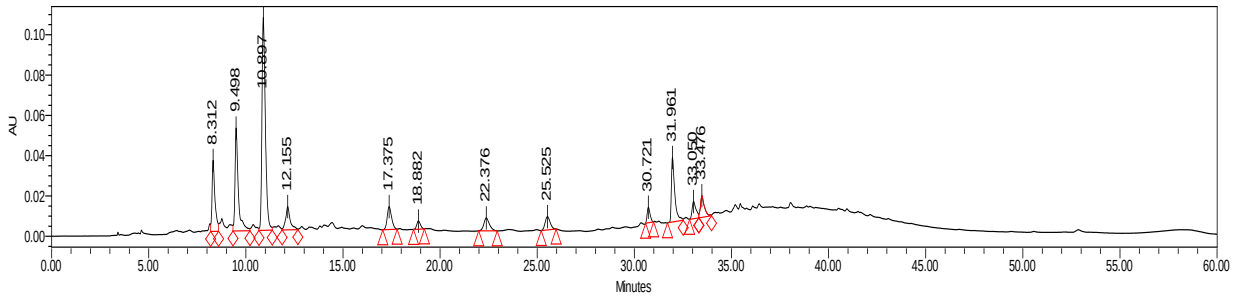


**Figure 305.** HPLC@280 nm, charred layer 6, reflux rep 2, run 2

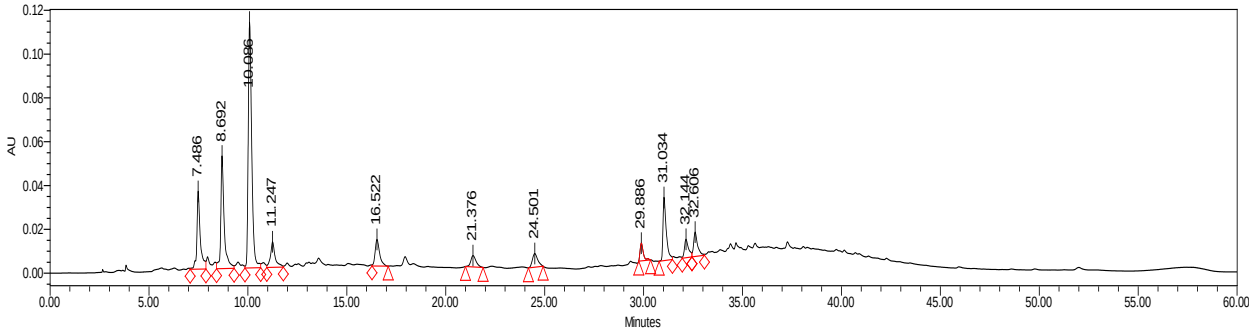


**Figure 306.** HPLC@280 nm, charred layer 6, control rep 1, run 1

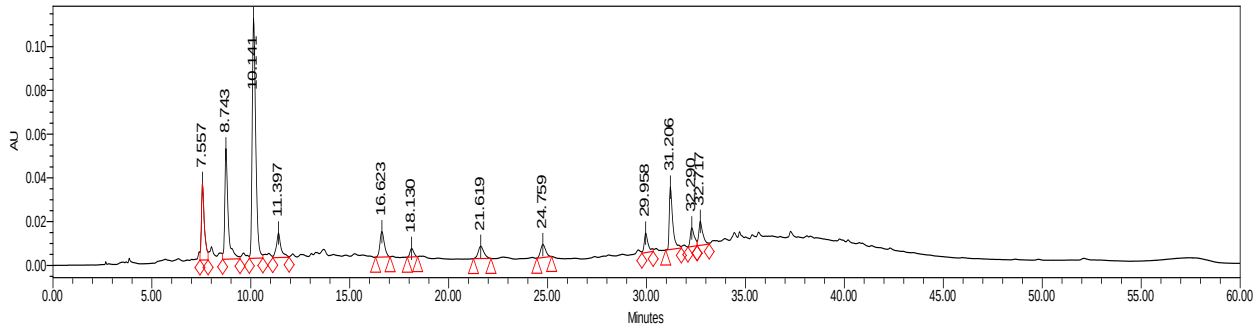


**Figure 307.** HPLC@280 nm, charred layer 6, control rep 1, run 2

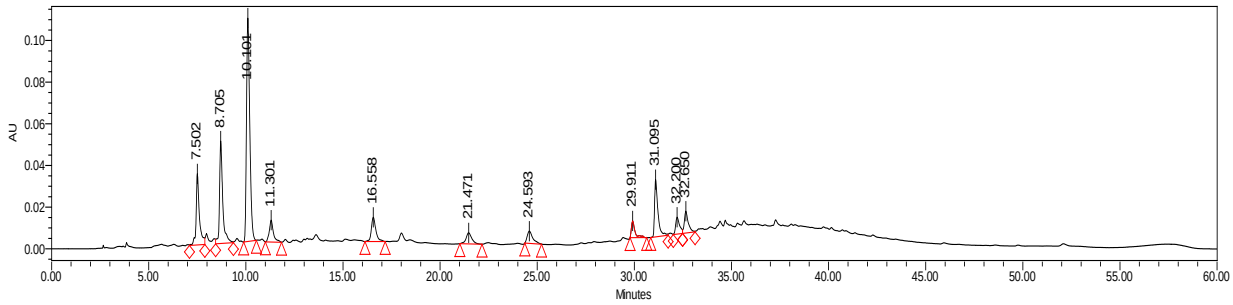


**Figure 308.** HPLC@280 nm, charred layer 6, control rep 2, run 1

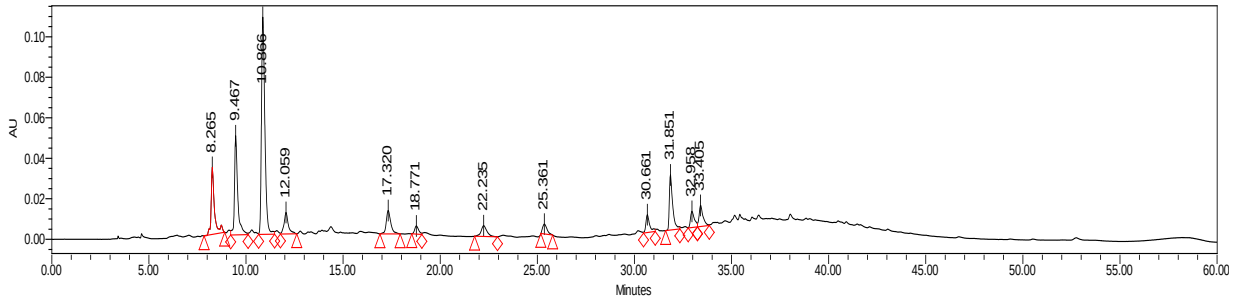


**Figure 309.** HPLC@280 nm, charred layer 6, control rep 2, run 2

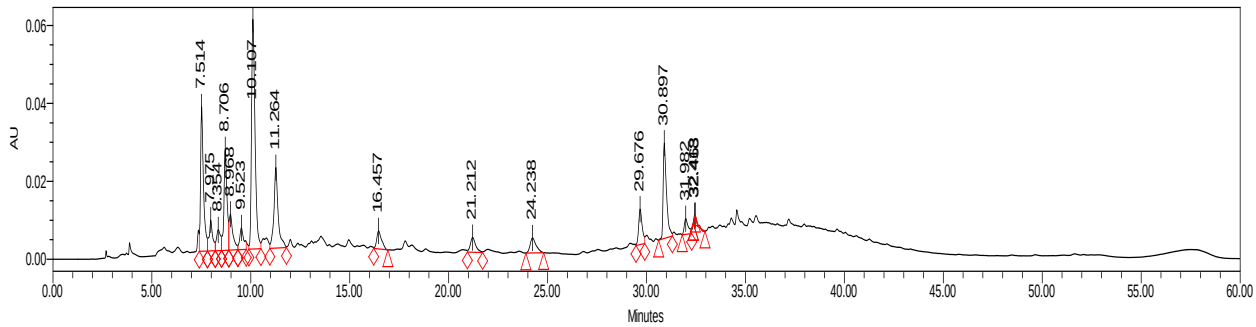


**Figure 310.** HPLC@280 nm, charred layer 7, sonicated rep 1, run 1

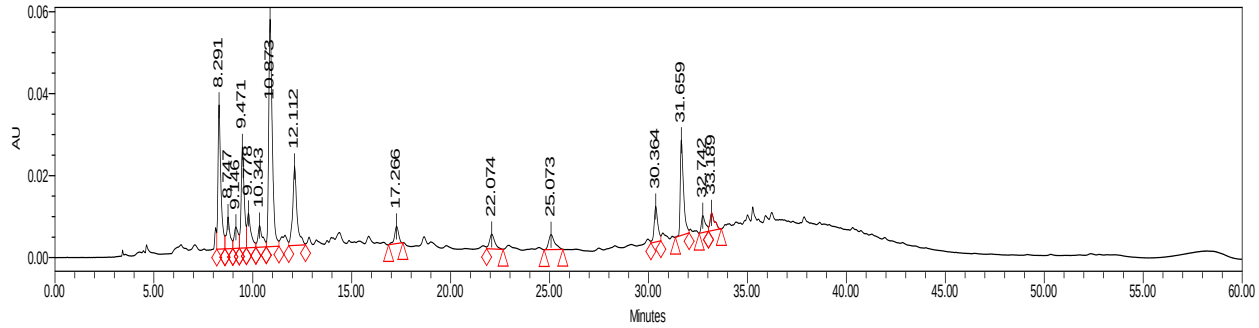


**Figure 311.** HPLC@280 nm, charred layer 7, sonicated rep 1, run 2

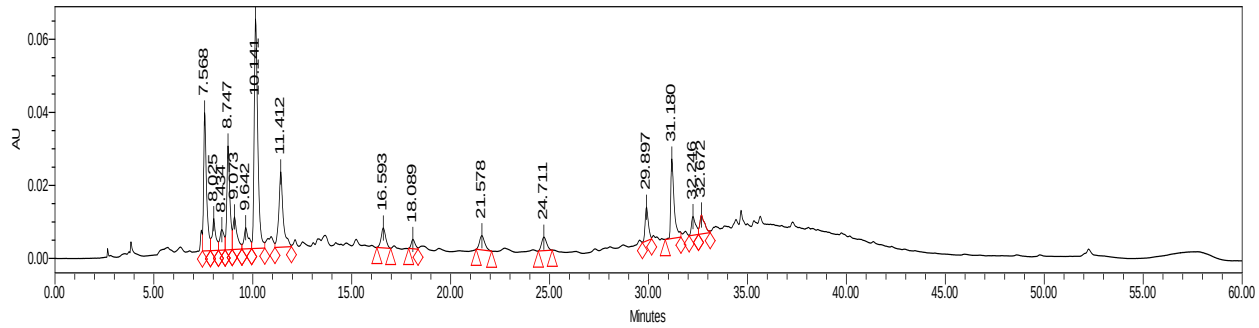


**Figure 312.** HPLC@280 nm, charred layer 7, sonicated rep 2, run 1

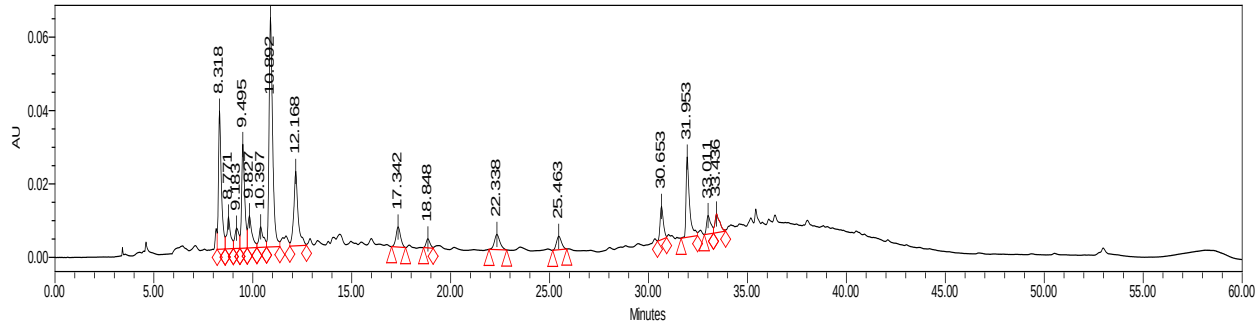


