Extraction, Characterization, and Monosaccharide Composition Comparison of Polysaccharides Extracted from *Cannabis sativa* Cherry Hemp Root and Fedora 17 Hemp Leaves

by

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ABSTRACT

Traditional medicine has used plants and plant extracts to treat inflammation, tumors, neurodegenerative diseases, arthritis, and in some cases the effects of radiation. While these plants tended to be effective, the reason behind their medicinal properties was unknown. There has now been extensive research on polysaccharides in many plants regarding their bioactivities. Hemp has been shown to have many bioactive properties including anti-anxiety, anti-nausea, anti-arthritic, anti-psychotic, and anti-inflammatory properties. While there has been much research done on the cannabinoids of hemp, there has been little previous research on the polysaccharides isolated from *Cannabis sativa*. This project focuses on purifying and characterizing the polysaccharides of *Cannabis sativa* from cherry hemp root and Fedora 17 leaves. The hemp polysaccharides were extracted using ethanol and water. The crude extract was then purified by anion-exchange chromatography and size exclusion chromatography. The pure polysaccharides were hydrolyzed, and the resulting monosaccharides were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) for HPLC analysis to determine the monosaccharide composition. Functional groups were determined using Fourier Transform Infrared spectroscopy.

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CHAPTER I: INTRODUCTION

The objective of this research is to purify and characterize the polysaccharides in different varieties and parts of hemp. Polysaccharides in other plants, such as ginseng, have bioactive properties. It is also known that other compounds in hemp, such as the cannabinoids, have many health benefits. The long use of hemp in Chinese medicine indicates that all parts of hemp hold bioactive properties. There are many varieties of hemp that may have different properties. The isolation and characterization of polysaccharides from hemp is the first step in understanding their bioactivities.

Carbohydrates are molecules made up of carbon, hydrogen, and oxygen. The generic formula for carbohydrates is $(CH_2O)_x$. Monosaccharides are the simplest form of carbohydrates consisting of an aldehyde or ketone and multiple hydroxy groups (Nelson, 2017). Monosaccharides containing an aldehyde are classified as aldoses, while those containing a ketone are classified as ketoses. Monosaccharides can also be sorted into categories according to the number of carbons. The most common are 5-carbon pentoses and 6-carbon hexoses. These monosaccharides can also form cyclic structures (Figure 1). The pentoses predominately form 5-membered furanose rings while the hexoses form 6-membered pyranose rings (Stylianopoulos, 2013). An equilibrium is maintained between the open and cyclic forms. To form more complex carbohydrates, monosaccharides are linked with a glycosidic bond. A glycosidic bond is formed between hydroxy groups of two monosaccharides with the loss of water. Polysaccharides are made up of multiple monosaccharides linked with glycosidic bonds (Figure 2).

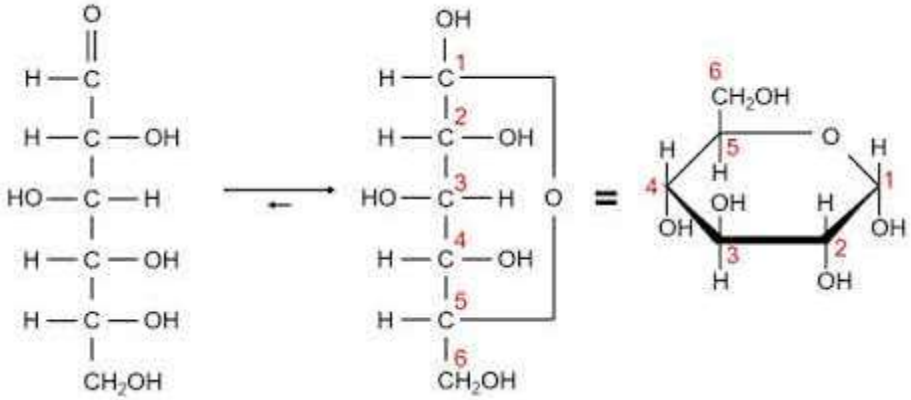


Figure 1. Cyclization of Glucose

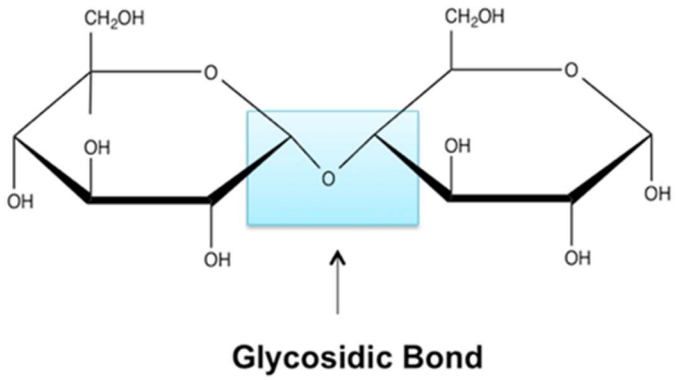


Figure 2. Glycosidic bond between two monosaccharides.

Bioactivites of Carbohydrates

In nature, polysaccharides perform many functions. Polysaccharides such as glycogen and starch are used for energy storage in cells. Cellulose and chitin are structural elements in plants and exoskeletons, respectively. Heparin is a blood thinner, and a peptidoglycan determines the classification of bacteria as gram-positive or gram-negative (Stick, 2001). Polysaccharides

have shown antioxidant activity, antitumor activity, immunostimulatory activity, and wound healing activity (Li et al, 2018).

Carbohydrates can form glycoconjugates with lipids or proteins (Taylor and Drickamer, 2006). Glycoconjugates are carbohydrates covalently bonded to a different type of molecule. These glycoconjugates perform many functions in living organisms such as facilitation of ligand binding on animal cell surfaces and intracellular signaling (Broussard & Boyce, 2019). Addition of carbohydrates to another biomolecule can alter the physical properties, such as water solubility, of these molecules (Varki et al., 2009). These molecules are also important for cell structure and interactions such as signaling and adhesion.

Chromophores and Derivatization

When extracting molecules for analysis, they must be detectable. Many detectors, such as UV-Vis, rely on the absorbance of light at specific wavelengths. In order for the molecule to be detected, it must absorb light from the detector. This typically occurs with the existence of a chromophore. The chromophore is the part of the molecule that absorbs light. It absorbs all wavelengths of light except the color that can be seen.

Monosaccharides can be difficult to study due to the lack of a chromophore. In order for the molecules to be detected by most HPLC detectors, they must be derivatized. A widely known derivative used with monosaccharides is the 1-phenyl-3-methyl-5-pyrazolone (PMP) derivative (Stepan & Staudacher, 2011) (Figure 3). The resulting compounds may be separated by reverse-phase HPLC and detected at 760 nm. Twenty-six different monosaccharides were determined in one HPLC run by Stepan and Staudacher. The derivatization was carried with the mix of sugars and methanolic PMP in aqueous sodium hydroxide. The reaction was then neutralized with

hydrochloric acid and washed with chloroform to remove the excess PMP before being loaded onto the column (Honda et al, 1989). A typical chromatogram is shown below (Figure 4).

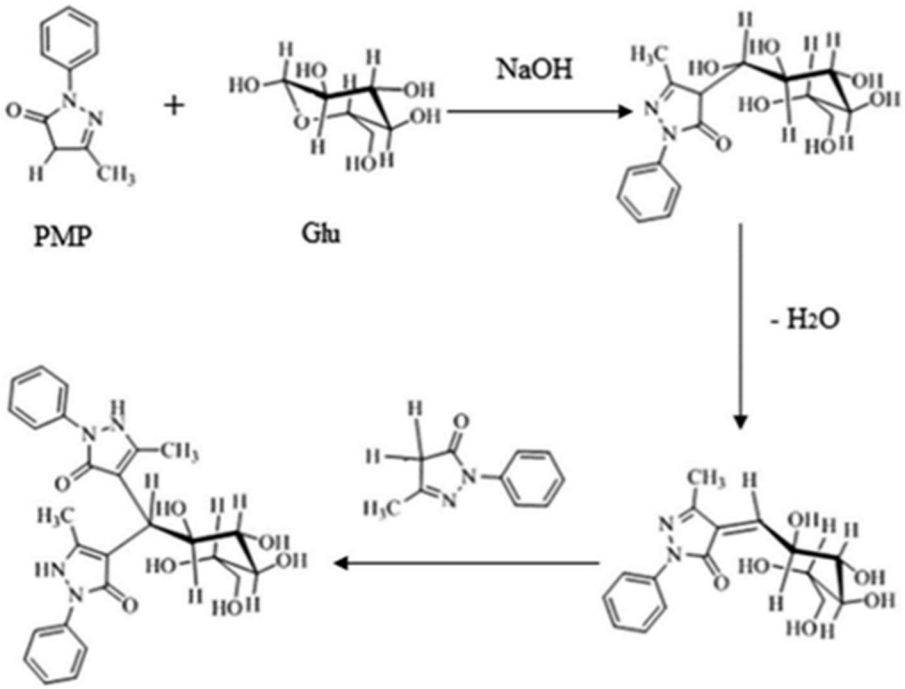
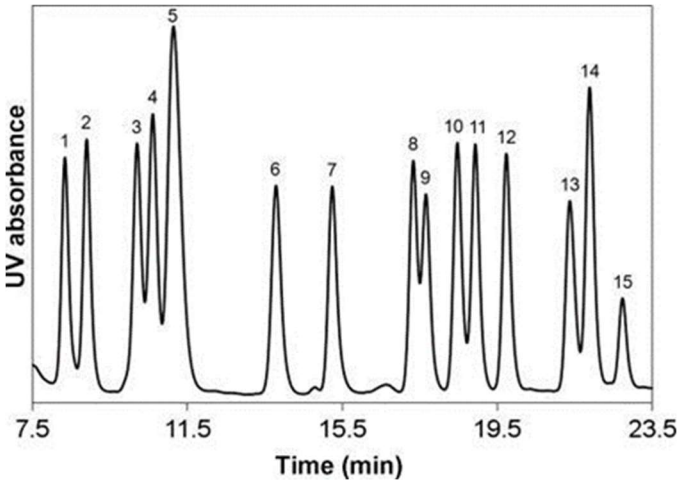


Figure 3. PMP derivatization of glucose (Xian et al., 2015)

Figure 4. Monosaccharides labeled with PMP and separated by reversed phase HPLC. Shown is the analysis of equal amounts (180 nmol) of Mannose (Man) (1), gulose (Gul) (2),Glucosamine (GlcN) (3), Mannosamine (ManN) (4), Ribose (Rib) (5), Galactosamine (GalN) (6), galacturonic acid (7), idose (8), Glucose (Glc) (9), 3-O-Methyl-Glucose (3-O-Me-Glc) (10), altrose (11), Xylose (Xyl) (12), Fucose (Fuc) (13), 4-O-Methyl-Galactose (4-O-Me-Gal) (14), and 2-O-Methyl-Galactose (2-O-Me-Gal) (15).



Cannabis sativa

In this research, the focus is on *Cannabis sativa*. Hemp and marijuana are both *Cannabis sativa*, although they can be readily distinguished. Legally, hemp must contain less than 0.3% tetrahydrocannabinol, or THC, content (Shipman, 2019) (Figure 5). THC is the psychoactive component found in this species. Hemp was cultivated by Native Americans as early as the 1500s. Hemp fibers were used to produce thread, rope, and cloth (Figure 6). In the 1600s and 1700s, Colonial America encouraged farmers to grow and process hemp (Nunley, 2019). For about 200 years, hemp was exported to England to be used for shoes, maps, books, sails (Figure 6), and tents. Throughout the 1800s and early 1990s hemp became more of a staple in America, spreading toward the west coast (Nunley, 2019). In 1941, when the United States joined World War II, the need for hemp greatly increased. The government distributed 400,000 pounds of hemp seeds to encourage cultivation. For the next 5 years, approximately 42,000 tons of hemp fiber were produced annually (Nunley 2019). When the war ended, so did the need for hemp.

Figure 5. Marijuana strain of Cannabis (left); Hemp strain of Cannabis (right)

Figure 6. Ropes of hemp fiber (left); Boat with hemp sails and hemp rigging (right)

Restrictions on Hemp

In 1937, the Marijuana Tax Act restricted the sale and growth of all cannabis, making it difficult to legally grow hemp. As a result, production began to fall (Earleywine, 2005). Around the same time, synthetic fibers were beginning to be a source for manufacturers, reducing the need for hemp (Nunley, 2019). In 1970, the Controlled Substances Act increased regulation on all cannabis. Sterilized hemp seed, hemp fiber, and hemp seed oil were excluded from the regulations (Nunley 2019). Over 70 years later, the Agricultural Act of 2014 allowed state Departments of Agriculture and higher education systems to grow hemp for research (Hemp Industries Association, 2019). The 2018 Farm Bill removed hemp from the Controlled Substances Act allowing hemp to again be sold commercially, including CBD oil. The hemp CBD market is predicted to be worth $22 billion by 2022 (Nunley, 2019).

The cannabinoids in hemp have been studied extensively with various analytical methods (Raharjo and Verpoorte, 2004). The main cannabanoids in hemp are THC, cannabidiol (CBD), and cannabinol (CBN). CBD has been found in hemp seed oil that has demonstrated antimicrobial, anti-tremor, and anti-epileptic effects (Leizer et al., 2000). Hemp has also

demonstrated “potent bioactivites on human health” (Andre, 2016), such as anti-anxiety, anti-nausea, anti-psychotic, anti-inflammatory, and immunomodulatory properties (Andre, 2016).

CHAPTER II: MATERIALS AND METHODS

Materials and Reagents

Farm cherry hemp root was grown on a farm in Tennessee. Greenhouse cherry hemp root and Fedora 17 leaves were grown in the greenhouse on the Middle Tennessee State University campus and were supplied by High Grade Hemp Seeds and Schiavi Seeds, respectively.

Extraction of Polysaccharides

Fedora 17 hemp leaves, greenhouse cherry hemp root, and farm cherry hemp root underwent the same process of extraction. Approximately 10 grams of the material were ground in a seed and nut grinder. The ground material was refluxed in 500 mL of 95% ethanol for 4 hours. The ethanol extract was filtered and the supernatant removed. The residue was refluxed again in 500 mL of water overnight. The aqueous extract was filtered to remove remaining solid material. The clarified solution was concentrated under reduced pressure on a Rotavapor. To remove any protein, the concentrated extract was treated with 20 mL of Sevag's reagent (1:4 n-butanol:chloroform) and sonicated for 20 minutes (Sevag et al. 1938). After sonication, the solution was centrifuged at 4000 rpm for 10 minutes at 4 ^{o}C. The upper aqueous layer was then treated with Sevag's reagent, sonicated and centrifuged twice more. After removal of proteins was complete, the upper aqueous layer was transferred to a container with 4 volumes of 95% ethanol to precipitate the polysaccharides overnight at 4 ^{o}C. The solution was centrifuged at 4000 rpm, and the ethanol decanted. The pellet was dissolved in water, then lyophilized. The mass of crude polysaccharides was determined, and the percent polysaccharide calculated. The crude polysaccharide was dissolved in water for further purification. Due to the large amount of polysaccharide present in the Fedora 17 leaves, the crude extract was separated into 4 parts,

designated with #1, #2, #3, and #4. Further purification was only carried out on Fedora 17 #2 and Fedora 17 #3. Fedora 17 #1 and Fedora 17 #4 were saved for later analysis.

Purification of Polysaccharides

Polysaccharides were purified by anion ion-exchange chromatography on an AKTA FPLC system equipped with a HiPrep 16/10 DEAE-Sepharose column. The sample was loaded onto the column and eluted with a step gradient of increasing sodium chloride in water containing 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 1.0 M sodium chloride at a flow rate of 3 mL/min. As the column was washed, 5 mL fractions were collected. Fractions containing sugar were determined using a phenol sulfuric acid assay (Masuko et al. 2005). Related fractions containing sugar were pooled, dialyzed, and lyophilized. The lyophilized fractions were dissolved in water to a concentration of 5 mg/mL and stored at 4 ^{o}C for future analysis.

Determination of Molecular Weight and Purity

Each fraction was assayed for purity by high performance size exclusion chromatography (HPSEC) on a Dionex HPLC equipped with a TSK Gel PwXL G4000 analytical size exclusion column. Dextran standards of known molecular weights were used to determine the molecular weight of each fraction. The column was eluted with 0.1 M NaCl at a flow rate of 0.5 mL/min. Polysaccharides were detected with a refractive index detector.