**Figure 313.** HPLC@280 nm, charred layer 7, sonicated rep 2, run 2

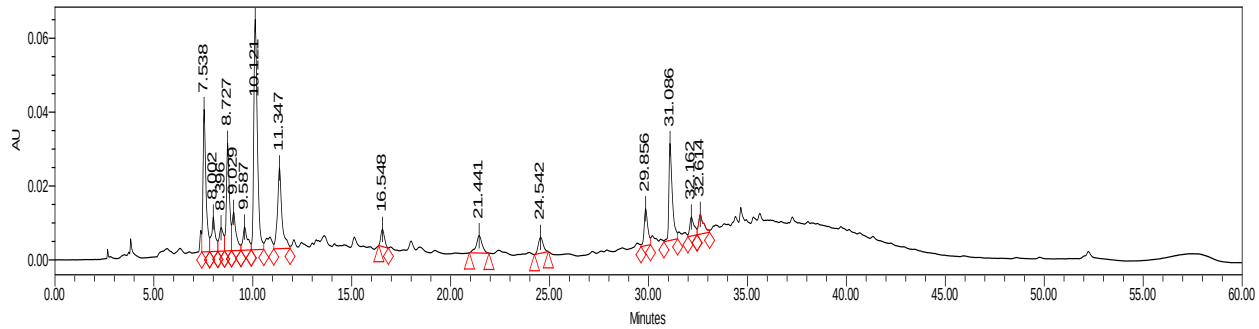


**Figure 314.** HPLC@280 nm, charred layer 7, reflux rep 1, run 1

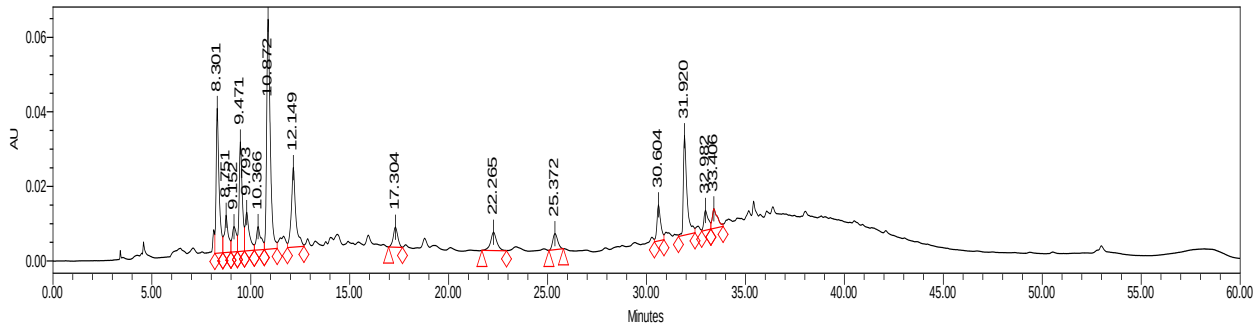


**Figure 315.** HPLC@280 nm, charred layer 7, reflux rep 1, run 2

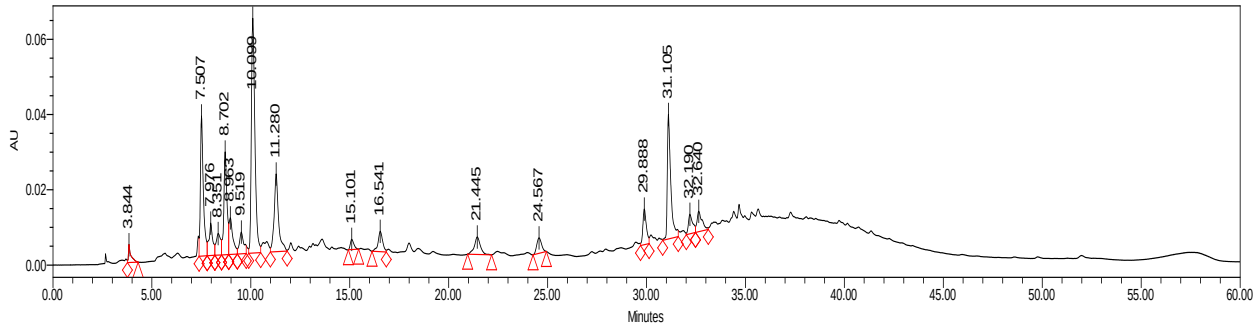


**Figure 316.** HPLC@280 nm, charred layer 7, reflux rep 2, run 1

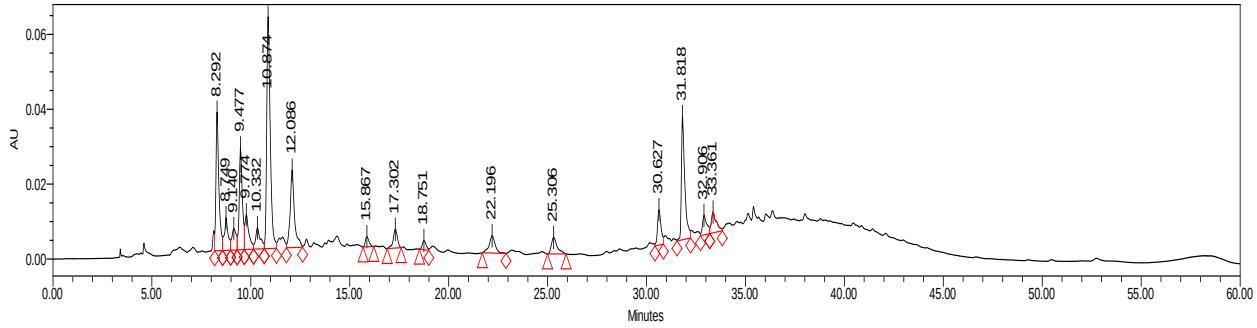


**Figure 317.** HPLC@280 nm, charred layer 7, reflux rep 2, run 2

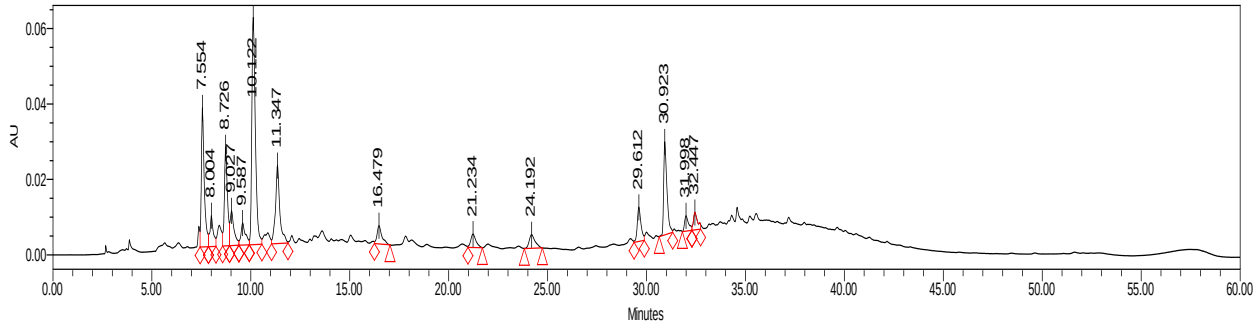


**Figure 318.** HPLC@280 nm, charred layer 7, control rep 1, run 1

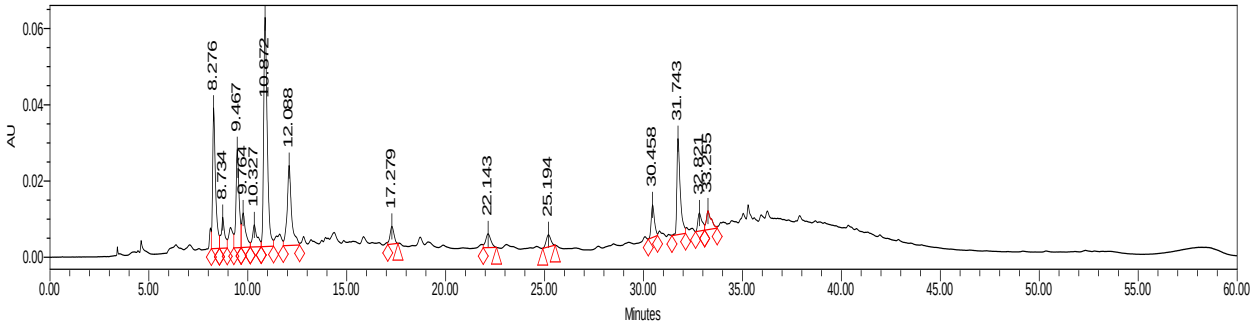


**Figure 319.** HPLC@280 nm, charred layer 7, control rep 1, run 2

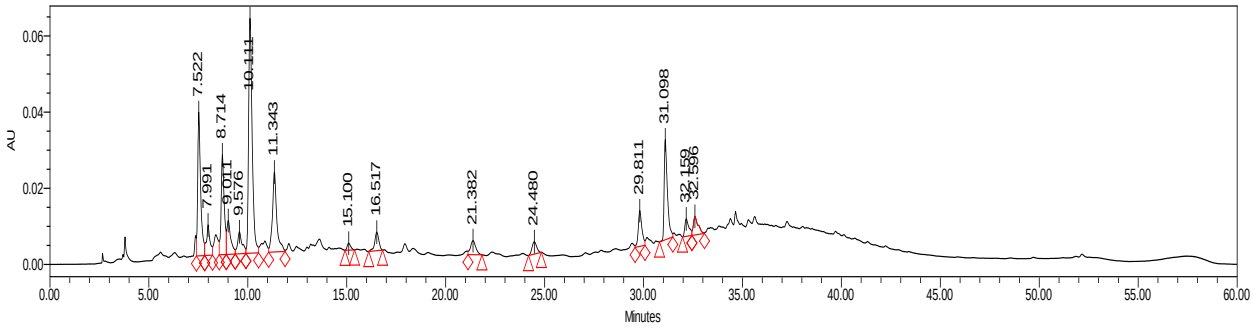


**Figure 320.** HPLC@280 nm, charred layer 7, control rep 2, run 1

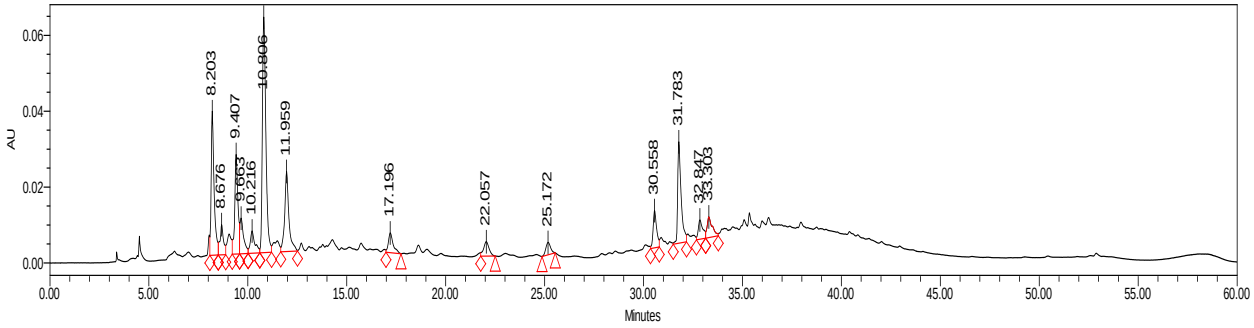


**Figure 321.** HPLC@280 nm, charred layer 7, control rep 2, run 2

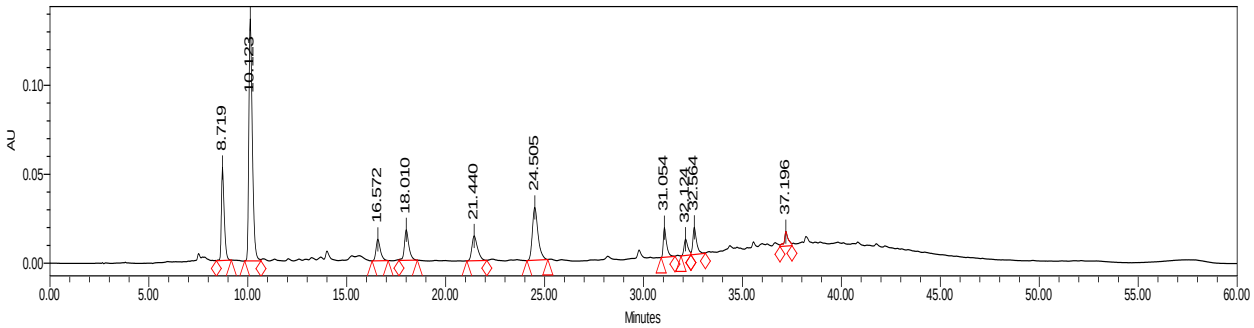


**Figure 322.** HPLC@280 nm, toasted layer 1, sonicated rep 1, run 1

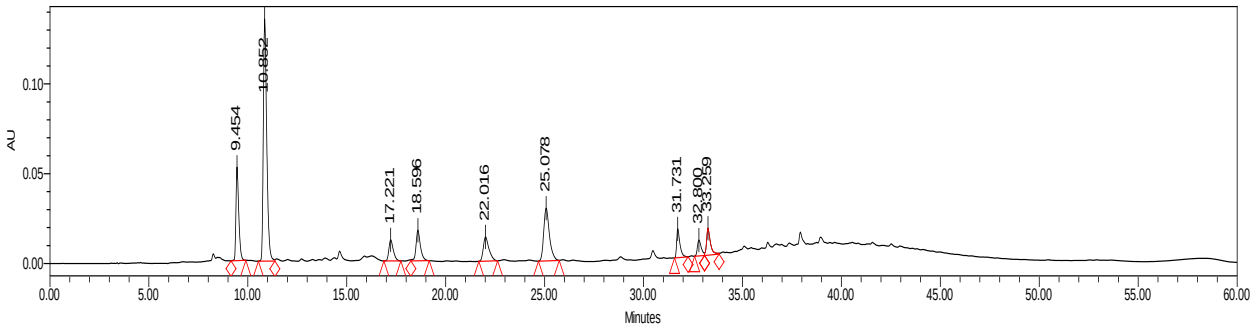


**Figure 323.** HPLC@280 nm, toasted layer 1, sonicated rep 1, run 2

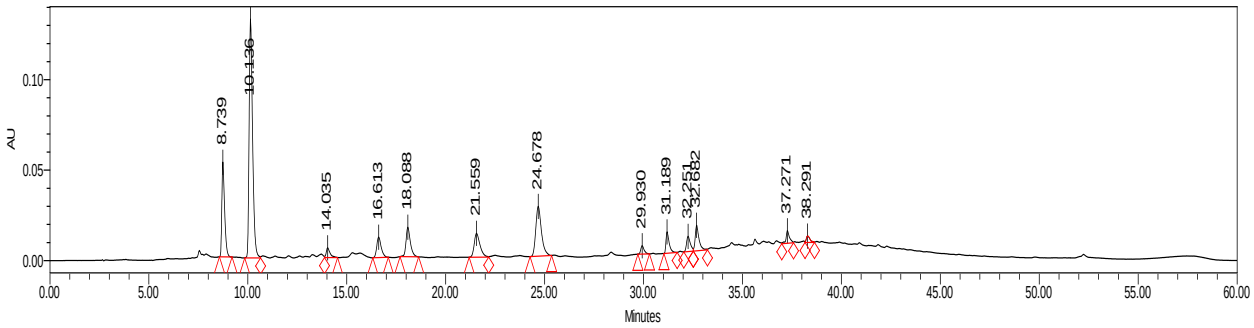


**Figure 324.** HPLC@280 nm, toasted layer 1, sonicated rep 2, run 1

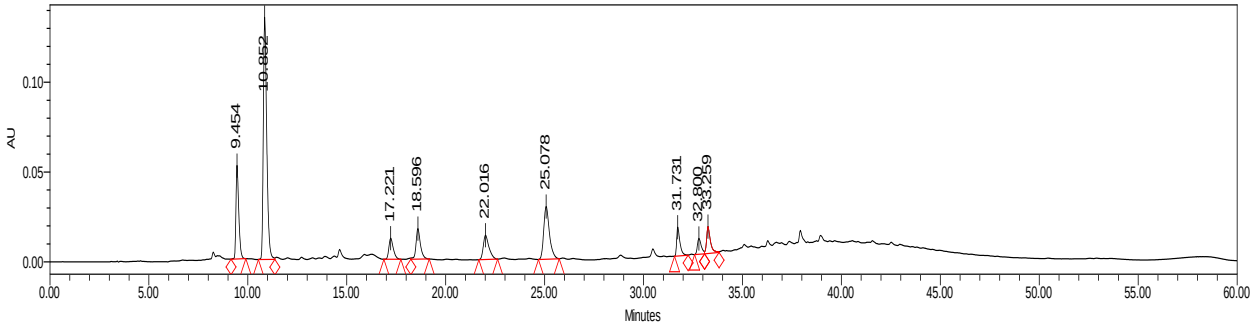


**Figure 325.** HPLC@280 nm, toasted layer 1, sonicated rep 2, run 2

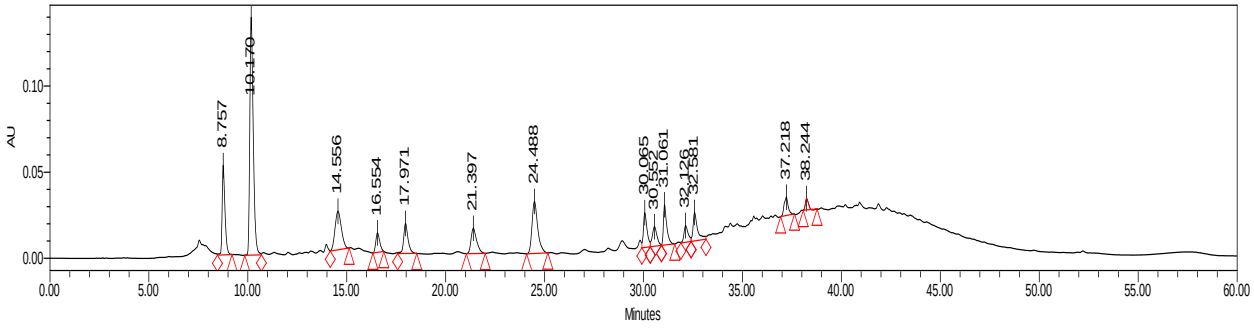


**Figure 326.** HPLC@280 nm, toasted layer 1, reflux rep 1, run 1

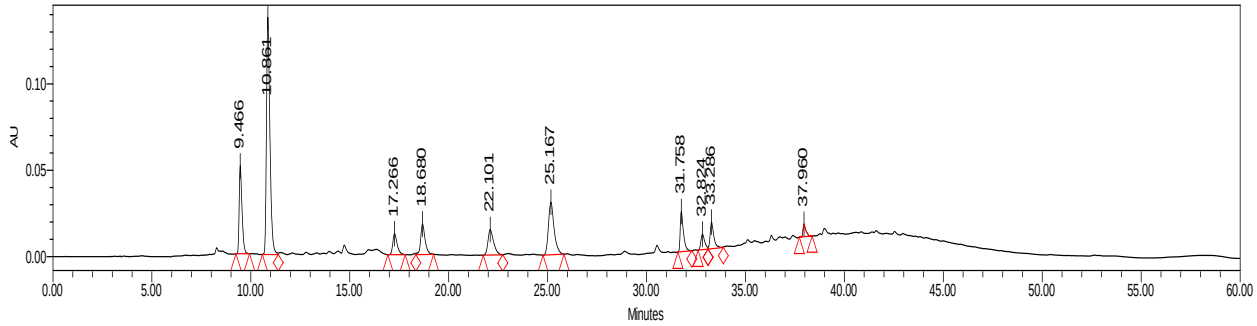


**Figure 327.** HPLC@280 nm, toasted layer 1, reflux rep 1, run 2

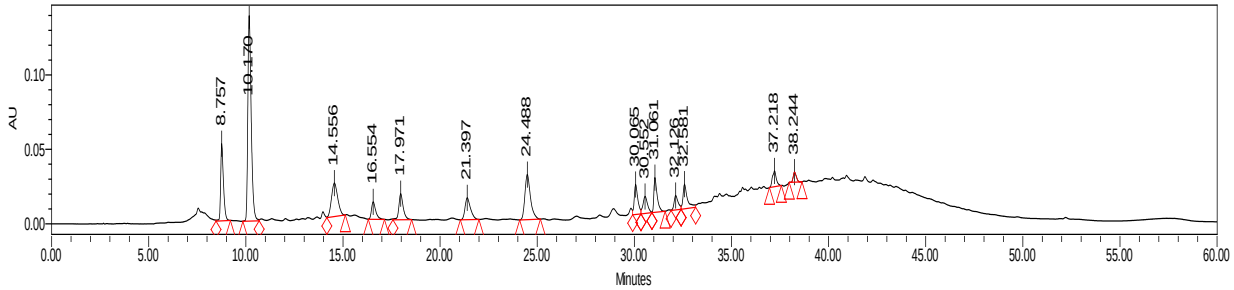


**Figure 328.** HPLC@280 nm, toasted layer 1, reflux rep 2, run 1

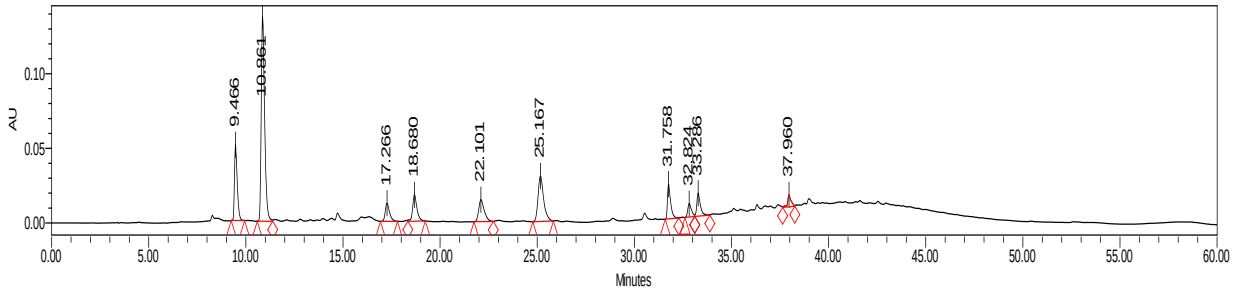


**Figure 329.** HPLC@280 nm, toasted layer 1, reflux rep 2, run 2

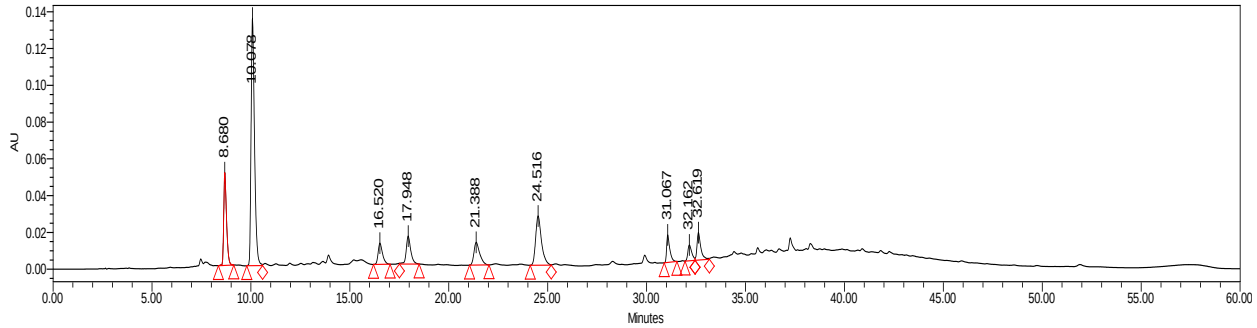