Monosaccharide Analysis by HPLC with PMP Derivatives

Between 1-2 mg of the samples were lyophilized and the glycosidic bonds hydrolyzed with 500 µL of 4M trifluoroacetic acid (TFA) for 5 hours at 100 ^{o}C. After hydrolysis, excess TFA was removed by blowing air into the sample in a heating block at 110 ^{o}C. The remaining dried sample was washed 3 times with 200 µL of methanol. The methanol was dried in the same manner that the excess TFA was removed. Then, 250 µL 1:5:1 of water:0.5 M NaOH:0.5 M

methanolic PMP were added to the vial, and the mixture was heated at 80 °C for 1 hour. Excess PMP was removed by extraction with HPLC grade chloroform (500 µL). The sample was vortexed, centrifuged, and the aqueous layer removed. The chloroform washing was performed a total of three times. After removal of excess PMP, 200 µL of aqueous sample was transferred to an HPLC vial, and HCl and NaOH were added to adjust the pH to 8, as needed.

The sample was analyzed by reverse phase HPLC on a Dionex UltiMate 3000 liquid chromatography system consisting of a quaternary solvent pump/mixer, temperature-controlled autosampler, temperature-controlled column compartment, and multiwavelength UV-Vis detector. Separation was carried out on a Phenomenex Hyperclone C18 (150 x 4.6 mm) column with a flow rate of 0.6 mL/min. The mobile phase consisted of two components: (A) 15% acetonitrile in 50 mM sodium phosphate pH 6.9 and (B) 40% acetonitrile in 50 mM sodium phosphate pH 6.9. At the beginning of the HPLC analysis, (A) was set at 100%, and (B) was set at 0%. After 15 minutes, (A) was decreased to 92% and (B) was increased to 8%. At 50 minutes, (A) was decreased to 80% and (B) was increased to 20%. At 58 minutes, (A) was returned to 100%, and the analysis was continued for an additional 7 minutes for a total analysis time of 65 minutes. The column temperature was 30 °C and the UV-Vis detector was set at 245 nm. Standard samples of 8 monosaccharides were used to determine the retention times of PMP derivatives. The retention time of each peak was used to determine the identity of the monosaccharides present.

Analysis of Functional Groups by FTIR

The polysaccharide samples were analyzed by Fourier Transform Infrared Spectroscopy on a Nicolet iS50R equipped with an iS50 attenuated total reflection sample holder (FTIR-ATR). The data was collected from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. A background of air

was collected, then the sample pellet was placed directly on the crystal and the FTIR spectrum was collected. A total of 108 scans were collected per sample.

CHAPTER III: RESULTS AND DISCUSSION

Fedora 17 and Cherry hemp are two different varieties of *Cannabis sativa*. Fedora 17 is a French industrial hemp. It contains 1.50-2.00% CBD and less than 0.12% THC in the buds. Cherry hemp is also an industrial hemp. Cherry hemp flowers are high in CBD with less than 0.3% THC content (USDA, 2016). Many previous studies on hemp have focused on the cannabinoids and their medicinal properties. This study focuses on polysaccharides in hemp.

Extraction

Greenhouse cherry hemp root (6.147 g) were extracted overnight, resulting in 49.3 mg of crude extract, a 0.802% yield. Farm cherry hemp root (6.076 g) was extracted overnight in ethanol resulting in 57.3 mg, a yield of 0.943%. Fedora 17 leaves (10.795 g) were also extracted overnight in ethanol resulting in 1.5297 g, a 14.17% yield, significantly higher than that isolated from roots. The large amount of extract from Fedora 17 leaves required the sample to be divided into roughly 4 equal parts. Fedora 17 #2 (276.3 mg) and Fedora 17 #3 (273.7 mg) were further purified. This step was not necessary with the samples extracted from roots, since the roots produce a much smaller yield. The low yield could be due to the difficulty in grinding the roots or just a lack of polysaccharide in the roots compared to leaves of cherry hemp. While most polysaccharides are isolated from leaves or flowers, some studies on root polysaccharides have been carried out. For example, Jiang et al. extracted polysaccharides from the roots of *Arctium lappa* L. resulting in yields at 6-9%. While that yield is low, the yield from the Cherry hemp root was over 6 times smaller.

Anion Exchange Chromatography

After extraction, the resulting crude extract was loaded onto an AKTA FPLC system equipped with a HiPrep 16/10 DEAE-Sepharose anion exchange column. The column was eluted

with a step gradient of increasing sodium chloride concentration in water. The resulting fractions were assayed by a phenol-sulfuric acid assay. Two phenol sulfuric acid assays were done for every sample run through the column, and the results were averaged to produce the final profile. Fedora 17 #2 and Fedora 17 #3 were from the same extraction, but two separate columns were run. The Fedora 17 #2 and Fedora 17 #3 hemp leaves extraction samples were expected to have the same profile since they were from the same extraction (Figure 7). Fedora 17 #2 and #3 contained 17.55% and 1.50% of the eluted sugars in the 0 M fraction, 17.40% and 27.74% in the 0.1 M fraction, 47.50% and 49.88% in the 0.2 M fraction, and 17.55% and 20.92% in the 0.3 M fraction, respectively.

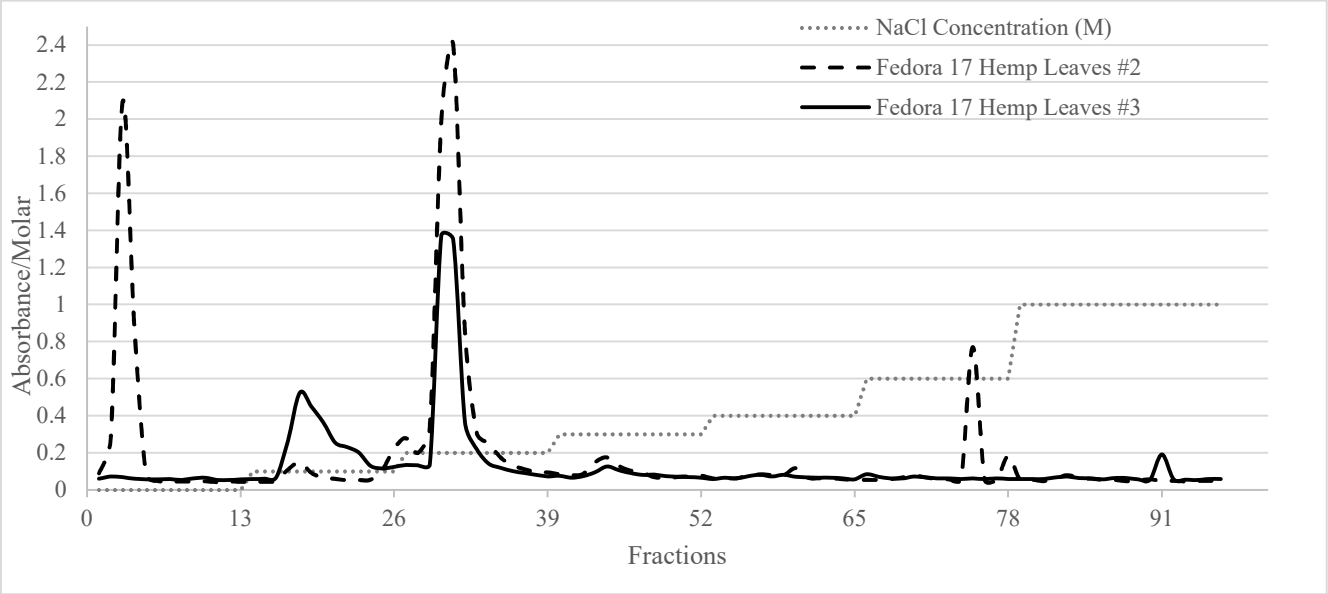


Figure 7. Phenol sulfuric acid assay profile of Fedora 17 samples

The hemp root extractions have similar elution profiles (Figure 8). The greenhouse and farm hemp roots contained 24.88% and 28.26% of the eluted sugars in the 0.1 M fraction and 18.31% and 34.40% in the 0.2 M fraction, respectively. The greenhouse roots contained 3.76% in the 0 M fraction and 37.09% in the 0.6 M fraction, while the farm hemp root had no

carbohydrate present in those fractions. The farm root did have 37.35% of the eluted sugar in the 1 M fraction. There is no previous research comparing the profiles of polysaccharides in greenhouse versus farm-grown plants.

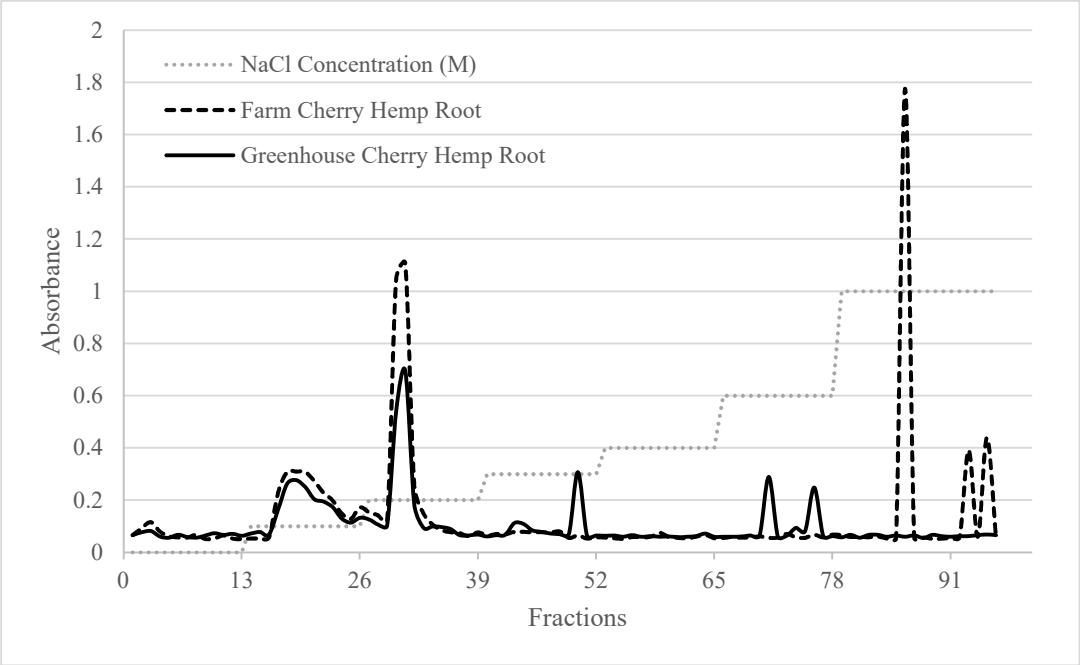


Figure 8. Elution profile of extractions of Cherry Hemp roots from DEAE ion exchange column. The presence of polysaccharide was determined using phenol-sulfuric acid assay.

The elution position of a polysaccharide is the result of a number of complex interactions between the polysaccharide and the column. One factor is the more negatively charged molecules will elute in fractions with higher NaCl concentrations. Uronic acids contribute a negative charge to polysaccharides, so the 0.6 M fraction from the greenhouse cherry hemp root is expected to contain uronic acids. The majority of the carbohydrates eluted in the 0.2 M fraction for all samples, as expected, due to the relatively neutral nature of carbohydrates.

Determination of Purity and Molecular Weight

All samples from the anion exchange column were loaded onto a Dionex HPLC equipped with a TSK Gel PwXL G4000 analytical size exclusion column and refractive index detector.

This column is used to analyze water-soluble linear polymers with molecular weights up to 300,000 Daltons. The size exclusion analysis showed a single peak for most fractions, indicating that the fractions are pure (Appendix I). The exception is the 0.2 M fractions of the Fedora 17 #2 and #3 samples that contained two distinct peaks.

In addition to the determination of purity, size exclusion chromatography can be used to determine the molecular mass of a polysaccharide. Dextrans are complex glucose polymers of microbial origin. They have a backbone of varying length of glucose with mainly α-D-(1-6) linkages, producing a molecular weight of at least 1000 Daltons. A series of dextran standards with known molecular weights ranging from 5 kDa to 410 kDa was used to determine a calibration curve. The retention time (x axis) was plotted against the log of the molecular weight (y axis) resulting in the equation shown below ($r^2 = 0.9956$):

$$y = -0.3264x + 7.024$$

where x is the retention time (min) and y is the log of the molecular weight (Figure 9). The molecular weight of each fraction was determined using this calibration curve (Table 1).

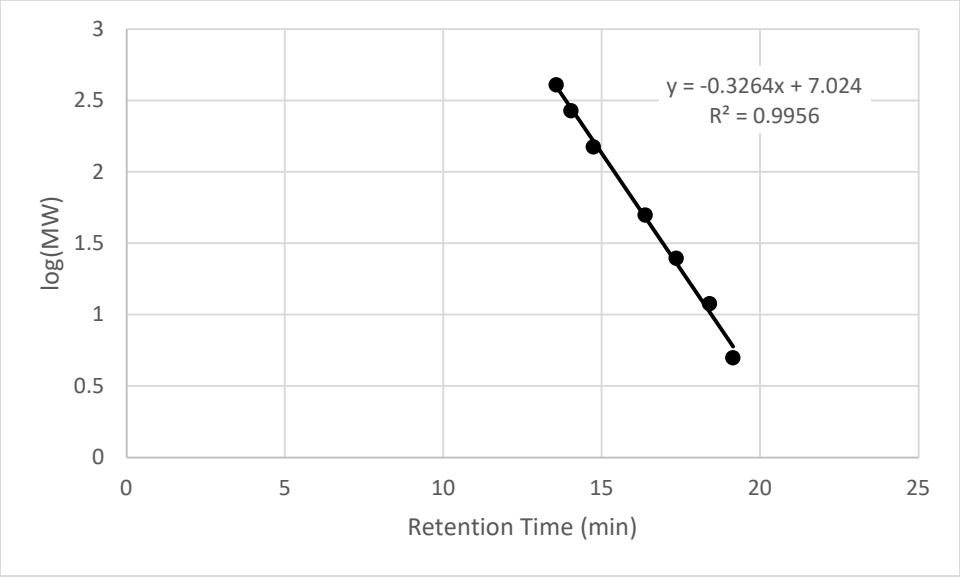


Figure 9. Calibration curve of dextran standards using a refractive index detector

	MOLECULAR WEIGHT (kDa)	1	2
CHERRY HEMP ROOTS	FARM 0.1 M	6.810	---
	FARM 0.2 M	13.855	---
	GREENHOUSE 0.1 M	5.460	---
	GREENHOUSE 0.2 M	6.919	---
	GREENHOUSE 0.3 M	5.374	---
FEDORA 17 LEAVES	FEDORA 17 #2 0 M	6.418	---
	FEDORA 17 #2 0.1 M	---	---
	FEDORA 17 #2 0.2 M	53.156	5.501
	FEDORA 17 #2 0.3 M	---	---
	FEDORA 17 #3 0.1 M	4.160	---
	FEDORA 17 #3 0.2 M	47.382	4.434
	FEDORA 17 #3 0.3 M	4.705	---

Table 1. Molecular weights of polysaccharides isolated from anion exchange chromatography in kDa

The molecular weights are comparable to polysaccharides isolated from sources such as frankincense and ginger (Hosain et al 2019) (Wang et al 2018). The Fedora 17 #2 0.1 M and 0.3 M fractions did not show any presence of sugar in the size exclusion analysis, so were not analyzed further.

Monosaccharide Analysis with PMP Derivatives

A set of 8 monosaccharide standards were derivatized, and the retention times were used to determine the monosaccharides present in each fraction (Appendix II). A chromatogram of the mix of eight standards is shown below (Figure 10). Xylose and arabinose elute closely, making it difficult to distinguish between the two.