**Figure 330.** HPLC@280 nm, toasted layer 1, control rep 1, run 1

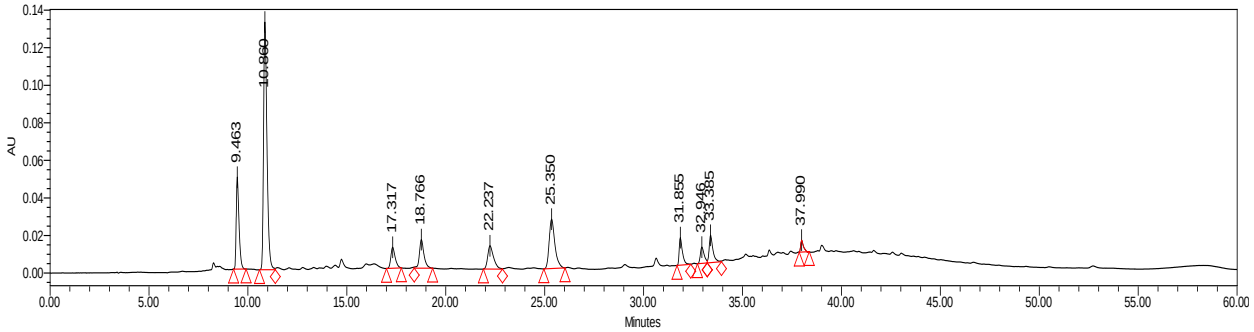


**Figure 331.** HPLC@280 nm, toasted layer 1, control rep 1, run 2

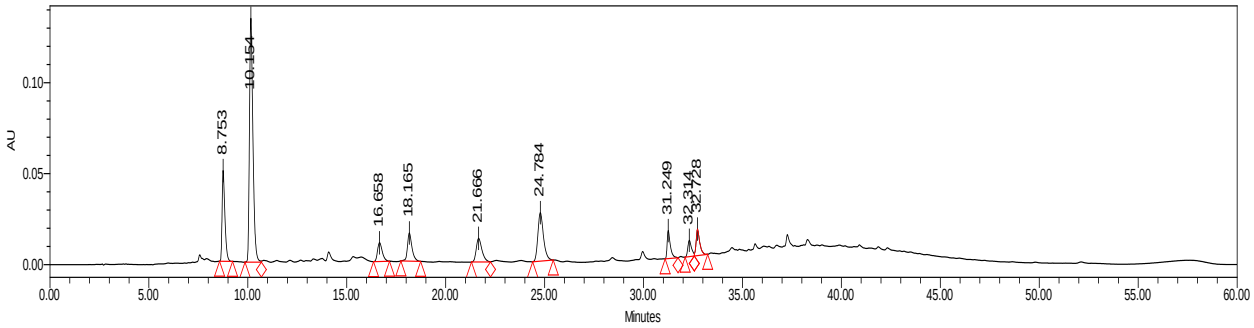


**Figure 332.** HPLC@280 nm, toasted layer 1, control rep 2, run 1

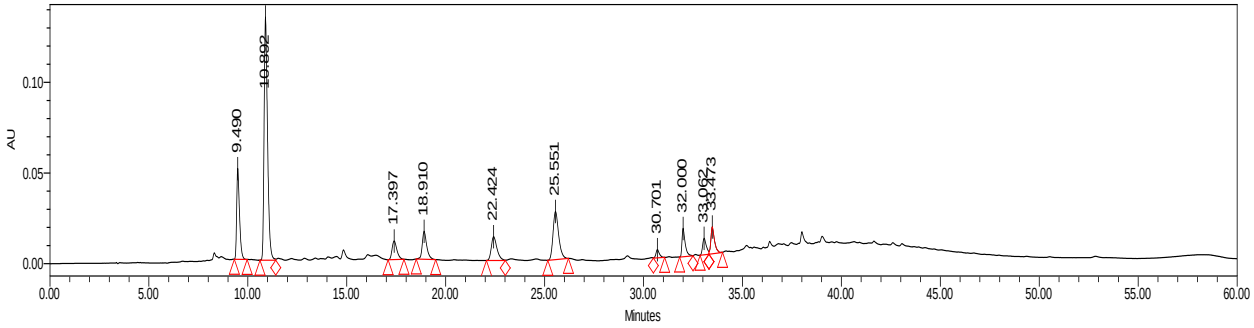


**Figure 333.** HPLC@280 nm, toasted layer 1, control rep 2, run 2

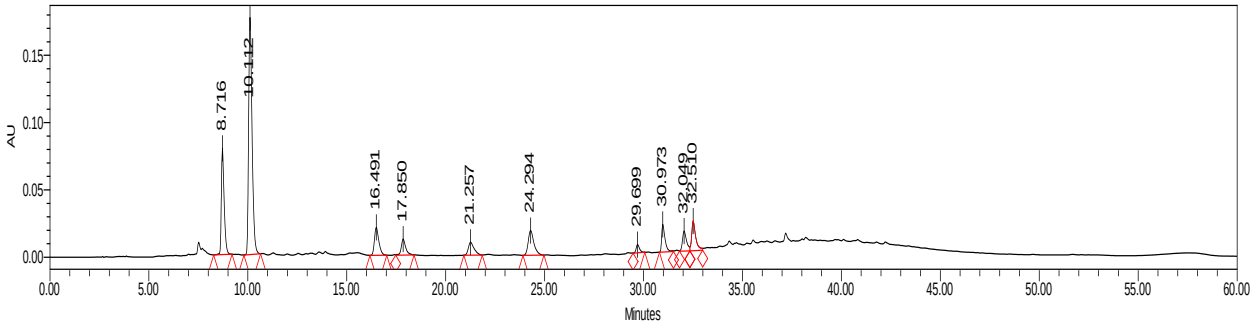


**Figure 334.** HPLC@280 nm, toasted layer 2, sonicated rep 1, run 1

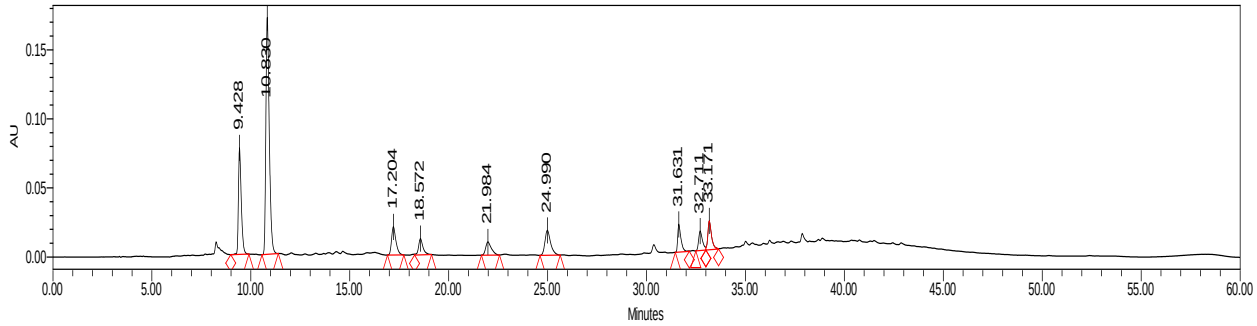


**Figure 335.** HPLC@280 nm, toasted layer 2, sonicated rep 1, run 2

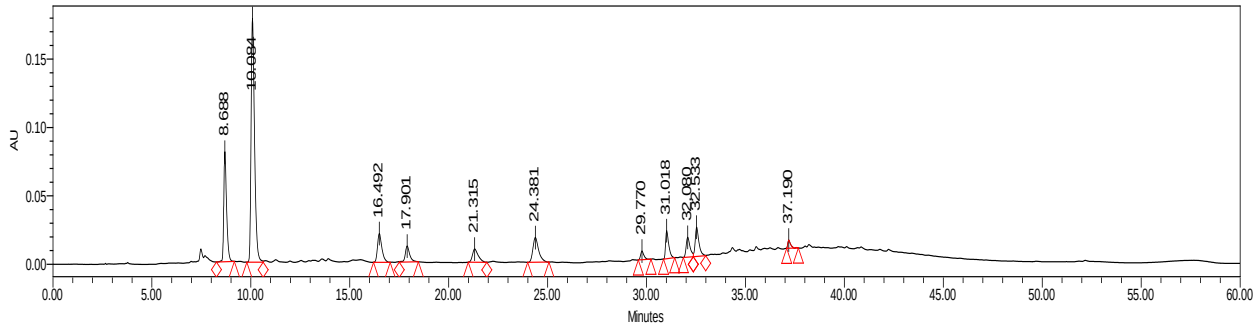


**Figure 336.** HPLC@280 nm, toasted layer 2, sonicated rep 2, run 1

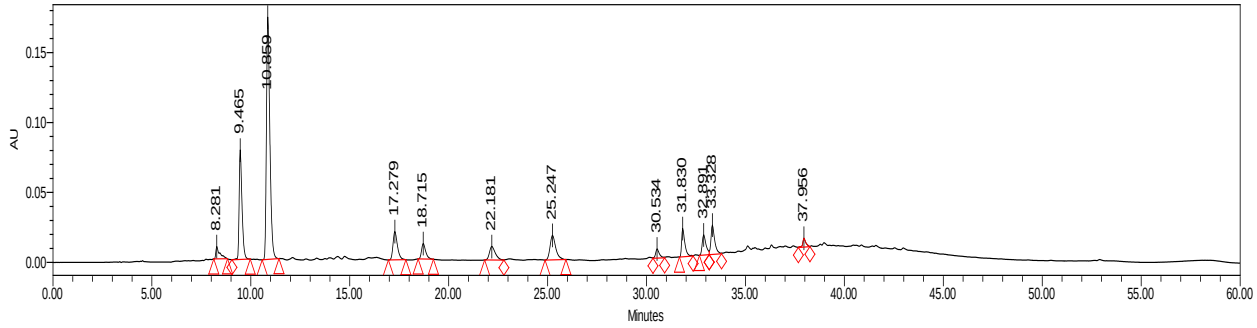


**Figure 337.** HPLC@280 nm, toasted layer 2, sonicated rep 2, run 2

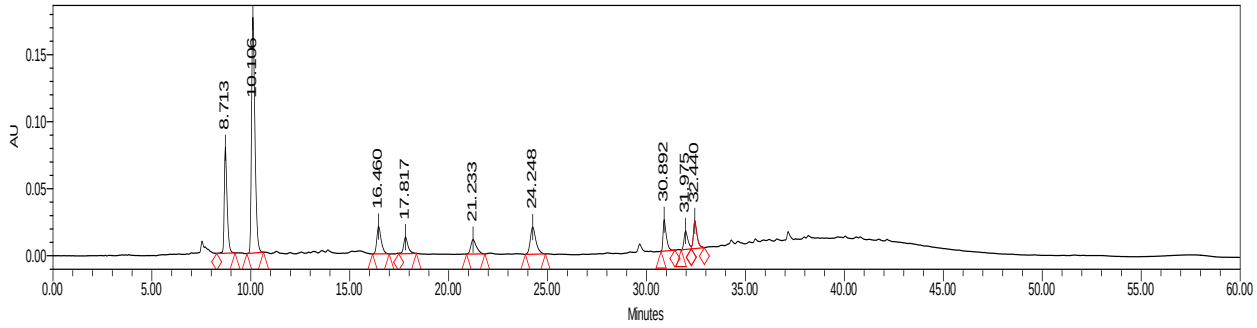


**Figure 338.** HPLC@280 nm, toasted layer 2, reflux rep 1, run 1

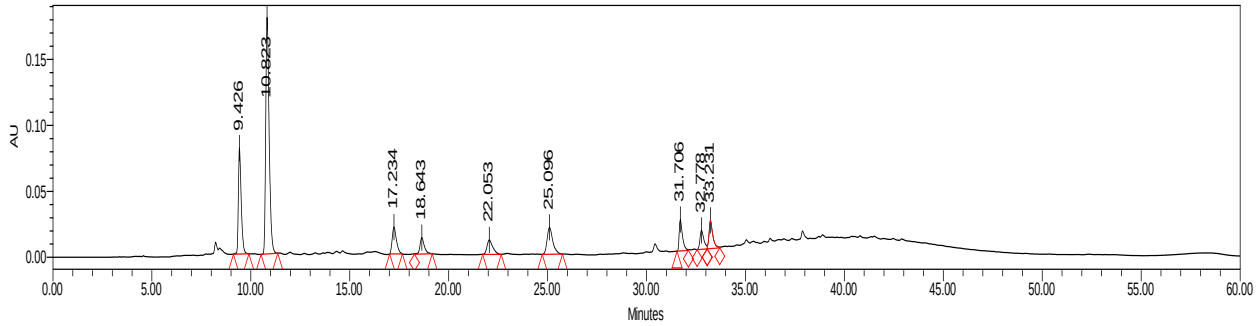


**Figure 339.** HPLC@280 nm, toasted layer 2, reflux rep 1, run 2

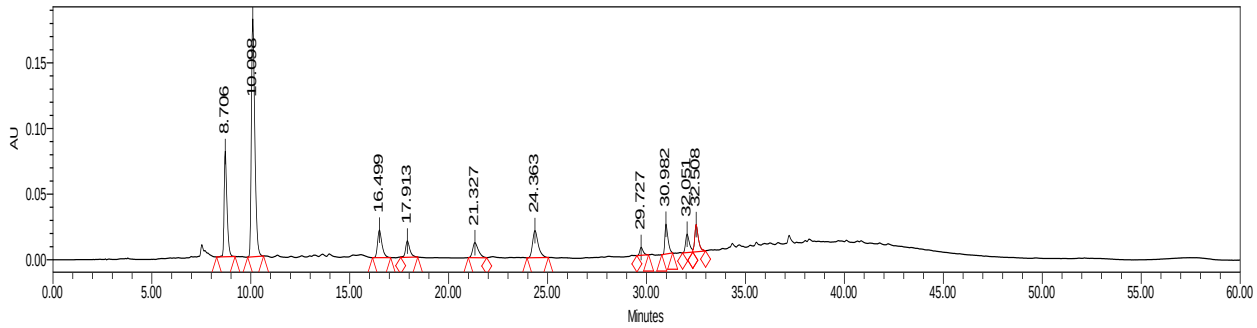


**Figure 340.** HPLC@280 nm, toasted layer 2, reflux rep 2, run 1

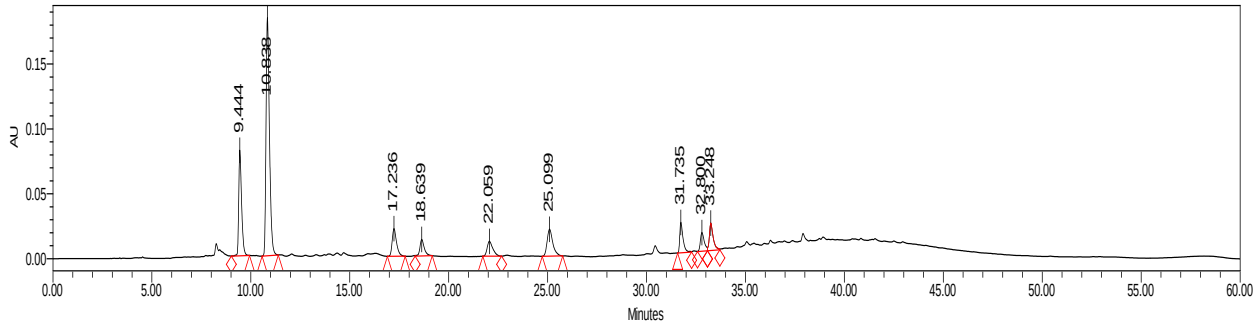


**Figure 341.** HPLC@280 nm, toasted layer 2, reflux rep 2, run 1

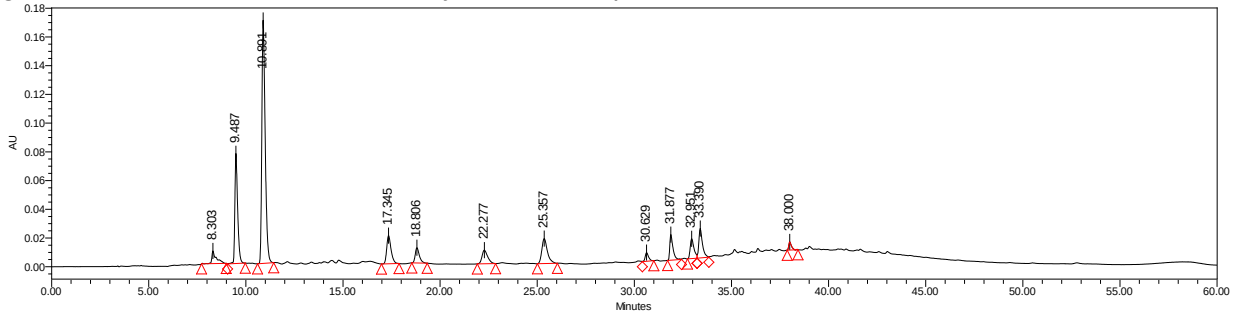


**Figure 342.** HPLC@280 nm, toasted layer 2, control rep 1, run 1

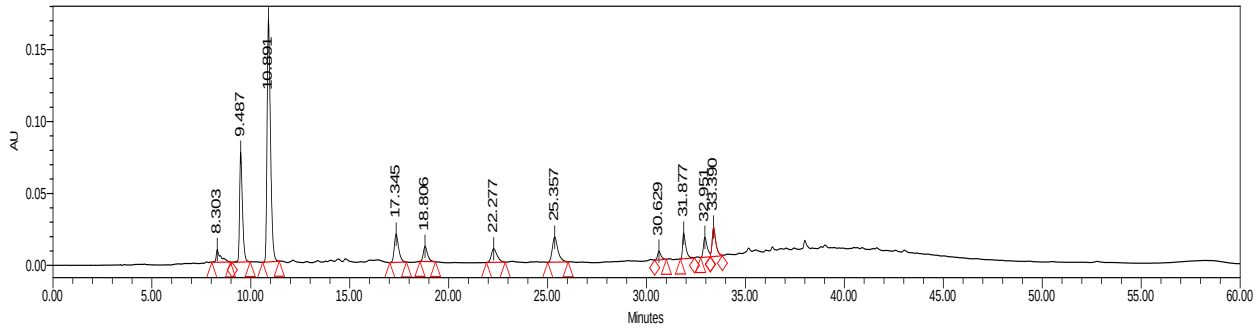


**Figure 343.** HPLC@280 nm, toasted layer 2, control rep 1, run 2

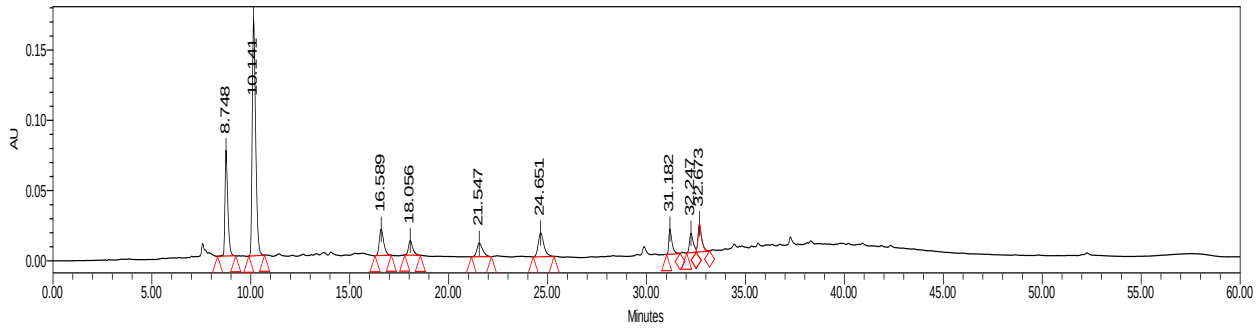


**Figure 344.** HPLC@280 nm, toasted layer 2, control rep 2, run 1

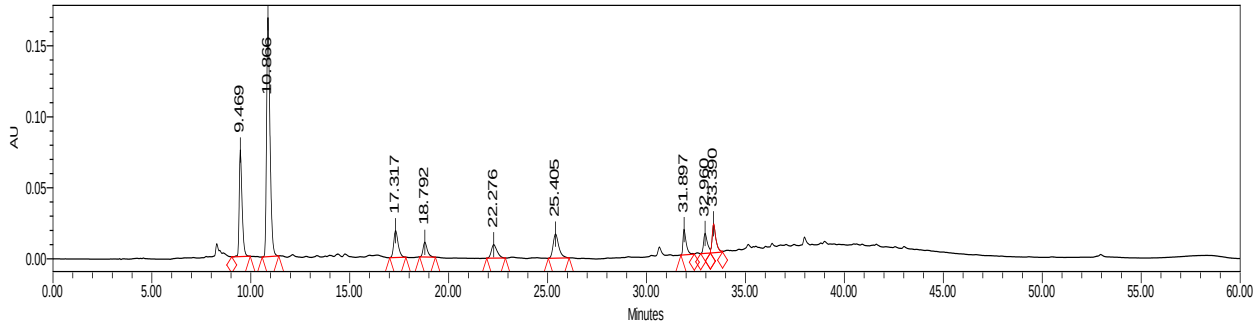


**Figure 345.** HPLC@280 nm, toasted layer 2, control rep 2, run 2

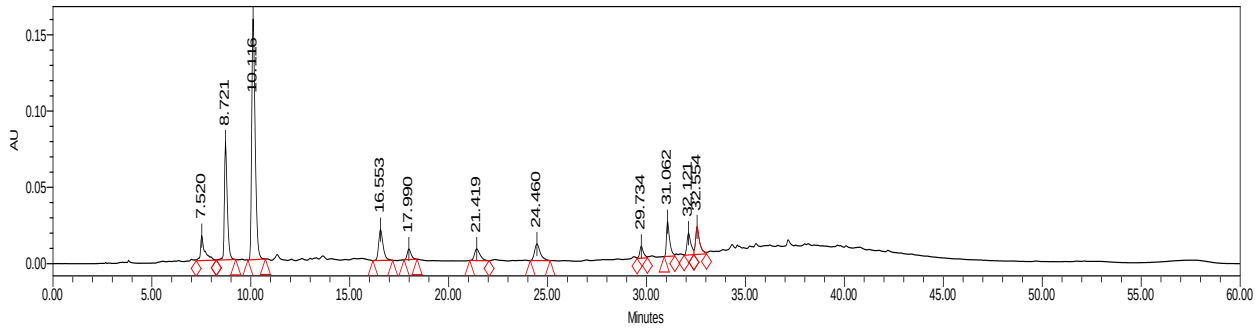


**Figure 346.** HPLC@280 nm, toasted layer 3, sonicated rep 1, run 1

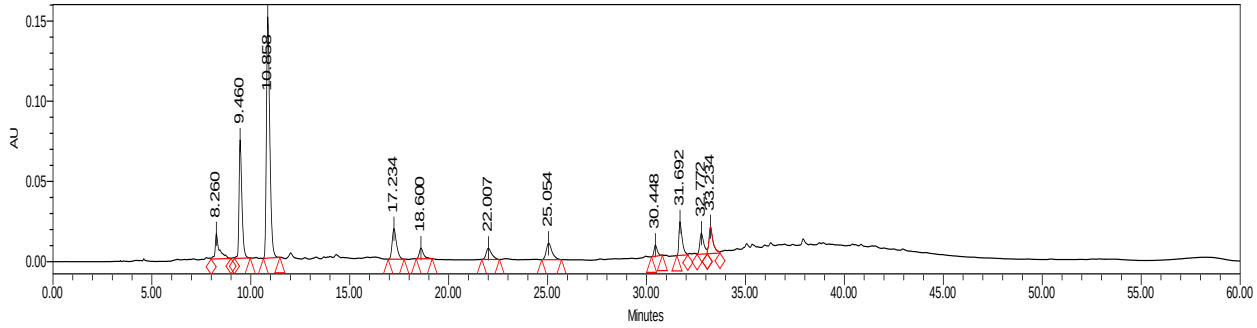