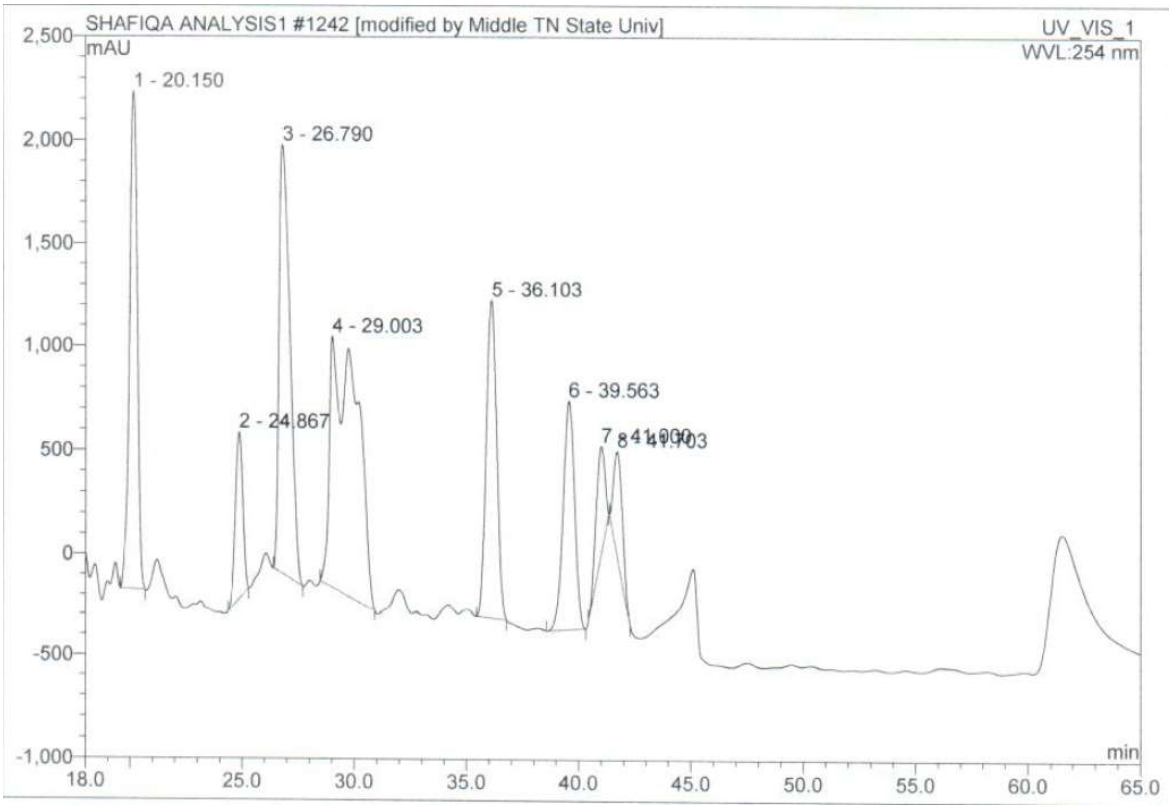


Figure 10. Monosaccharide standards labeled with PMP and separated by reversed phase HPLC. Shown is the analysis of Mannose (1), Rhamnose (2), Glucuronic acid (3), Galacturonic acid (4), Glucose (5), Galactose (6), Xylose (7), Arabinose (8).

The area of each peak was used to determine the percentage of each monosaccharide present in each fraction of the Fedora 17 leaves samples (Table 2) and the greenhouse and farm hemp samples (Table 3). This was not a comprehensive selection of standards, so the total composition for each fraction will not be 100%. Most of the fractions including the standards showed a significant peak at a high retention time of ~60 min that could greatly contribute to the low total percentage represented by the sugars tested. As this peak is present in all samples including the standard it most likely represents a highly retained reagent.

There has been a previous GC-MS analysis of greenhouse cherry hemp root 0.2 M fraction in an earlier extraction (Dyer, 2019). The composition of those two sets of data are

similar (Table 4). The sugars present are the same, except the HPLC data showed a small amount of glucose present. The GC-MS data had slightly higher percentages of each sugar present, resulting in a total of 82.66% total instead of the 66.68% in this HPLC analysis.

	FED 17 #2 0M	**FED 17 #2 0.1M**	**FED 17 #2 0.2M**	**FED 17 #3 0.2M**
MANNOSE	3.08	1.78	1.33	1.46
RHAMNOSE	4.31	----------	10.44	13.88
GLUCOSE	10.62	19.28	----------	----------
GALACTOSE	23.78	1.53	15.27	18.51
GLUCURONIC ACID	----------	----------	----------	----------
GALACTURONIC ACID	----------	----------	5.39	5.95
XYLOSE/ ARABINOSE	26.94	2.73	13.44	17.58
TOTAL	**68.73**	**25.32**	**35.87**	**57.38**

Table 2. Percent Monosaccharide Composition for Fedora 17 leaves fractions

	GH 0.1M	**GH 0.2M**	**GH 0.3M**	**GH 0.6M**	**FARM 0.1M**	**FARM 0.2M**	**FARM 1M**
MANNOSE	11.63	8.41	5.79	12.97	6.86	3.32	0.77
RHAMNOSE	4.31	5.44	----------	----------	----------	14.98	----------
GLUCOSE	9.01	5.8	6.36	5.98	----------	----------	----------
GALACTOSE	24.58	15.16	9.99	8.83	15.77	16.78	3.01
GLUCURONIC ACID	----------	----------	----------	----------	----------	----------	----------
GALACTURONIC ACID	----------	----------	----------	----------	----------	19.17	----------
XYLOSE/ ARABINOSE	37.7	31.87	41.88	42.88	54.39	22.54	8.01
TOTAL	**87.23**	**66.68**	**64.02**	**70.66**	**77.02**	**76.79**	**11.79**

Table 3. Percent Monosaccharide Composition for Greenhouse and Farm Cherry Hemp root fractions

	GC-MS ANALYSIS	HPLC ANALYSIS
MANNOSE	7.65	8.41
RHAMNOSE	7.66	5.44
GLUCOSE	----	5.80
GALACTOSE	21.93	15.16
GLUCURONIC ACID	----	----
GALACTURONIC ACID	----	----
XYLOSE/ARABINOSE	45.42	31.87
TOTAL	**82.66**	**66.68**

Table 4. Comparison of percent monosaccharide composition of greenhouse cherry hemp root 0.2 M fraction from GC-MS analysis of TMS derivatives and HPLC analysis of PMP derivatives

All of the samples except the Fedora 17 #2 0.1 M fraction and Greenhouse 0.6 M fraction contain mostly xylose/arabinose and galactose. The presence of arabinose and galactose is likely from arabinogalactans. A linkage analysis would be necessary to determine the type of arabinogalactan present. It is likely that these arabinogalactans are from arabinogalactan proteins (AGPs), which are commonly found in the cell membrane and cell wall of most plant species (Knoch et al, 2014).

There is not a significant difference between the monosaccharide composition of the leaves and roots. The monosaccharides present are the same with some variations in the percentage of each. When comparing the Fedora 17 0.2 M fractions and the Farm 0.2 M fractions, the percent compositions are very similar except galacturonic acid. Farm 0.2 M has a much higher percentage of galacturonic acid (19.17 %) than Fedora 17 #2 and #3 0.2 M (5.39 % and 5.95 %, respectively). Another extraction will need to be analyzed to determine if that amount of galacturonic acid is correct.

Greenhouse and farm cherry hemp roots also have similar monosaccharides present with varying percentages with the exception of glucose. All cherry hemp root fractions contain

mannose, galactose, and xylose/arabinose. The greenhouse root fractions all contain small amounts of glucose, while none of the farm root fractions contain glucose. Greenhouse 0.1 M contains rhamnose and glucose that is not present in the farm 0.1 M fraction. There is also overall more total monosaccharides present in the greenhouse 0.1 M than the farm 0.1 M. The greenhouse 0.2 M fraction contains significantly less rhamnose than the farm 0.2 M fraction. It also contains no galacturonic acid. Greenhouse 0.2 M is the only sample from that eluted in 0.2 M NaCl from the anion exchange that does not contain galacturonic acid.

Analysis of Functional Groups by FTIR

The interferograms of all the samples contained peaks characteristic of carbohydrates (Appendix III). All contained a broad peak from 3000 – 3600 cm^{-1} indicating the stretching of hydroxyl groups. Intense peaks around the range of 1025-1040 cm^{-1} are from C-O stretching. All Fedora 17 and cherry hemp root fractions contained peaks at ~2900 cm^{-1} and ~1600 cm^{-1} that are characteristic of the O-H and C=O stretching in the carboxyl group of uronic acids. These uronic acids do not appear in the PMP derivative data. There could be trace amounts of uronic acids in the fractions that cannot be detected using the PMP derivatives.

This data also shows that the fractions may not be totally pure. Peaks at both 3410 cm^{-1} and 1400 cm^{-1} are characteristic of C=O and N-H bending of the peptide amide bond. The peak at 1400 cm^{-1} is seen in the interferograms, but the 3410 cm^{-1} peak is not present or overpowered by the large carbohydrate peak. There is no peak at 1540 cm^{-1} for any of the fractions, which would be expected for N-H and C-N bending in the peptide. Since the peak at 1540 cm^{-1} is not present, the 1400 cm^{-1} peak is likely caused by O-H bending. This could have been left on the crystal from cleaning with methanol. This is a probable explanation since the refractive index detector did not show any impurities.

CHAPTER IV: CONCLUSIONS

Greenhouse cherry hemp roots contain polysaccharides ranging from 5.415 kDa to 6.868 kDa. Farm cherry hemp roots contain polysaccharides ranging from 6.760 kDa to 13.796 kDa. Fedora 17 leaves contain polysaccharides ranging from 4.393 kDa to 53.231 kDa. The polysaccharides in Fedora 17 contain mannose, rhamnose, glucose, galacturonic acid, and xylose/arabinose. Polysaccharides in greenhouse cherry hemp roots contain mannose, rhamnose, galactose, and xylose/arabinose. Polysaccharides in farm cherry hemp roots contain mannose, rhamnose, galactose, galacturonic acid, and xylose/arabinose. There is no significant difference between the greenhouse and farm grown root monosaccharide compositions, and there is no significant difference between the leaves and root samples. All samples contain mostly galactose and xylose/arabinose indicating the presence of arabinogalactans. FTIR analysis confirms the purity of the samples. Future linkage analysis will determine the type of arabinogalactans present, then the samples can be analyzed for immunostimulatory activity.

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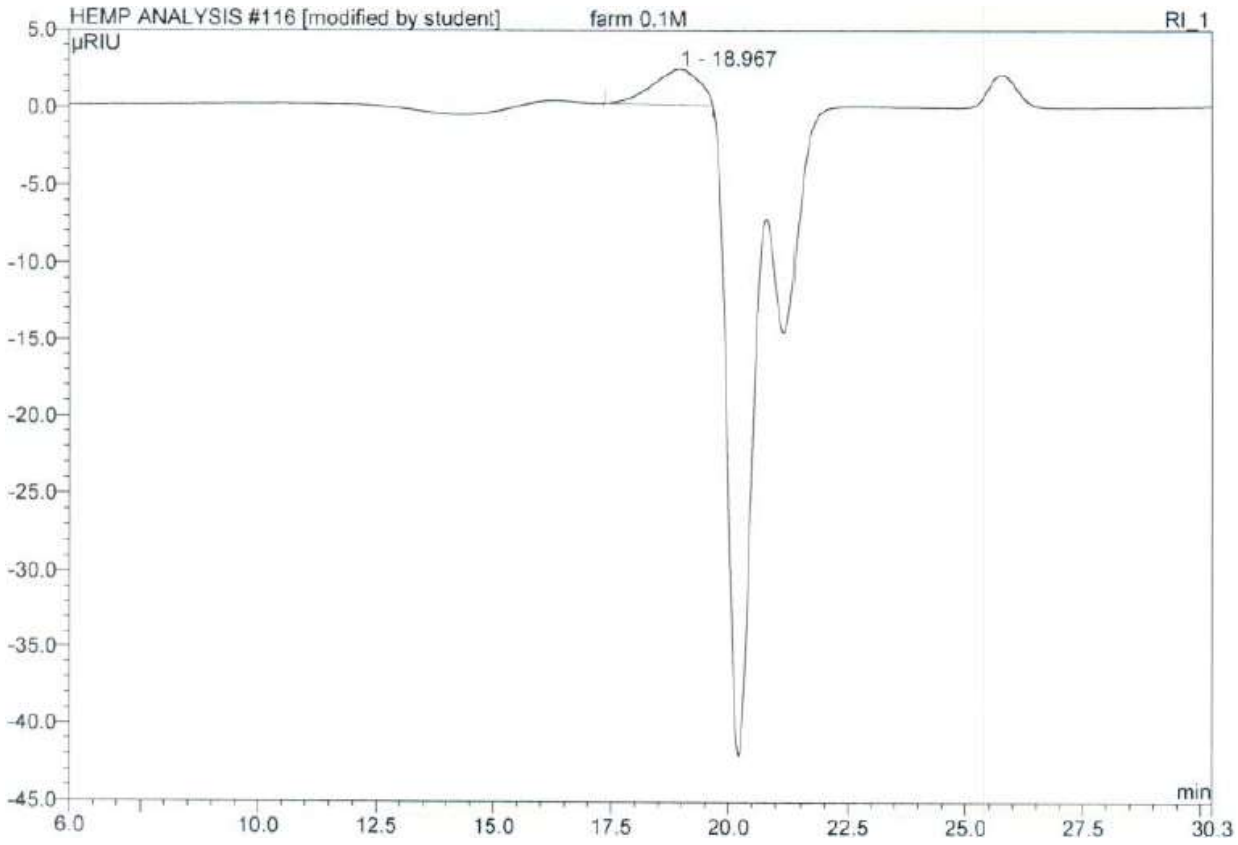
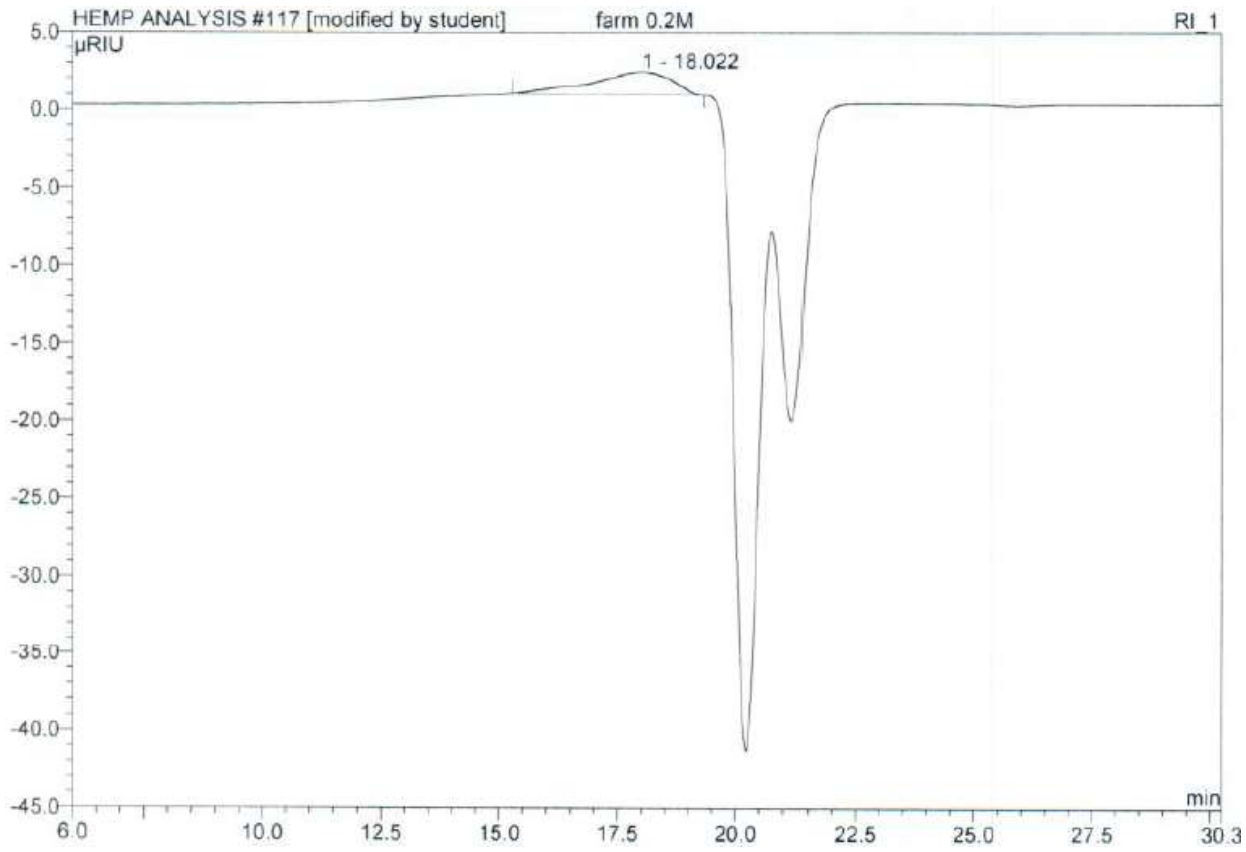
Appendix I. Chromatograms from the Size Exclusion Analysis

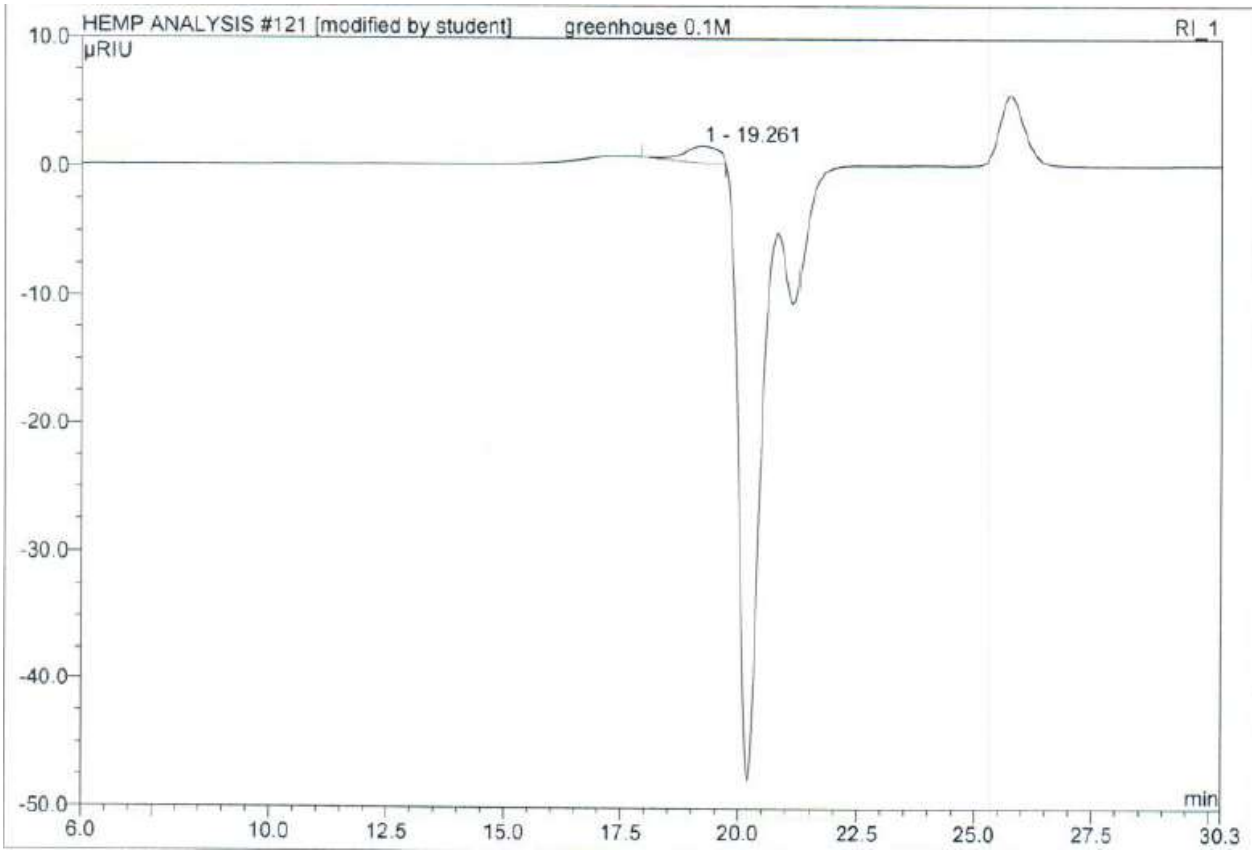
Farm 0.1 M



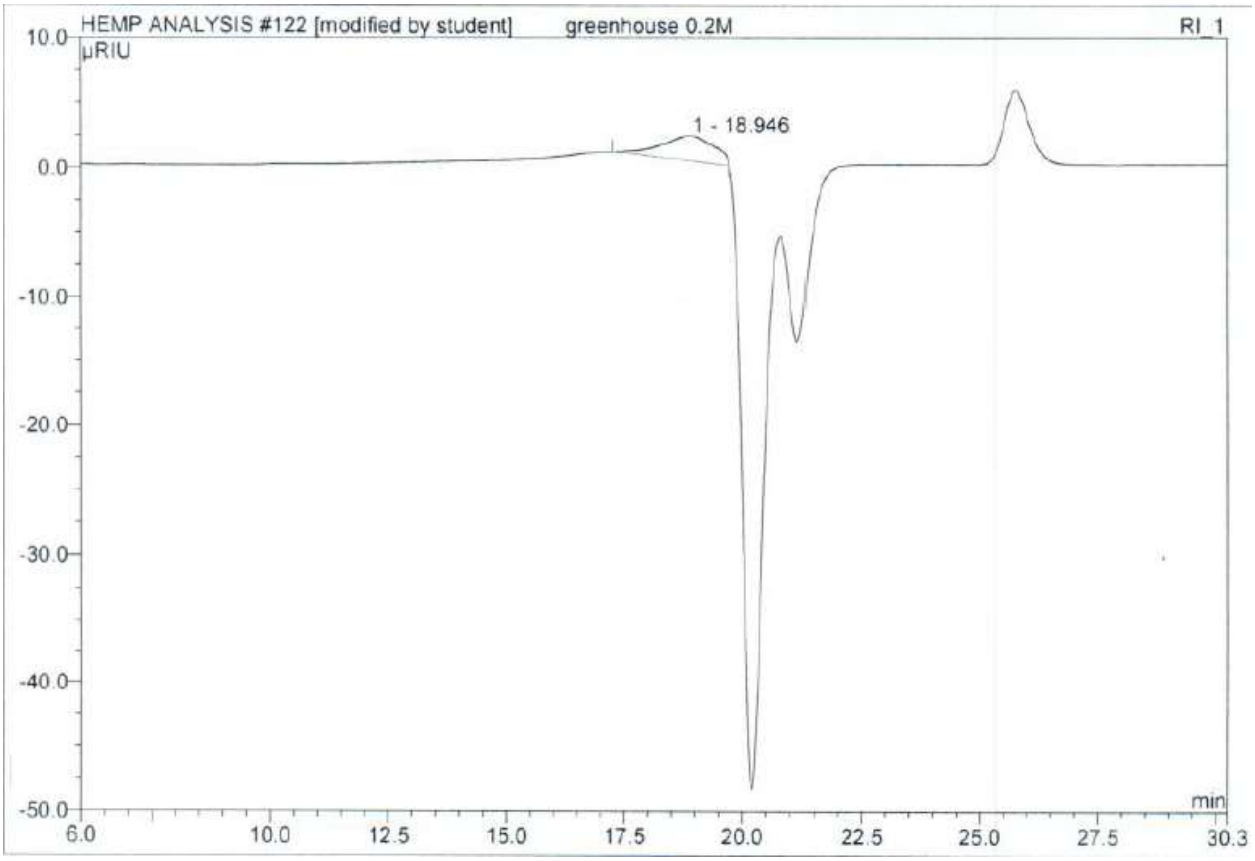
Farm 0.2 M


HEMP ANALYSIS #117 [modified by student]
farm 0.2M
RI_1
µRIU
1 - 18.022
5.0
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
-30.0
-35.0
-40.0
-45.0
min
6.0
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.3