**Figure 347.** HPLC@280 nm, toasted layer 3, sonicated rep 1, run 2

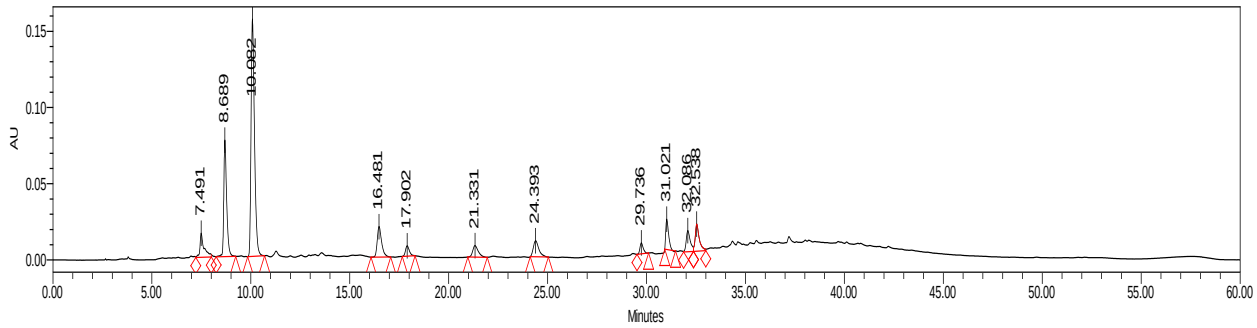


**Figure 348.** HPLC@280 nm, toasted layer 3, sonicated rep 2, run 1

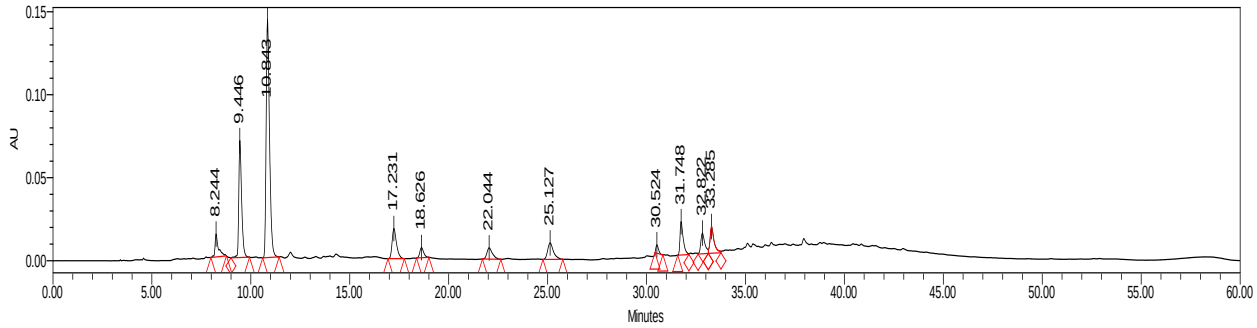


**Figure 349.** HPLC@280 nm, toasted layer 3, sonicated rep 2, run 2

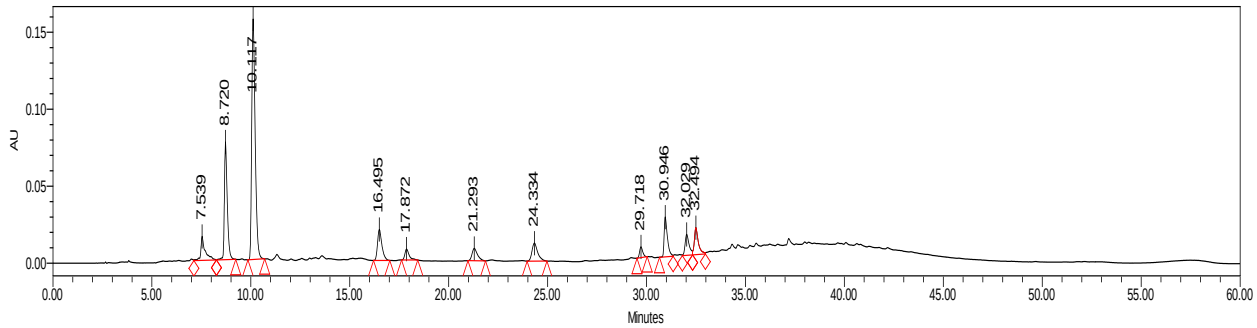


**Figure 350.** HPLC@280 nm, toasted layer 3, reflux rep 1, run 1

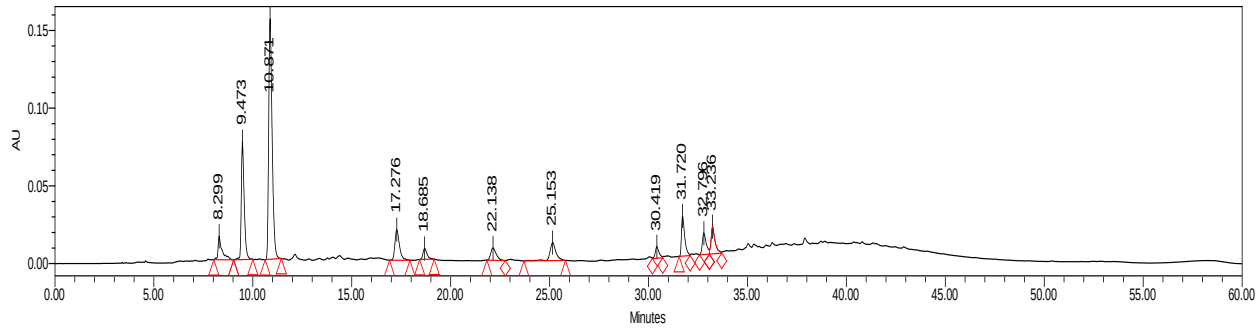


**Figure 351.** HPLC@280 nm, toasted layer 3, reflux rep 1, run 2

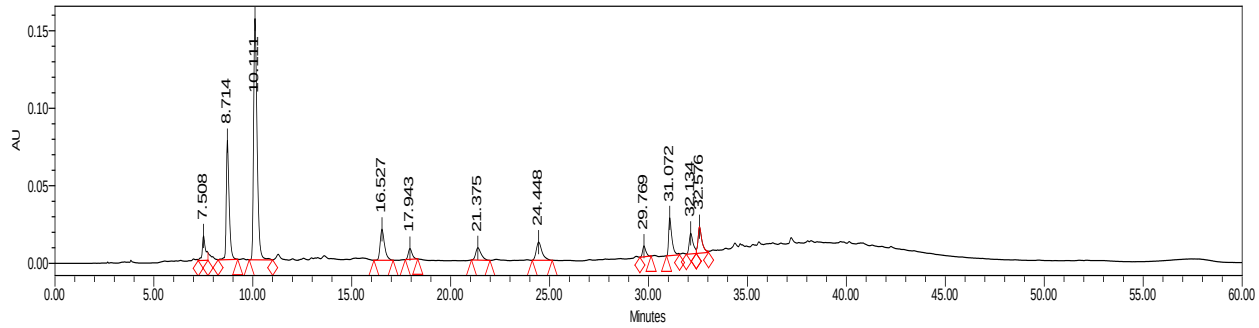


**Figure 352.** HPLC@280 nm, toasted layer 3, reflux rep 2, run 1

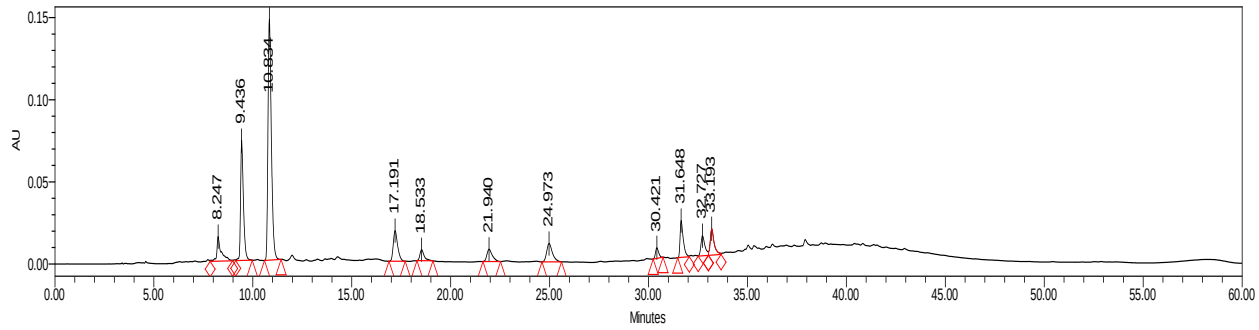


**Figure 353.** HPLC@280 nm, toasted layer 3, reflux rep 2, run 2

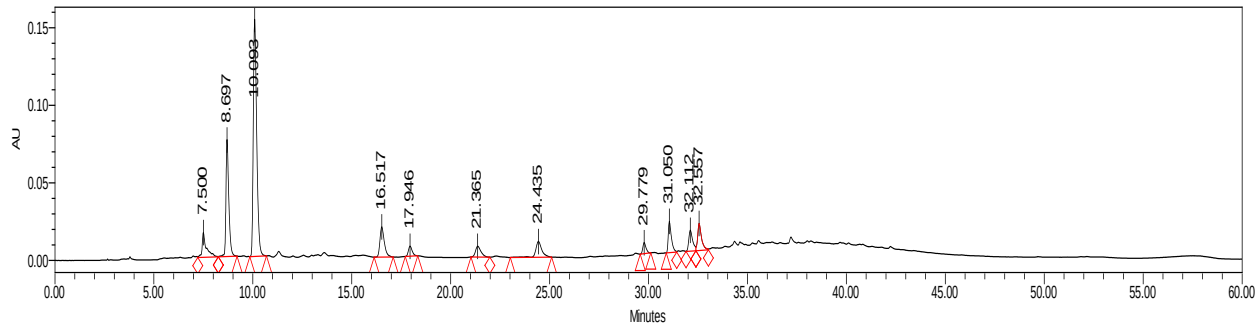


**Figure 354.** HPLC@280 nm, toasted layer 3, control rep 1, run 1

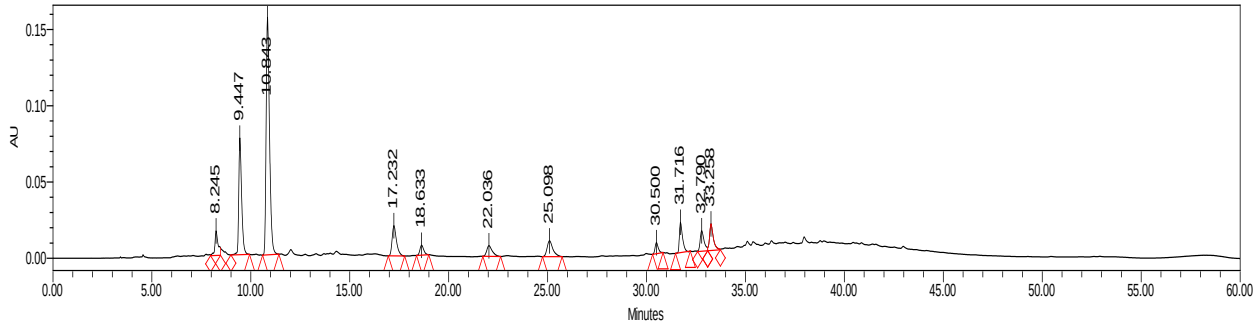


**Figure 355.** HPLC@280 nm, toasted layer 3, control rep 1, run 2

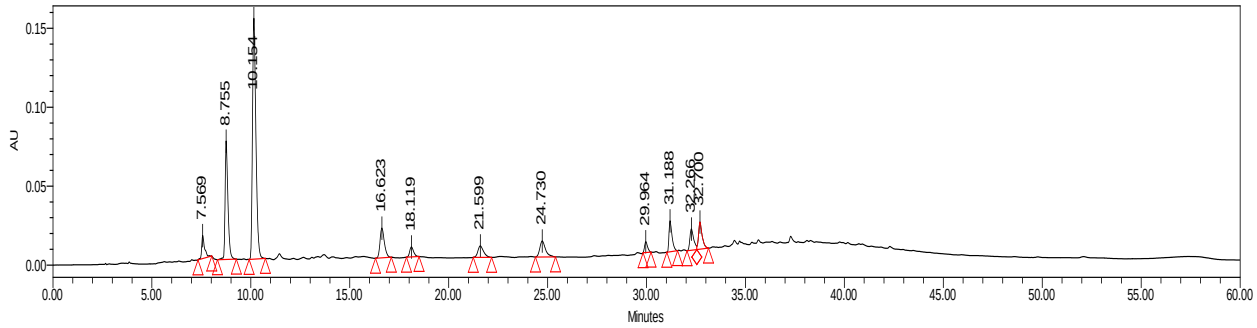


**Figure 356.** HPLC@280 nm, toasted layer 3, control rep 2, run 1

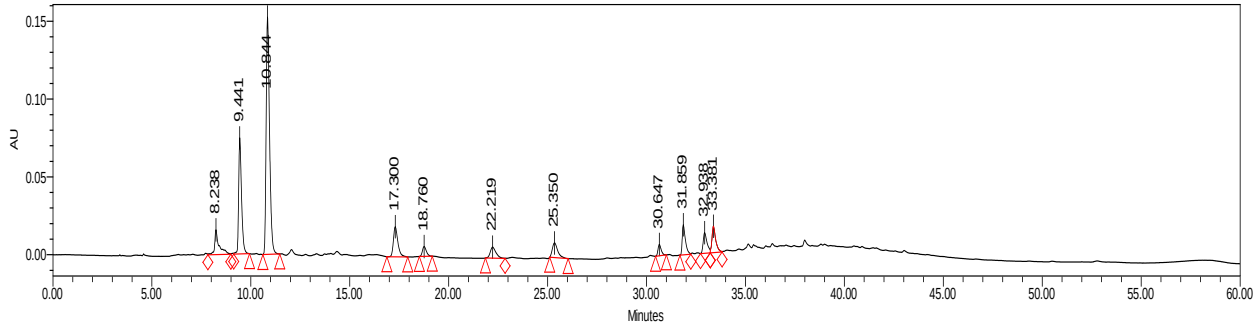


**Figure 357.** HPLC@280 nm, toasted layer 3, control rep 2, run 2

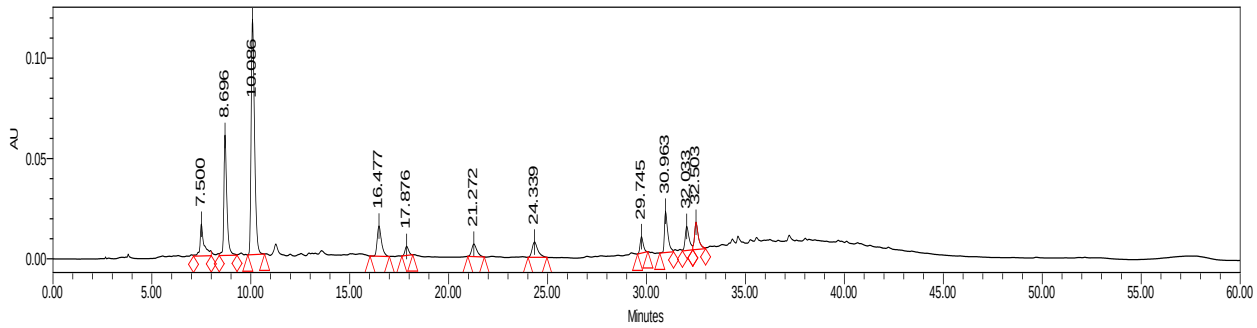


**Figure 358.** HPLC@280 nm, toasted layer 4, sonicated rep 1, run 1

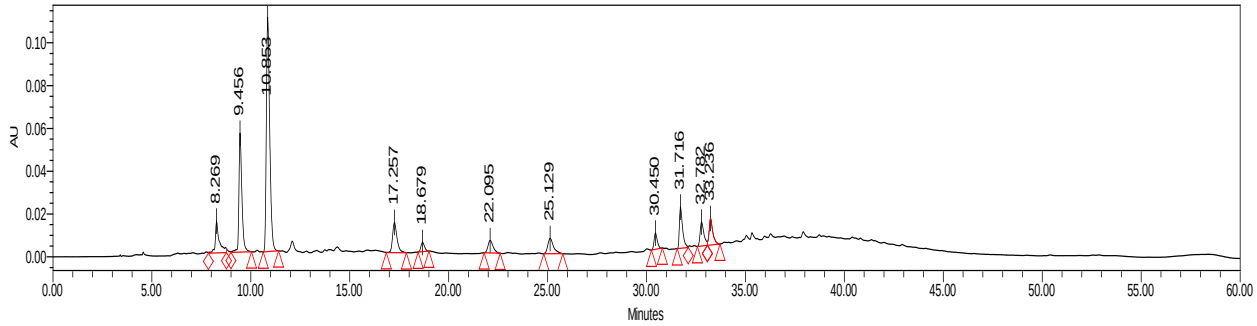


**Figure 359.** HPLC@280 nm, toasted layer 4, sonicated rep 1, run 2

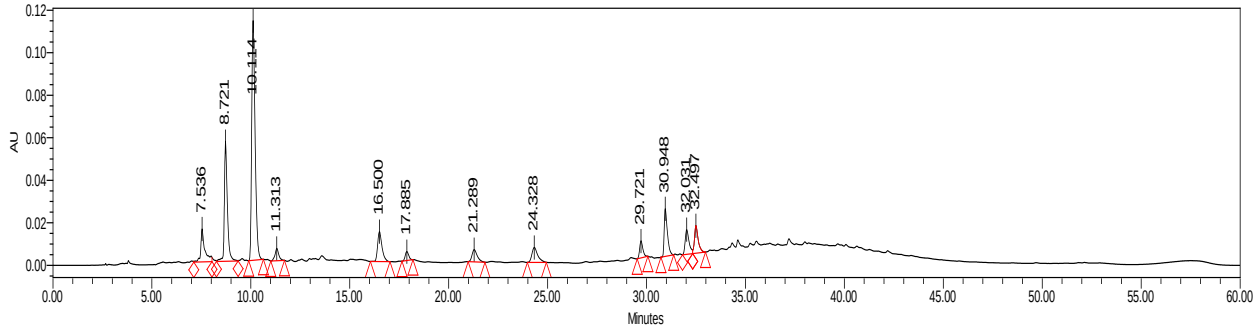


**Figure 360.** HPLC@280 nm, toasted layer 4, sonicated rep 2, run 1

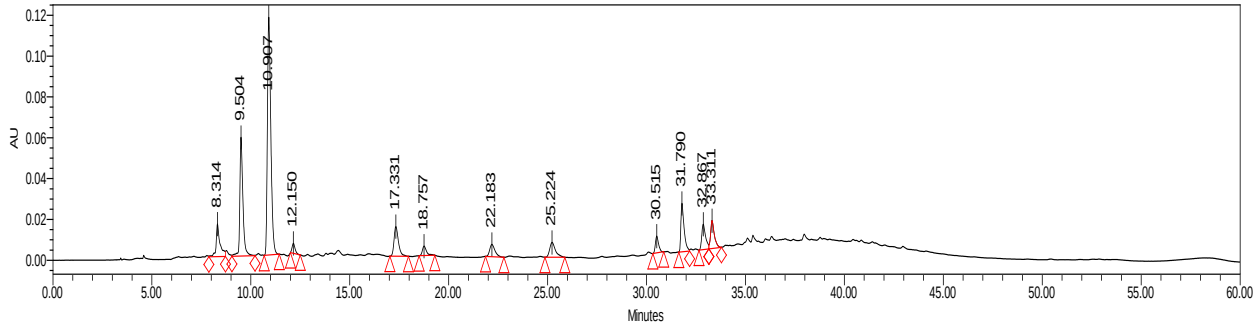


**Figure 361.** HPLC@280 nm, toasted layer 4, sonicated rep 2, run 2

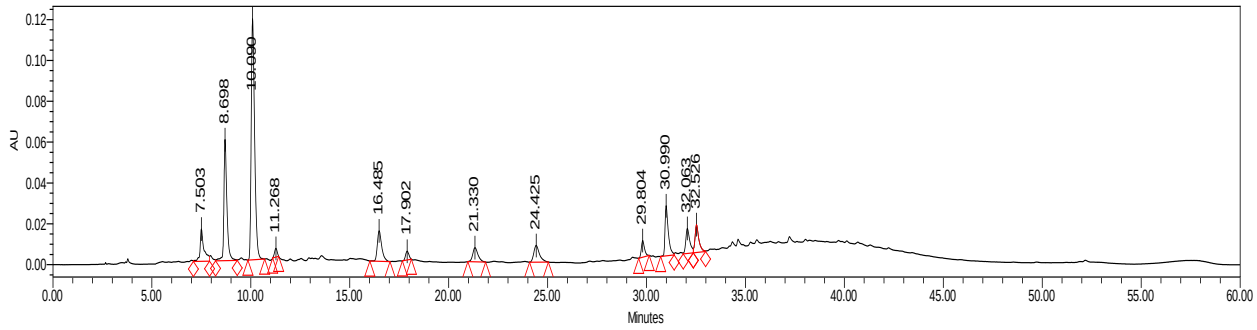


**Figure 362.** HPLC@280 nm, toasted layer 4, reflux rep 1, run 1

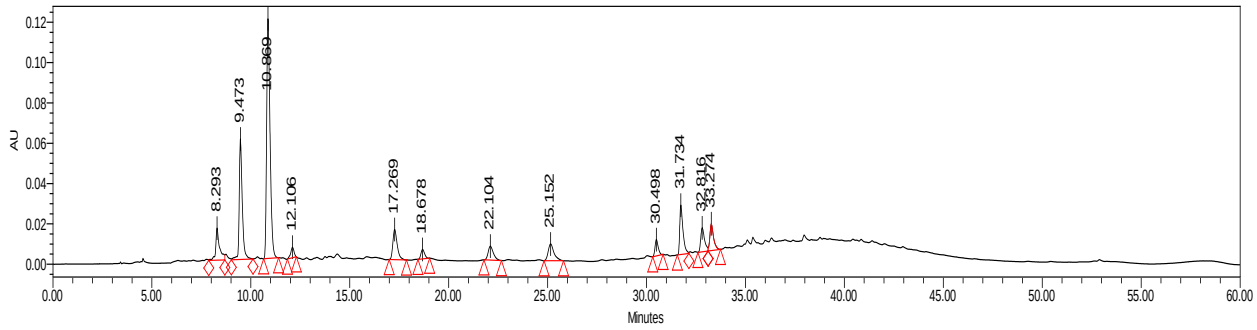


**Figure 363.** HPLC@280 nm, toasted layer 4, reflux rep 1, run 2

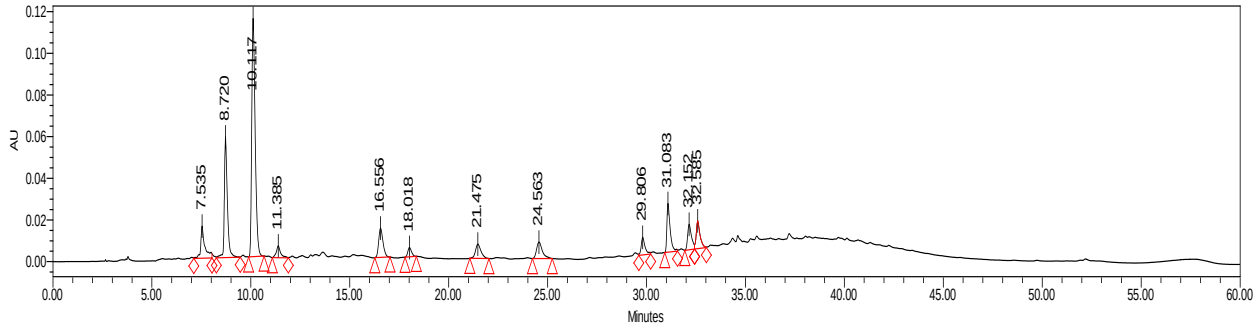


**Figure 364.** HPLC@280 nm, toasted layer 4, reflux rep 2, run 1

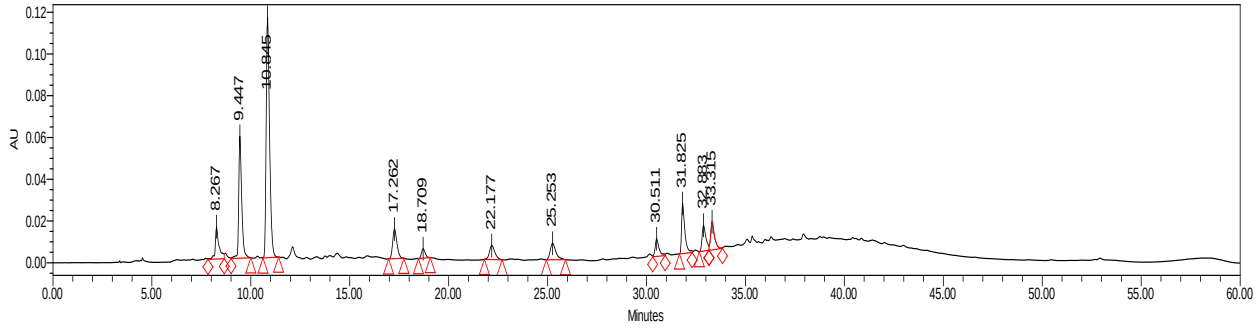