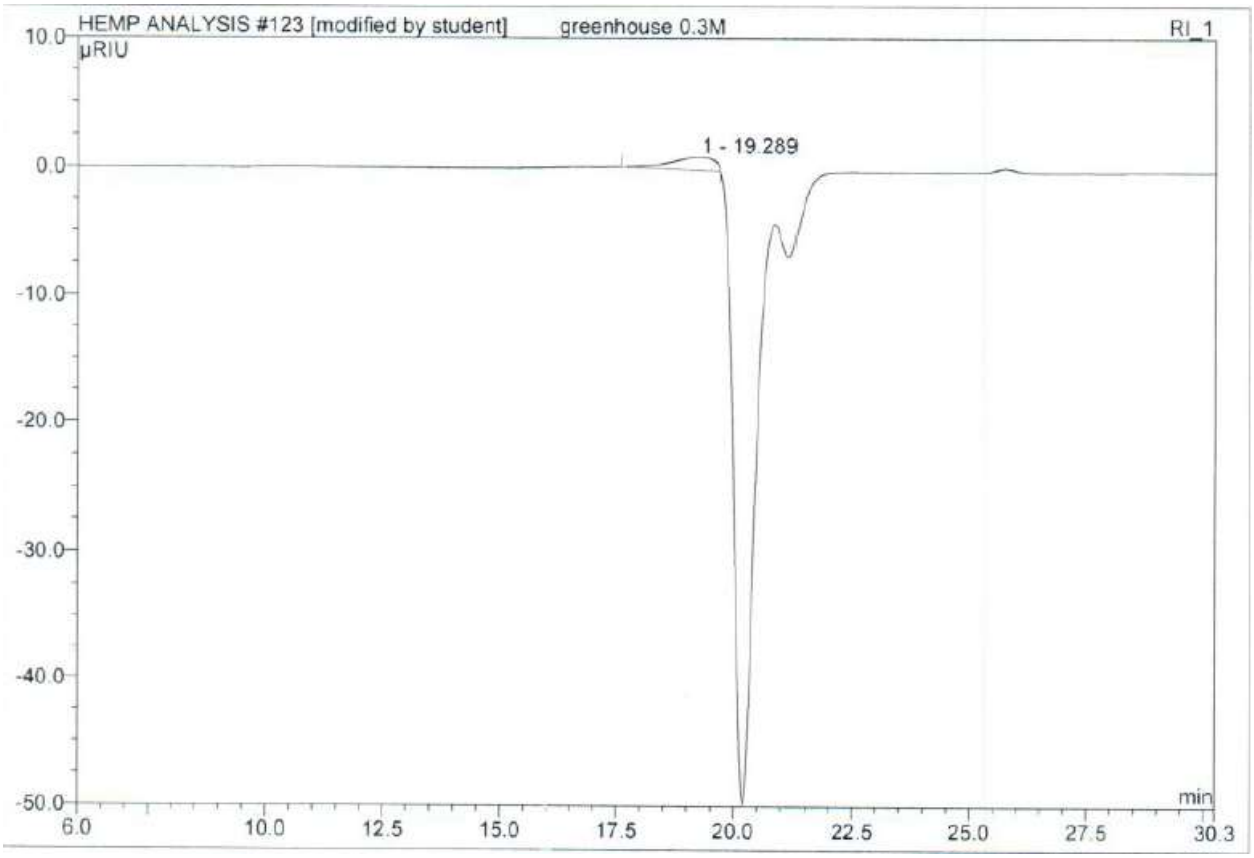
Greenhouse 0.1 M



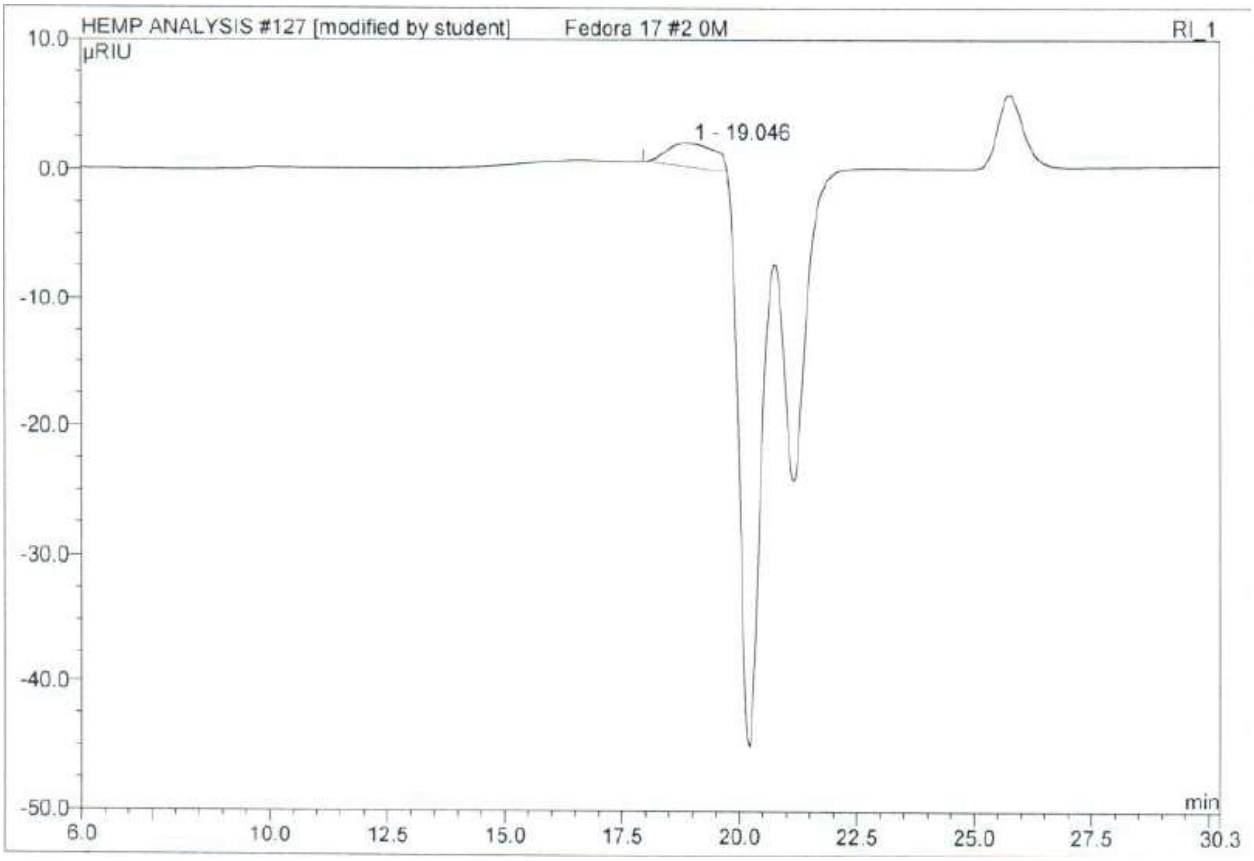
Greenhouse 0.2 M



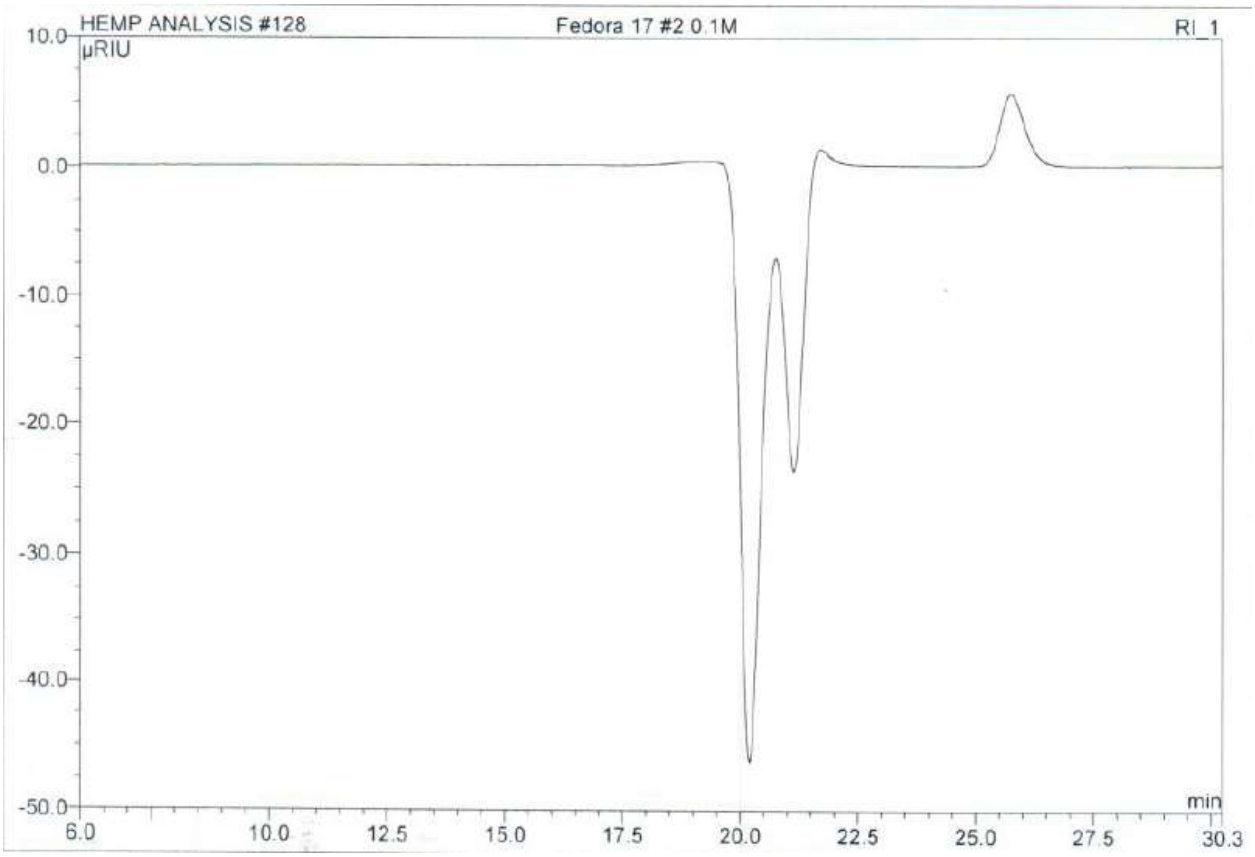
Greenhouse 0.3 M



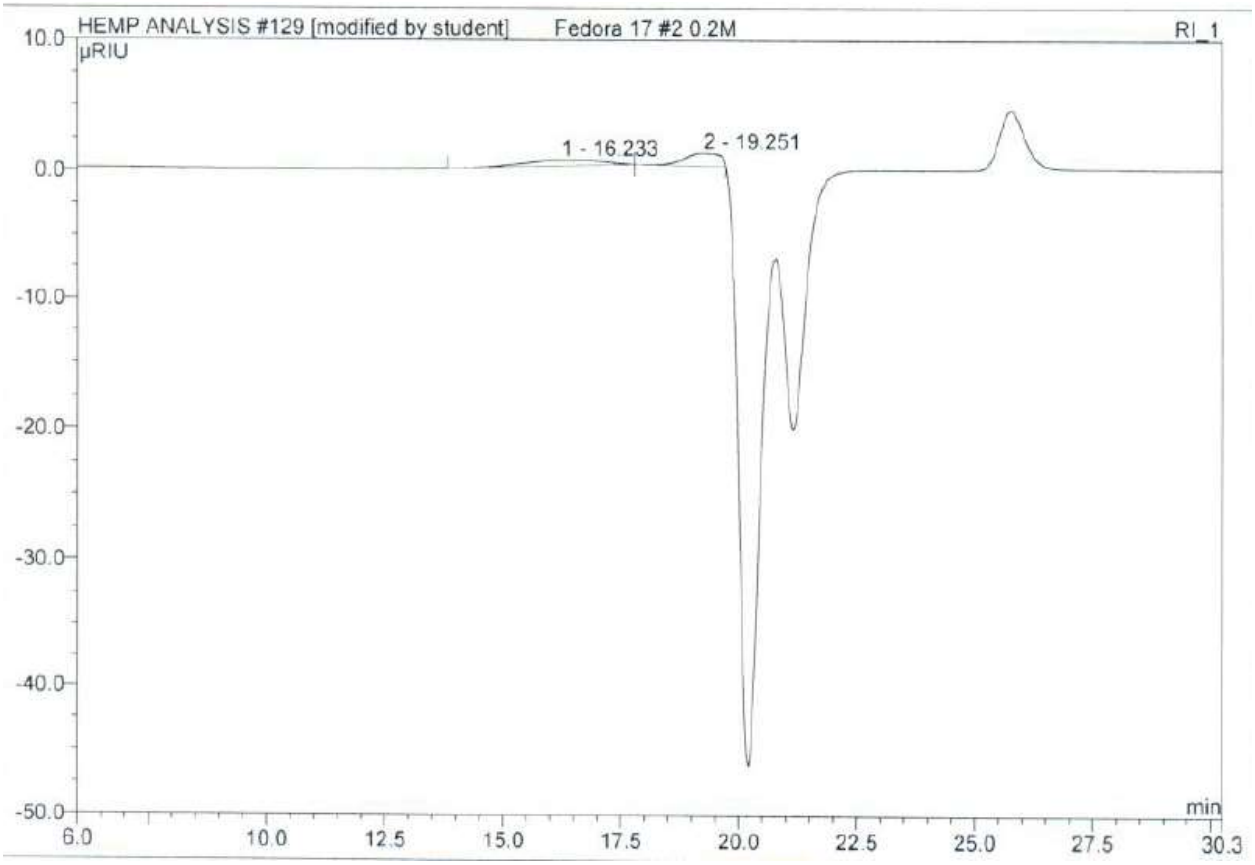
Fedora 17 #2 0 M



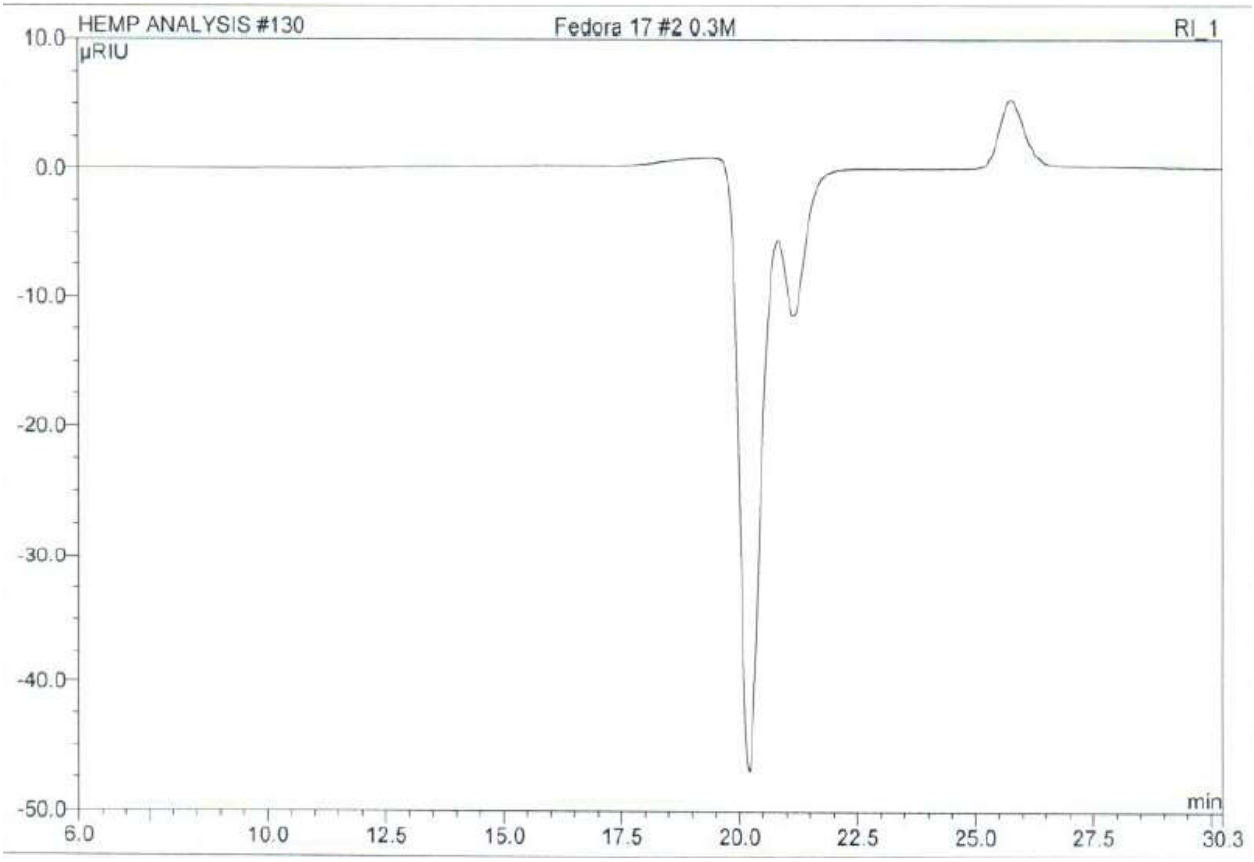
Fedora 17 #2 0.1 M



Fedora 17 #2 0.2 M



Fedora 17 #2 0.3 M



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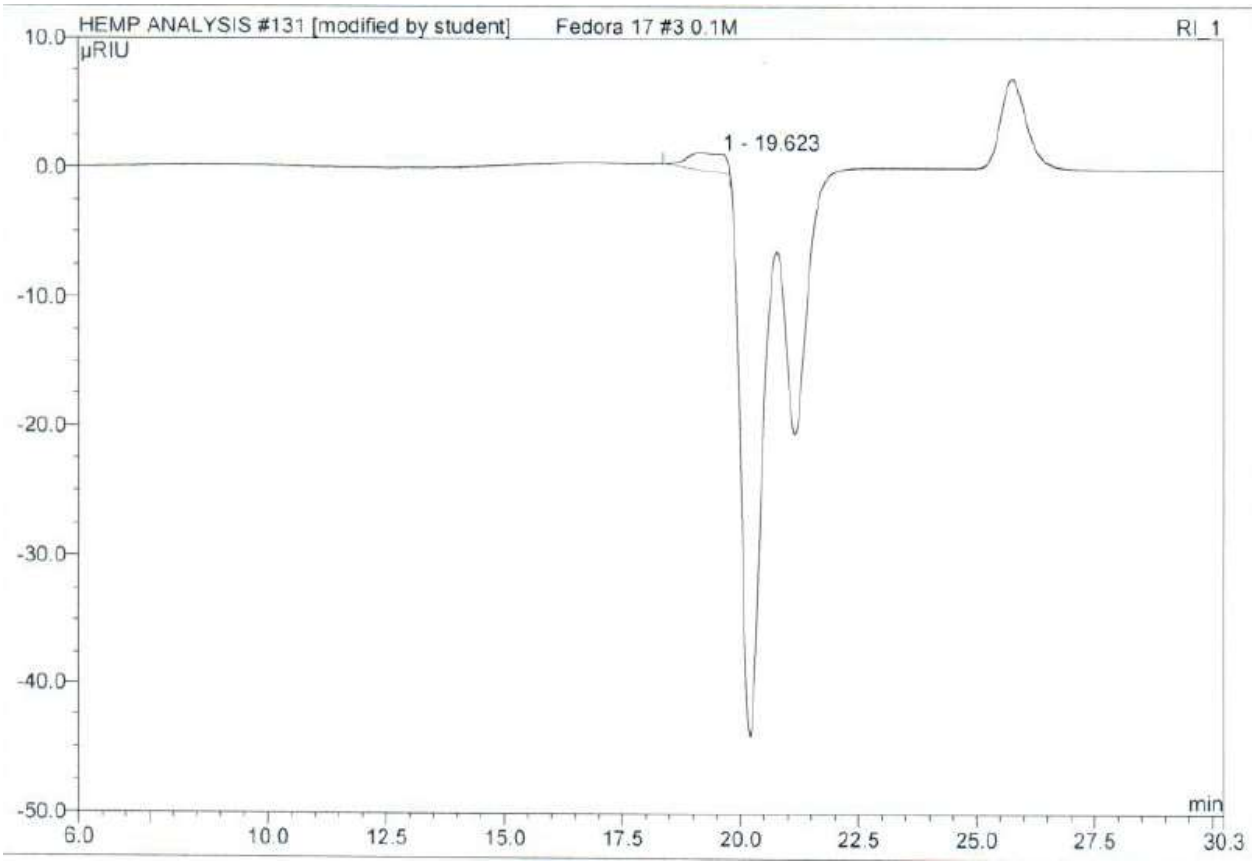
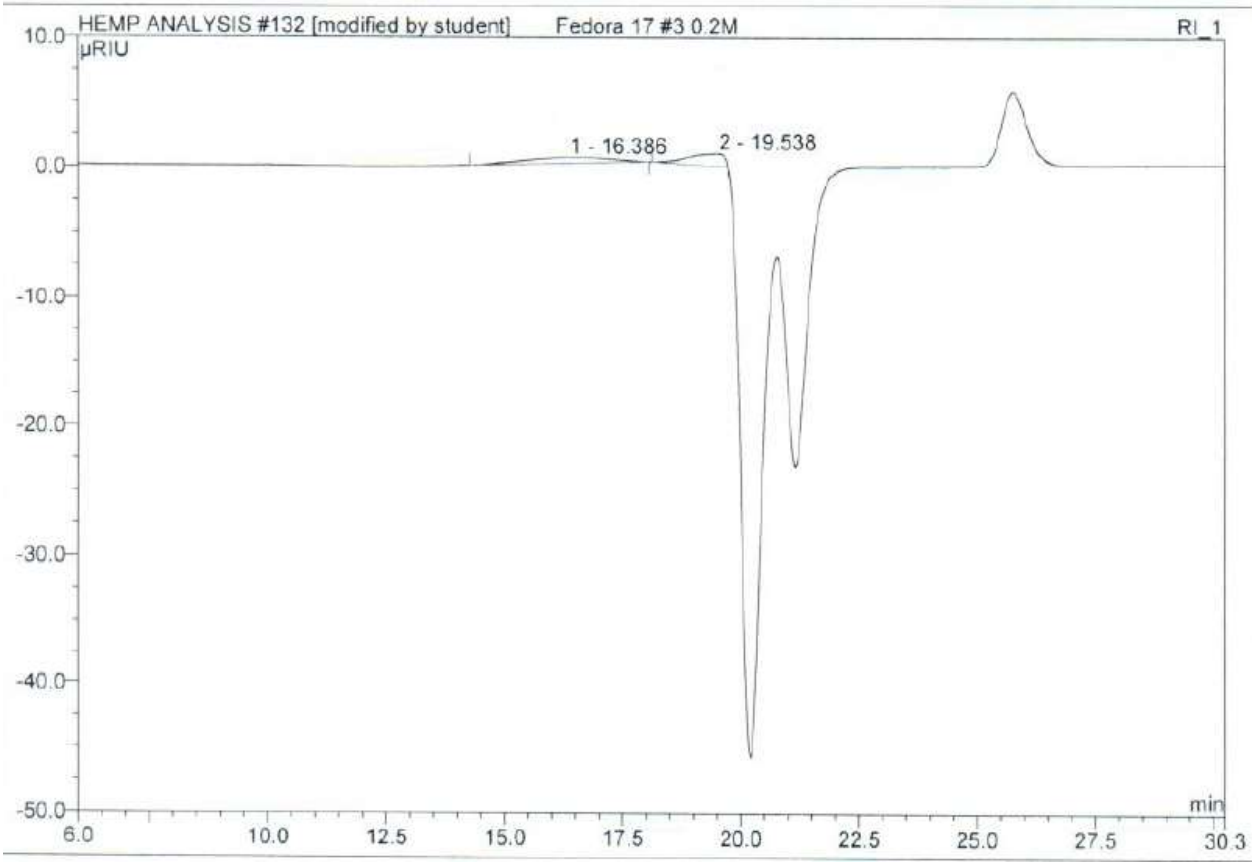
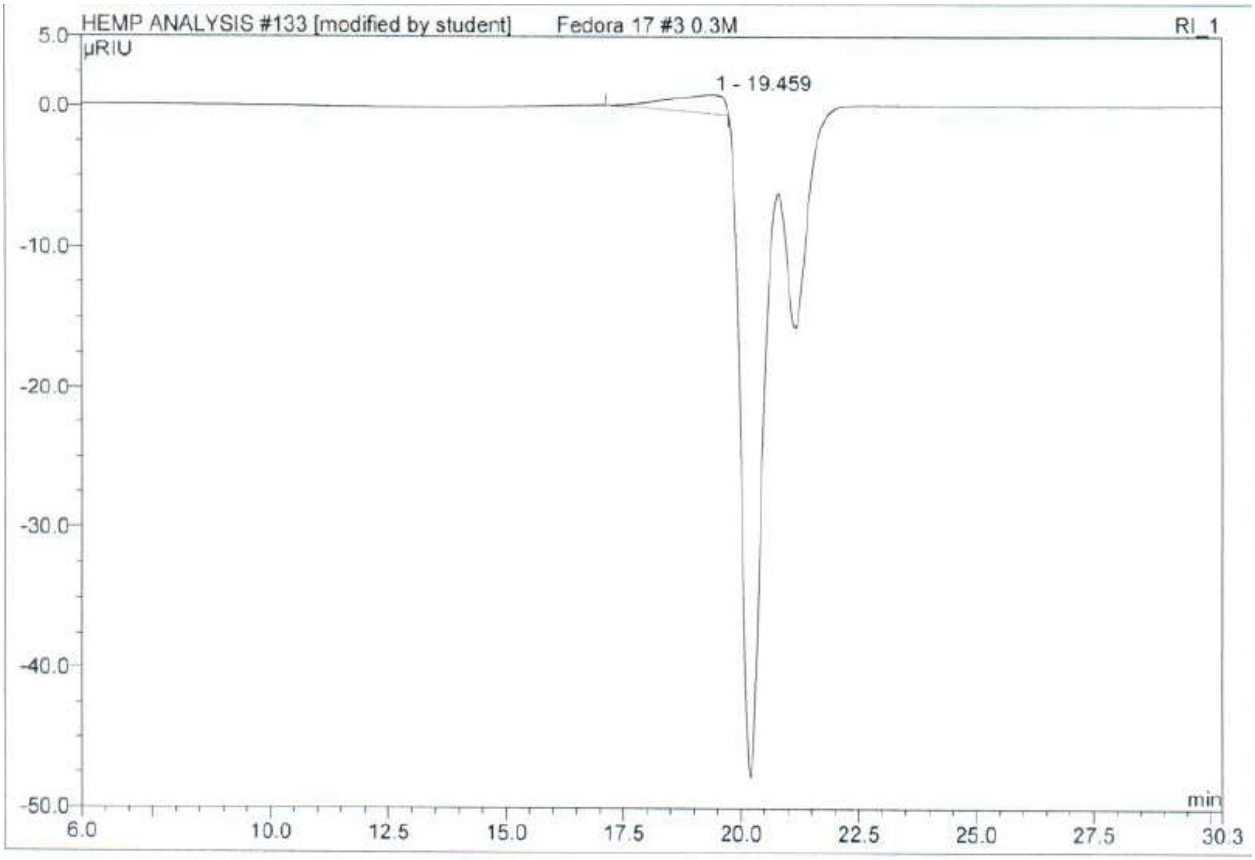