**Figure 365.** HPLC@280 nm, toasted layer 4, reflux rep 2, run 2

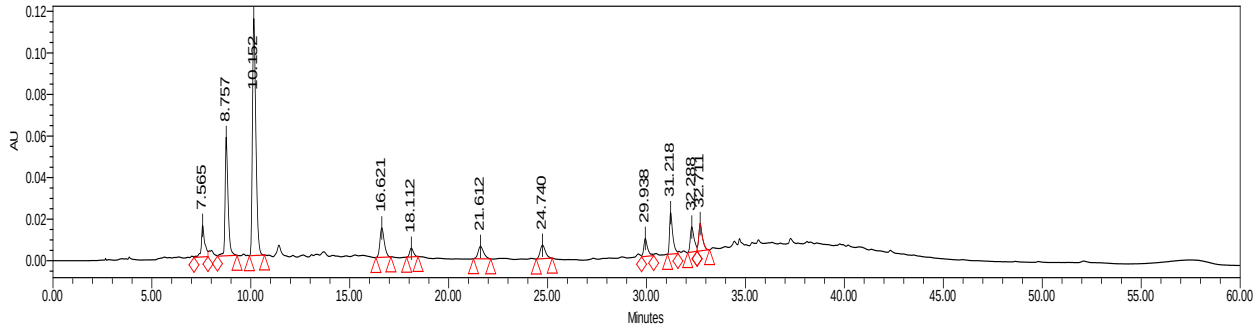


**Figure 366.** HPLC@280 nm, toasted layer 4, control rep 1, run 1

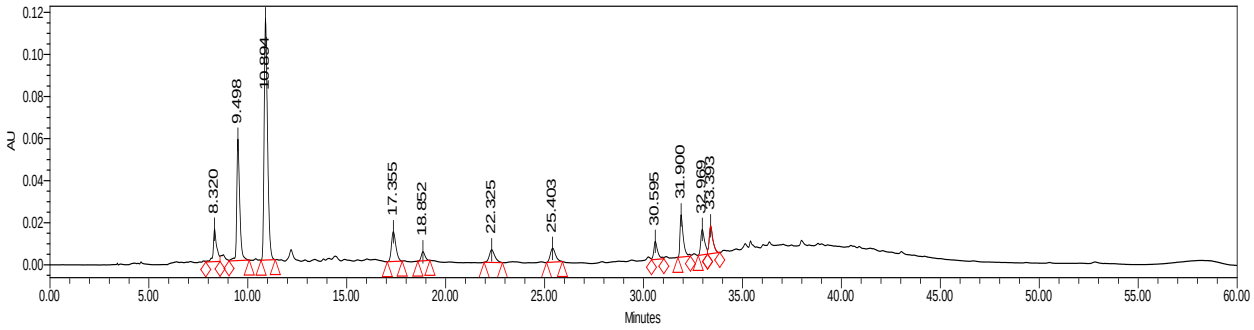


**Figure 367.** HPLC@280 nm, toasted layer 4, control rep 1, run 2

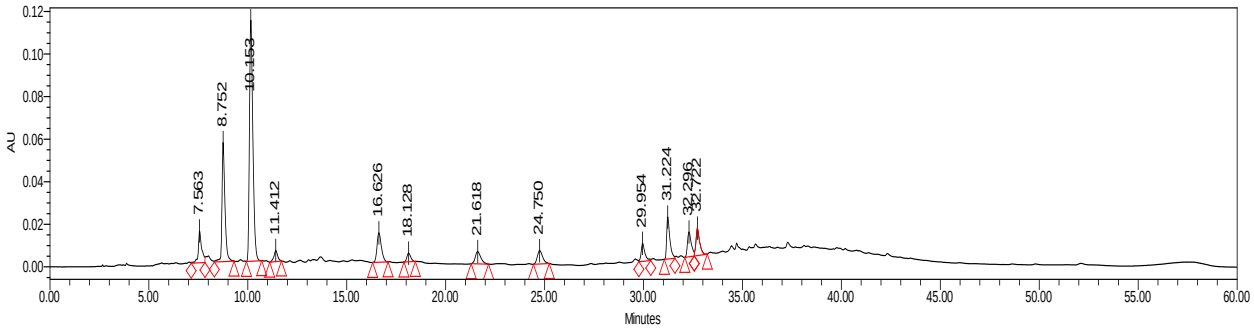


**Figure 368.** HPLC@280 nm, toasted layer 4, control rep 2, run 1

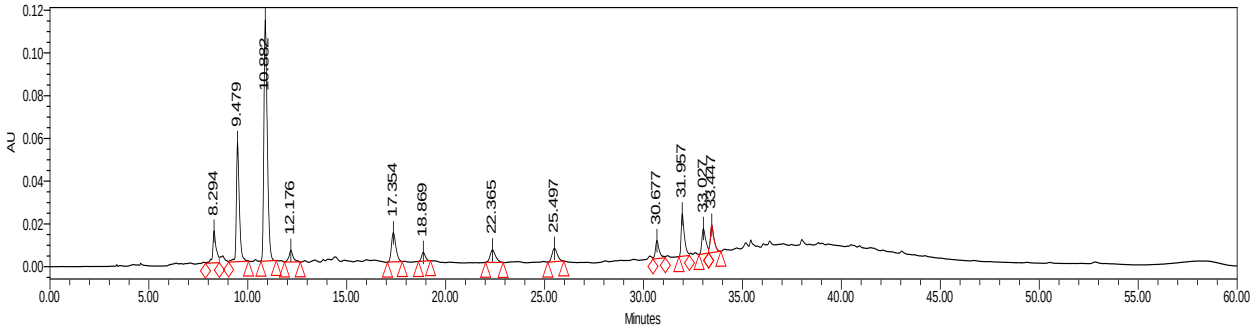


**Figure 369.** HPLC@280 nm, toasted layer 4, control rep 2, run 2

## VITA

Lindsay Elizabeth Rogerson was born on February 11, 1991, in Bethesda, MD, to William T. Rogerson Jr. and Lorna J. Rogerson. She became a student at the University of Tennessee in spring 2011 and completed a B.S. in Food Science in 2014. Lindsay immediately entered into the Graduate School at the University of Tennessee and will graduate with an M.S. in Food Science spring 2016.