Fedora 17 #3 0.2 M

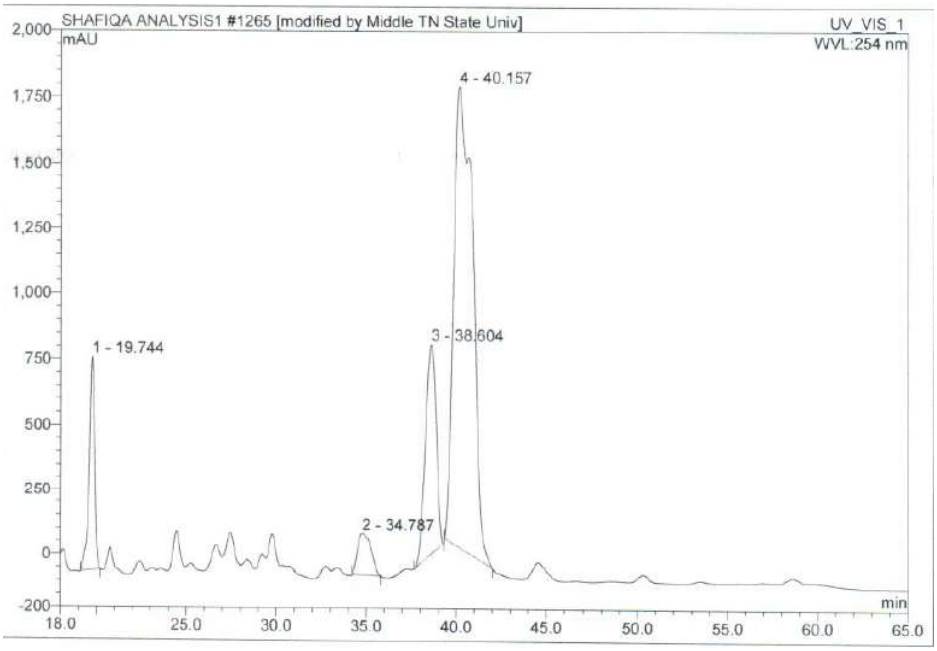


Fedora 17 #3 0.3 M



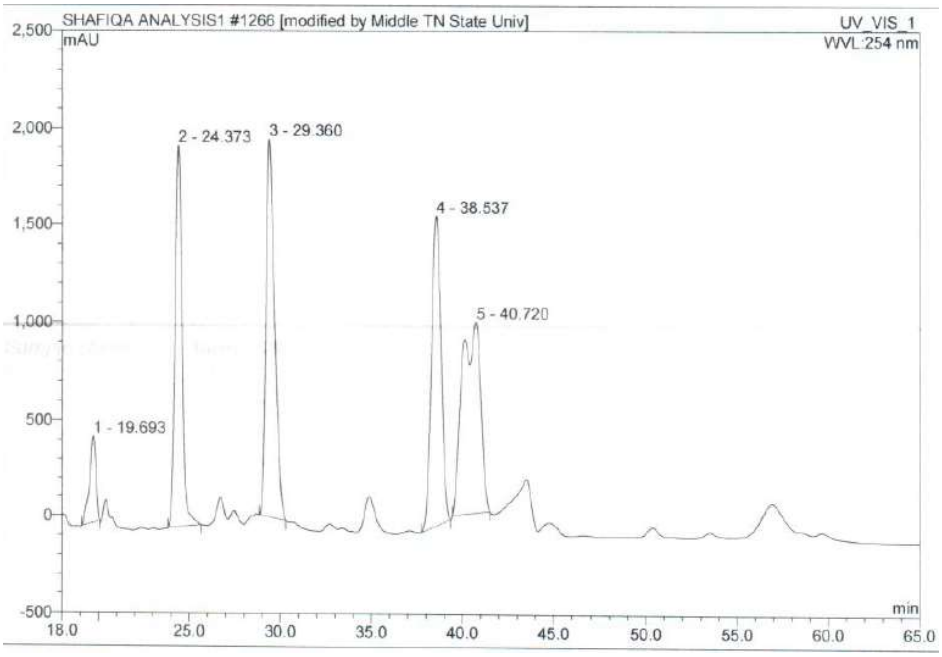
Appendix II. Chromatograms from the HPLC analysis with PMP derivatives

Farm 0.1 M



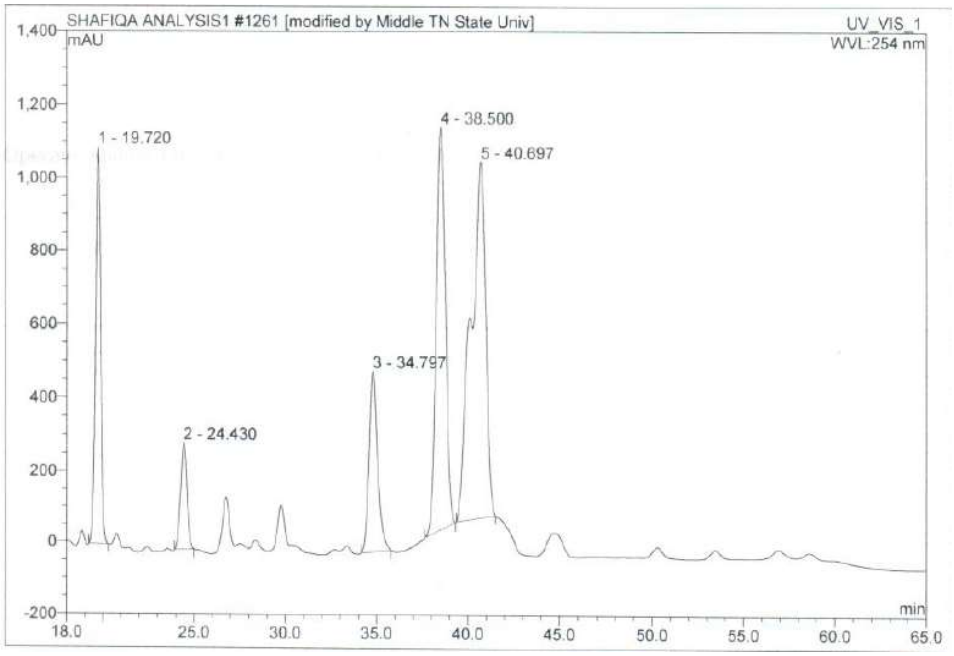
Mannose (1), Galactose (3), Xylose/Arabinose (4)

Farm 0.2 M



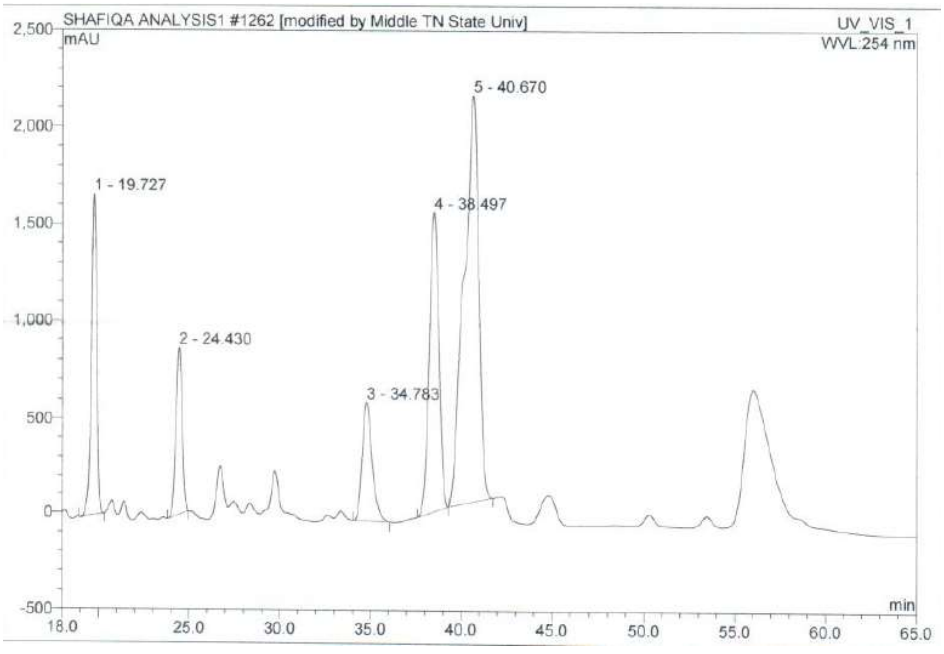
Mannose (1), Rhamnose (2), Galactose (3), Galacturonic Acid (4), Xylose/Arabinose (5)

Greenhouse 0.1 M



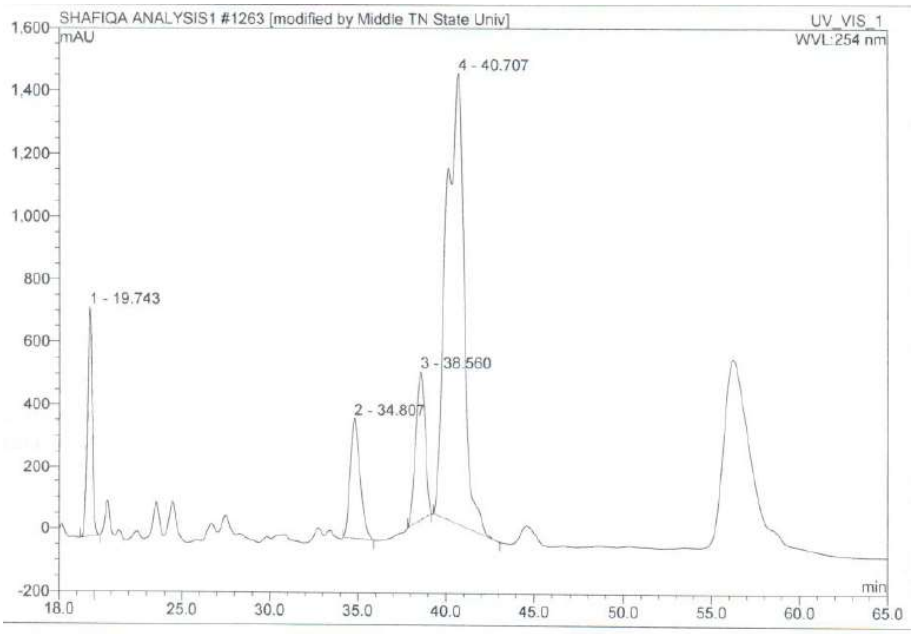
Mannose (1), Rhamnose (2), Glucose (3), Galactose (4), Xylose/Arabinose (5)

Greenhouse 0.2 M



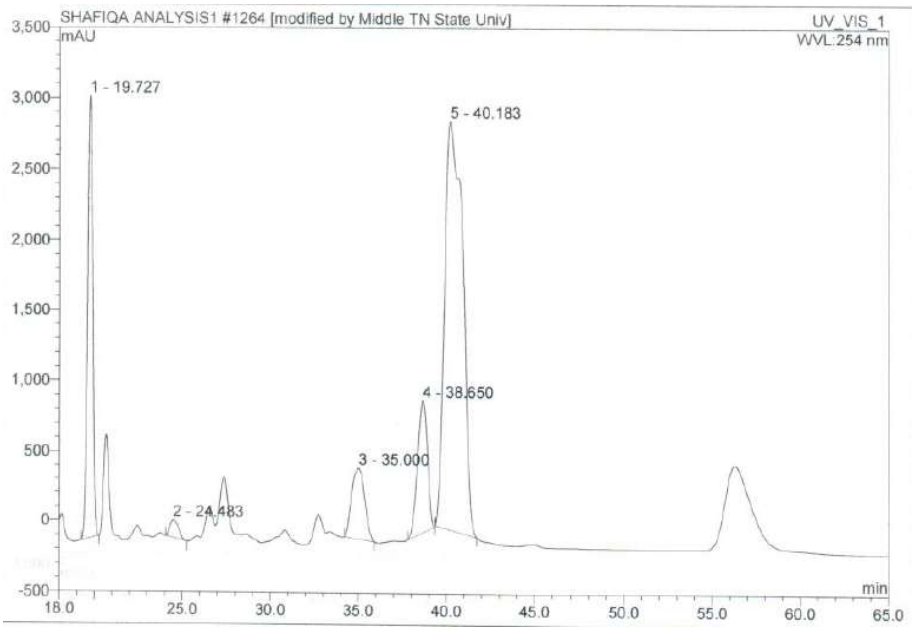
Mannose (1), Rhamnose (2), Glucose (3), Galactose (4), Xylose/Arabinose (5)

Greenhouse 0.3 M



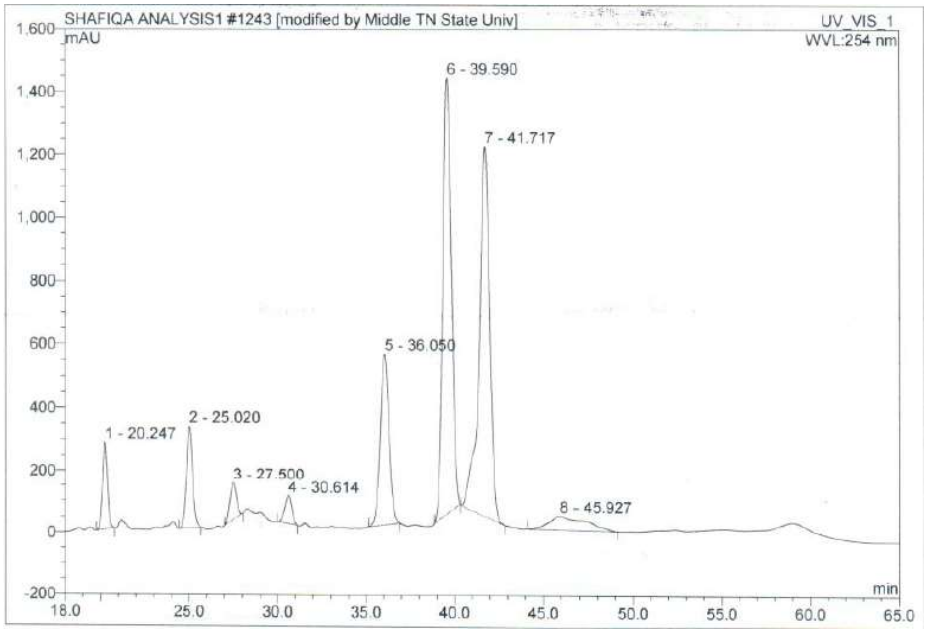
Mannose (1), Glucose (2), Galactose (3), Xylose/Arabinose (4)

Greenhouse 0.6 M



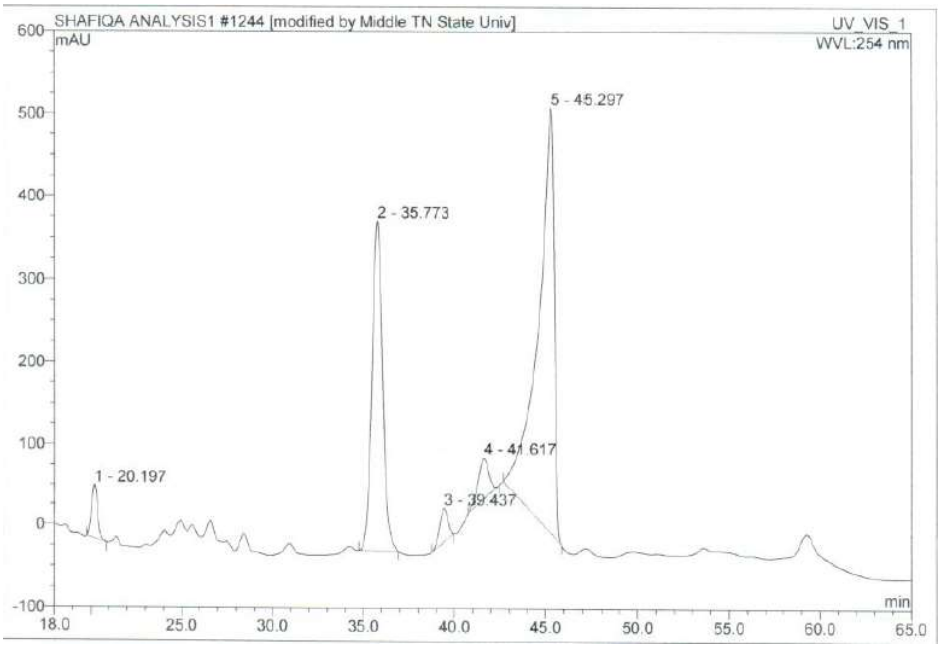
Mannose (1), Glucose (3), Galactose (4), Xylose/Arabinose (5)

Fedora 17 #2 0 M



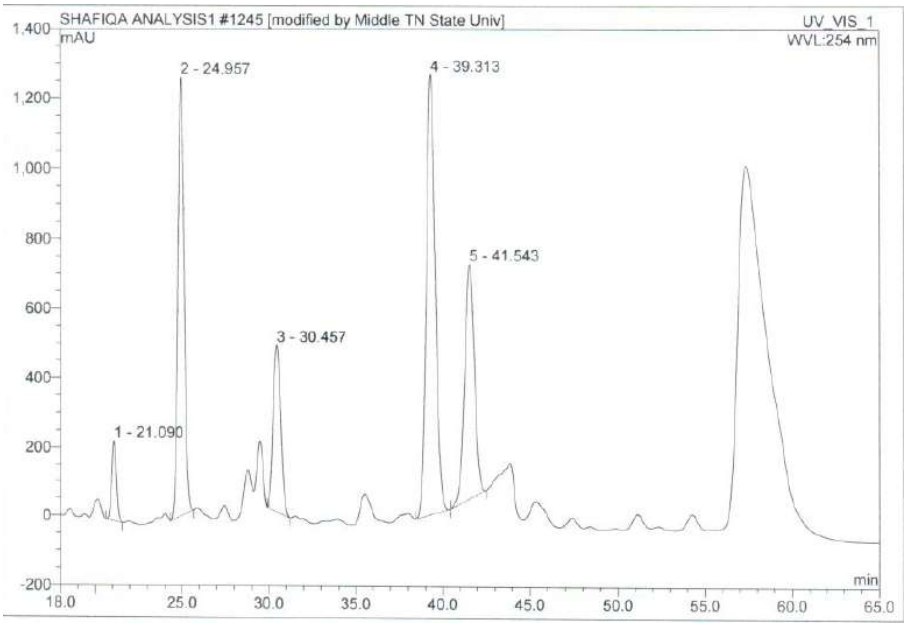
Mannose (1), Rhamnose (2), Glucose (5), Galactose (6), Xylose/Arabinose (7)

Fedora 17 #2 0.1 M



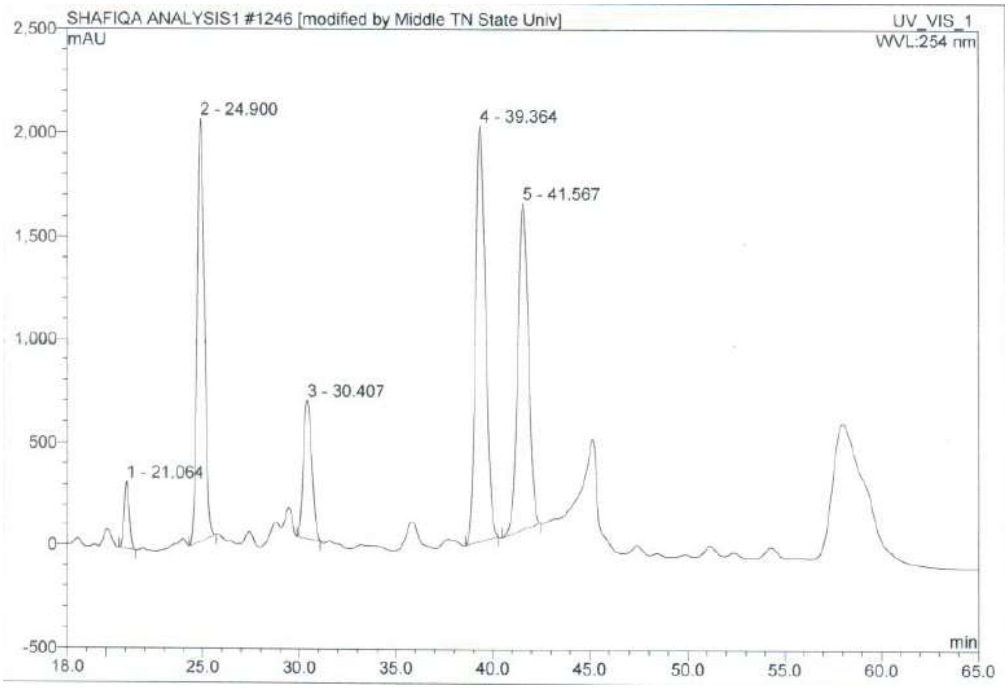
Mannose (1), Glucose (2), Galactose (3), Xylose/Arabinose (4)

Fedora 17 #2 0.2 M



Mannose (1), Rhamnose (2), Galacturonic Acid (3), Galactose (4), Xylose/Arabinose (5)

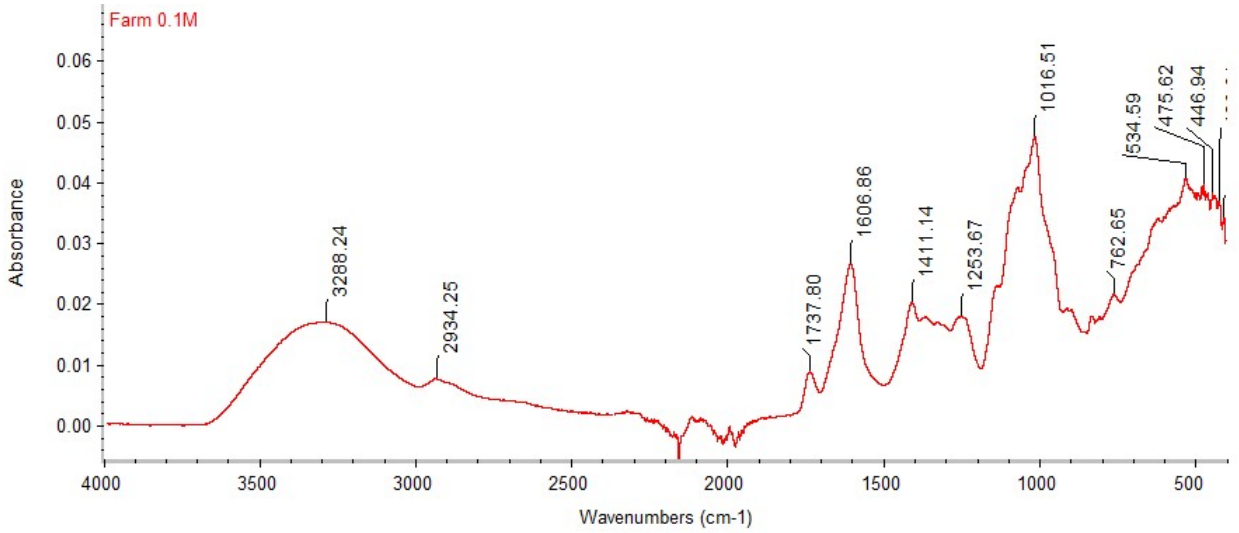
Fedora 17 #3 0.2 M



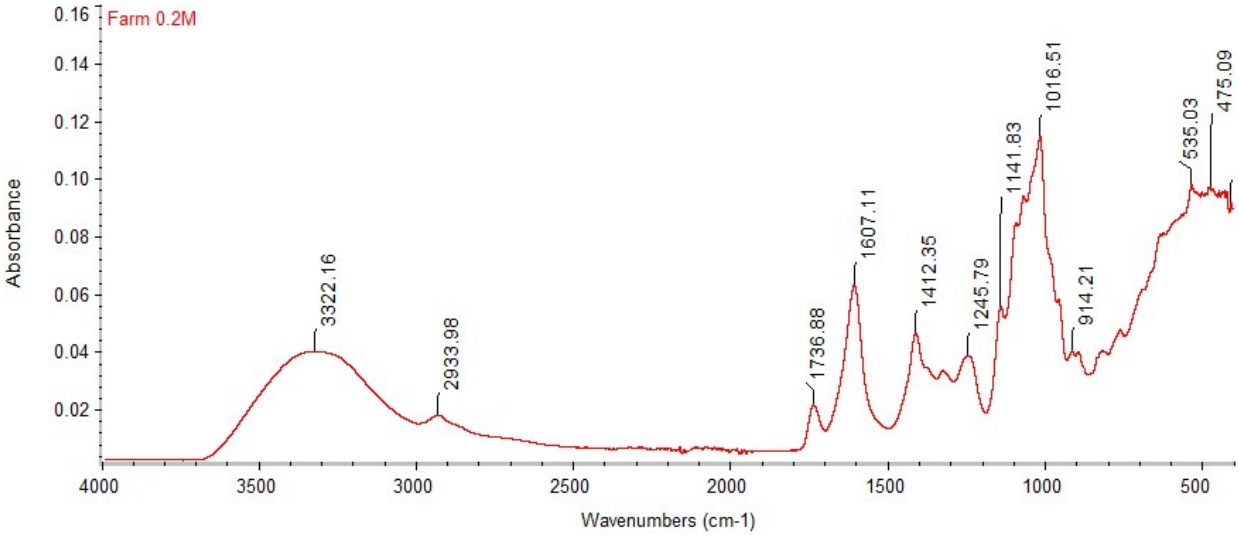
Mannose (1), Rhamnose (2), Galacturonic Acid (3), Galactose (4), Xylose/Arabinose (5)

Appendix III. Interferograms from the FTIR-ATR analysis

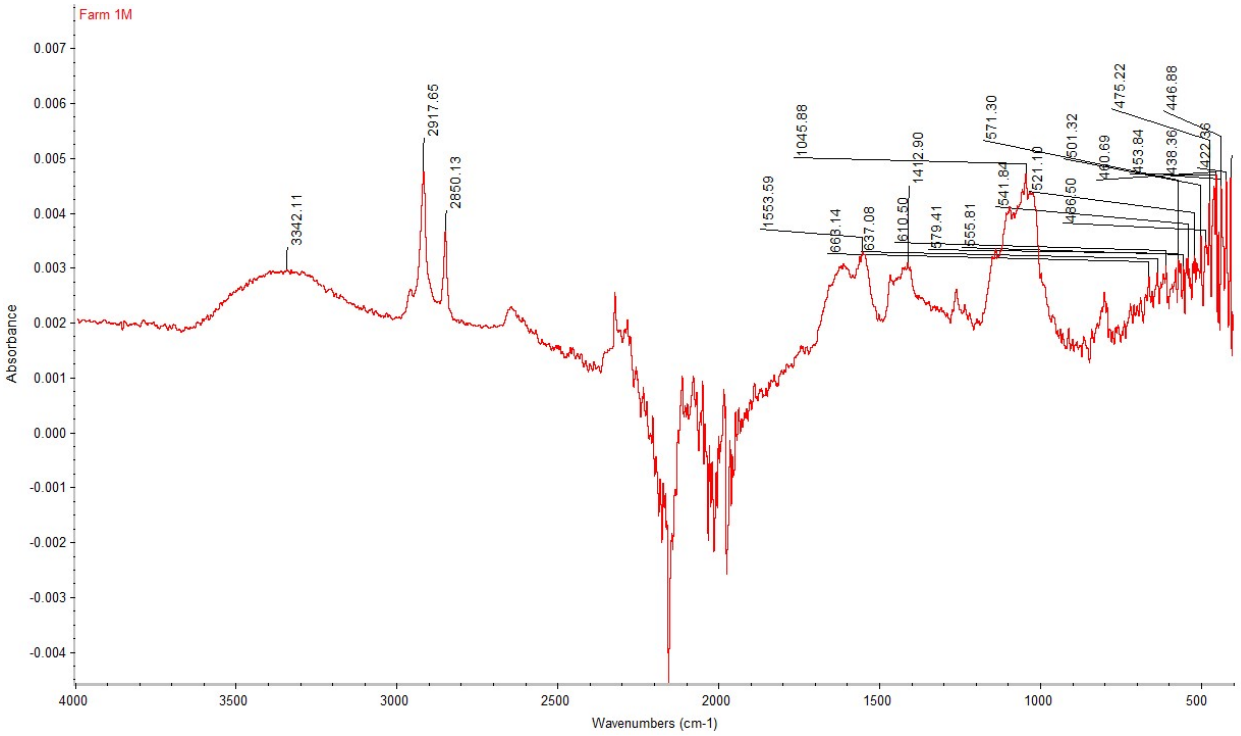
Farm Cherry Hemp Root



Peak at 3288.24 cm^{-1} is O-H stretching from carbohydrate. Peak at 1016.51 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2934.25 cm^{-1} and 1606.86 cm^{-1} are O-H and C=O stretching from uronic acids.

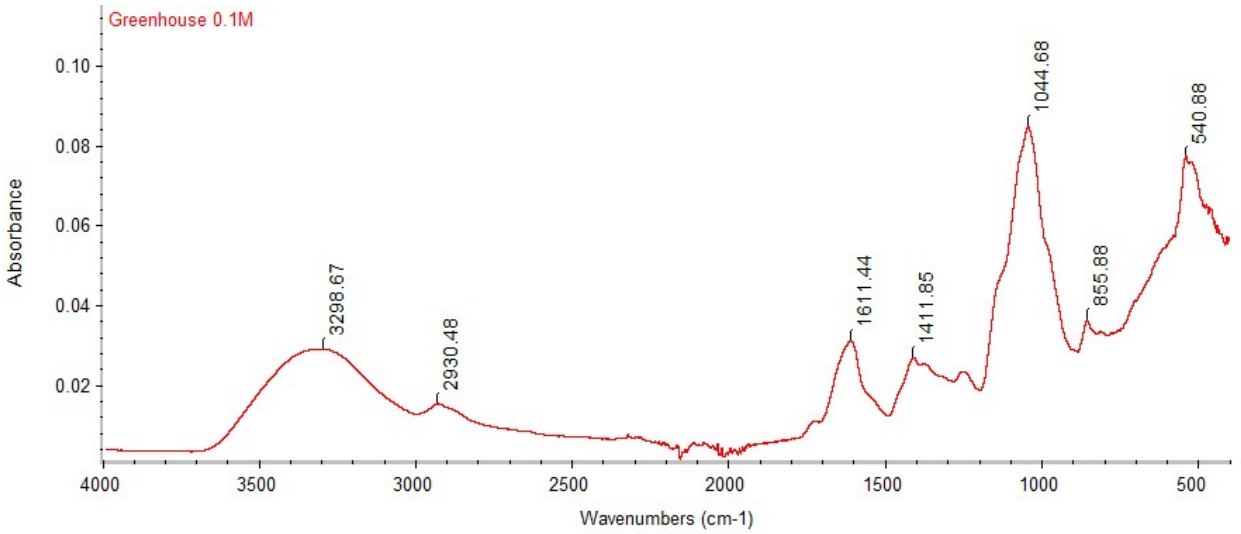


Peak at 3322.16 cm^{-1} is O-H stretching from carbohydrate. Peak at 1016.51 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2933.98 cm^{-1} and 1607.11 cm^{-1} are O-H and C=O stretching from uronic acids.

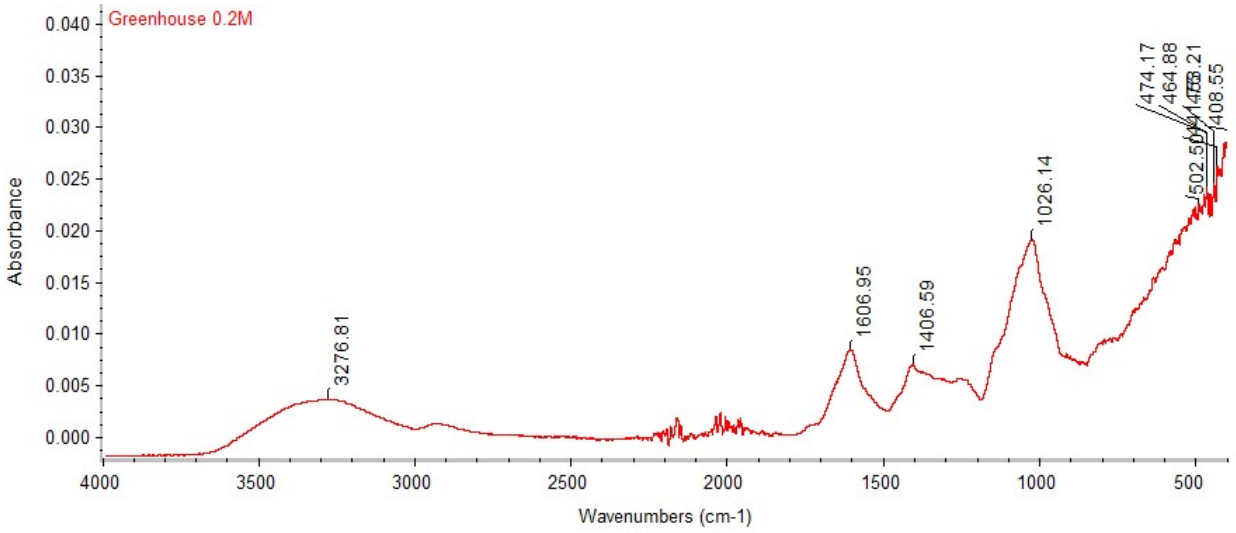


Peak at 3342.11 cm^{-1} is O-H stretching from carbohydrate. Peak at 1045.88 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2917.65 cm^{-1} and 1610.50 cm^{-1} are O-H and C=O stretching from uronic acids.

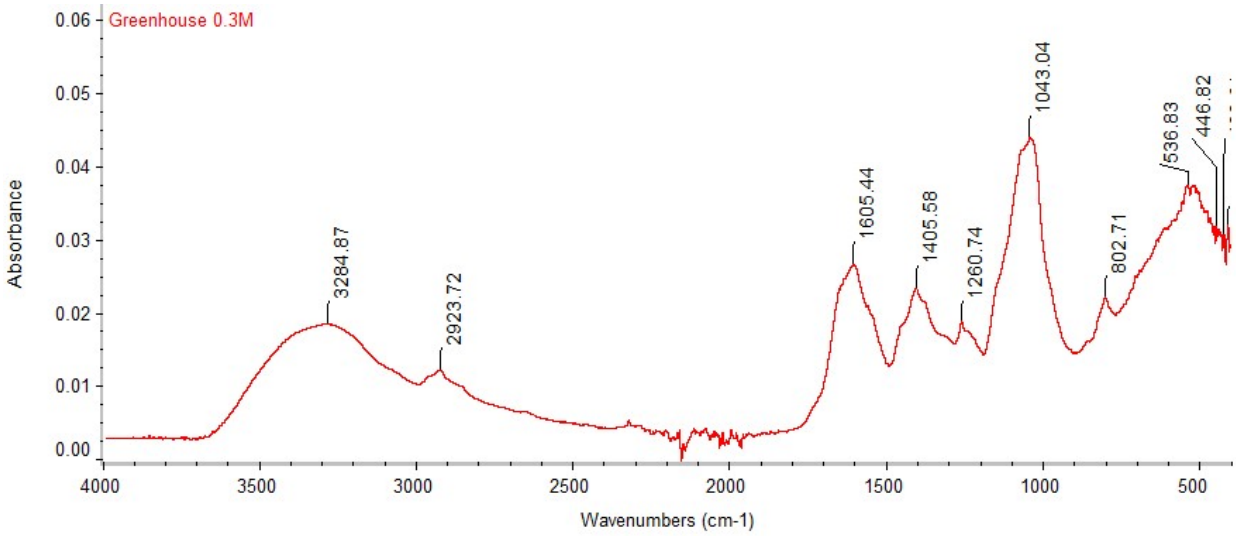
Greenhouse Cherry Hemp Root



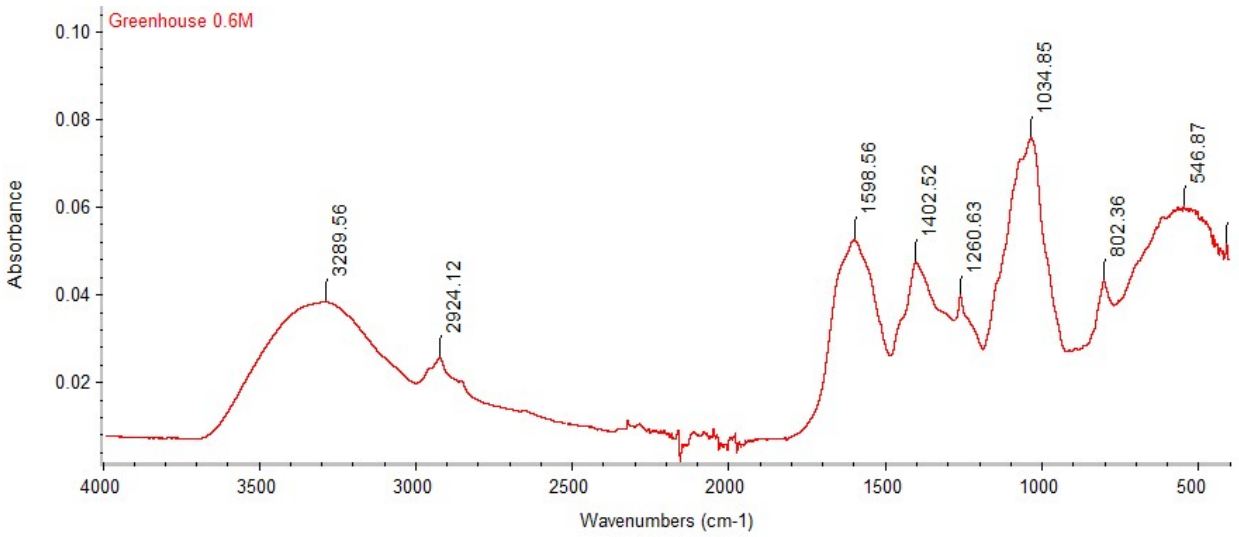
Peak at 3298.67 cm^{-1} is O-H stretching from carbohydrate. Peak at 1044.68 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2930.48 cm^{-1} and 1611.44 cm^{-1} are O-H and C=O stretching from uronic acids.



Peak at 3276.81 cm^{-1} is O-H stretching from carbohydrate. Peak at 1026.14 cm^{-1} is C-O stretching from carbohydrate. Peak at 1606.95 cm^{-1} is C=O stretching from uronic acids.

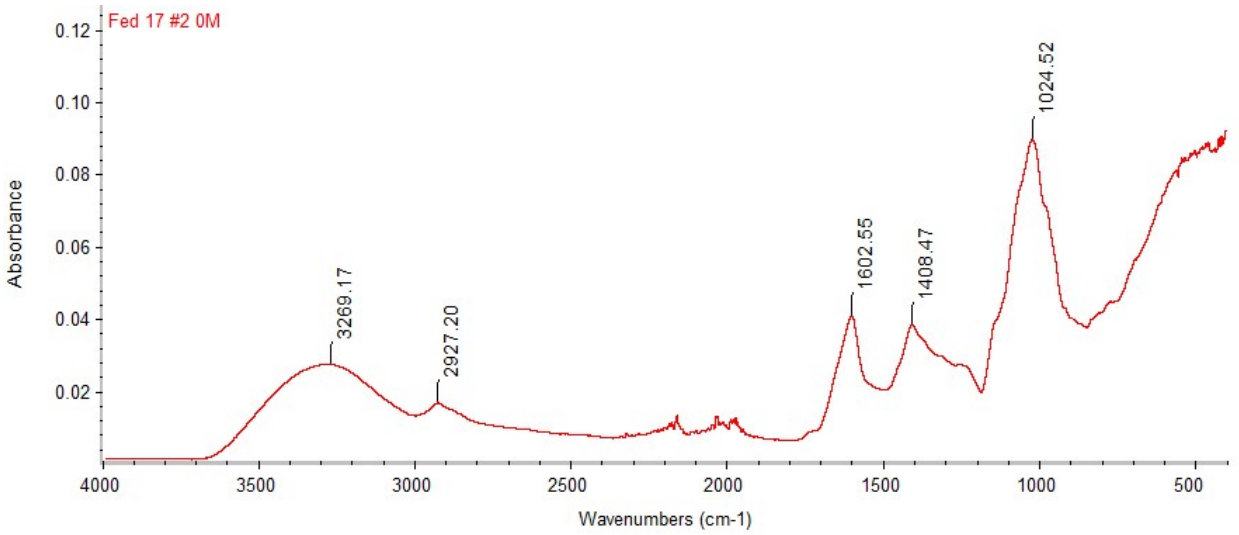


Peak at 3264.87 cm^{-1} is O-H stretching from carbohydrate. Peak at 1043.04 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2923.72 cm^{-1} and 1605.44 cm^{-1} are O-H and C=O stretching from uronic acids.

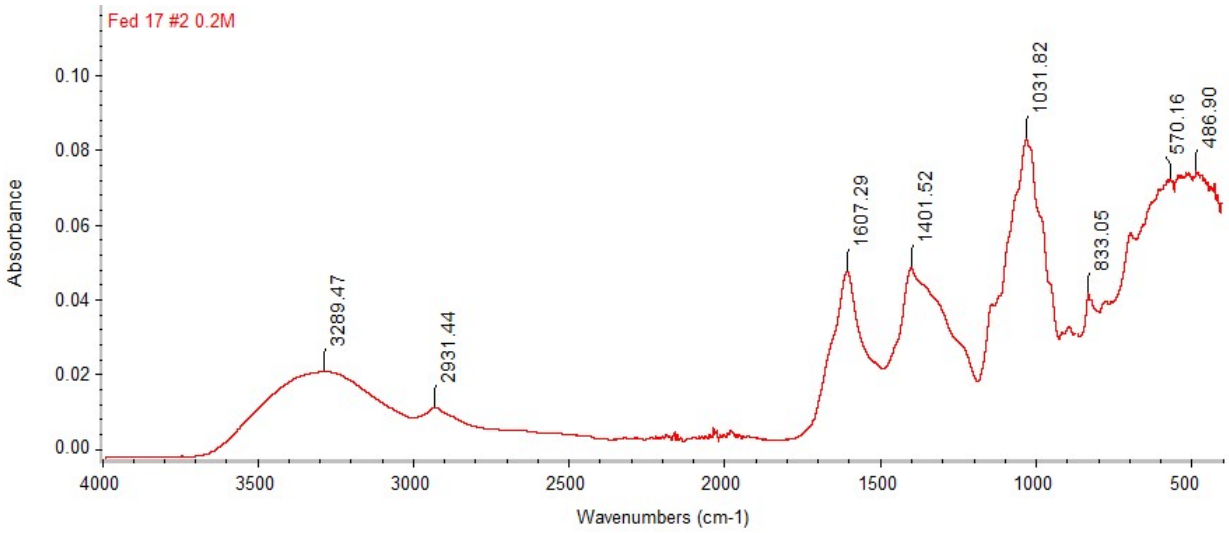


Peak at 3289.56 cm^{-1} is O-H stretching from carbohydrate. Peak at 1034.85 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2924.12 cm^{-1} and 1598.56 cm^{-1} are O-H and C=O stretching from uronic acids.

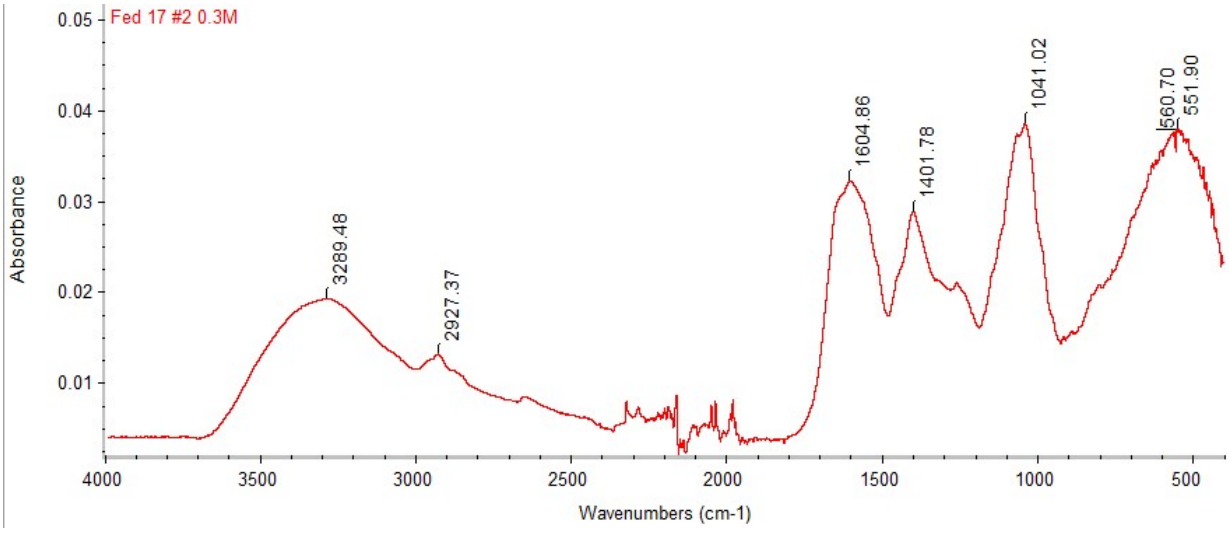
Fedora 17 #2



Peak at 3269.17 cm^{-1} is O-H stretching from carbohydrate. Peak at 1024.52 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2927.20 cm^{-1} and 1602.86 cm^{-1} are O-H and C=O stretching from uronic acids.

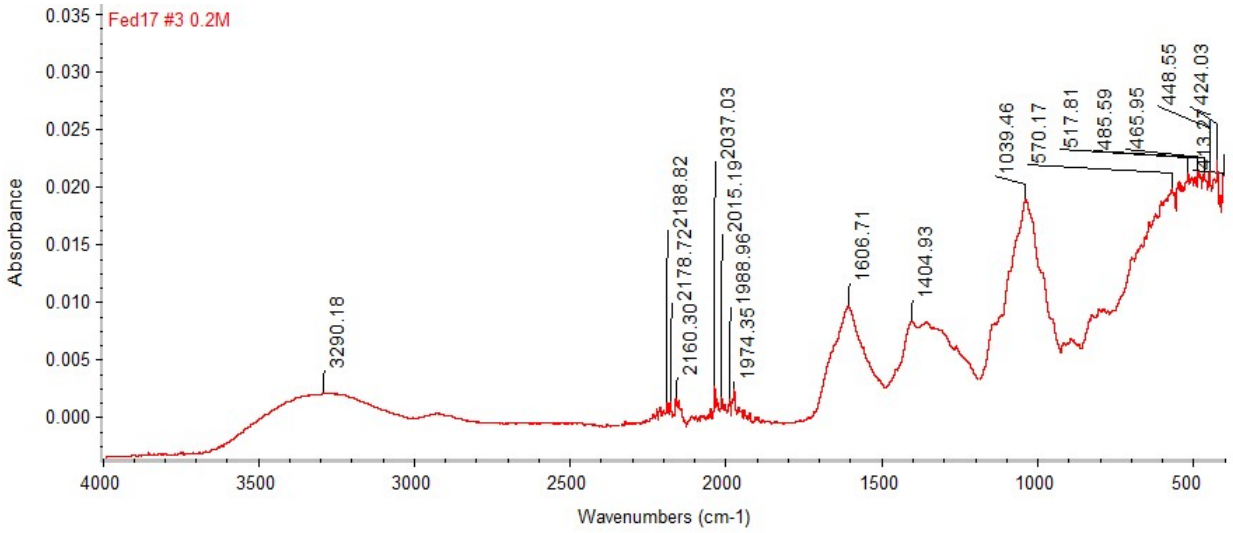


Peak at 3289.47 cm^{-1} is O-H stretching from carbohydrate. Peak at 1031.82 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2931.44 cm^{-1} and 1607.29 cm^{-1} are O-H and C=O stretching from uronic acids.

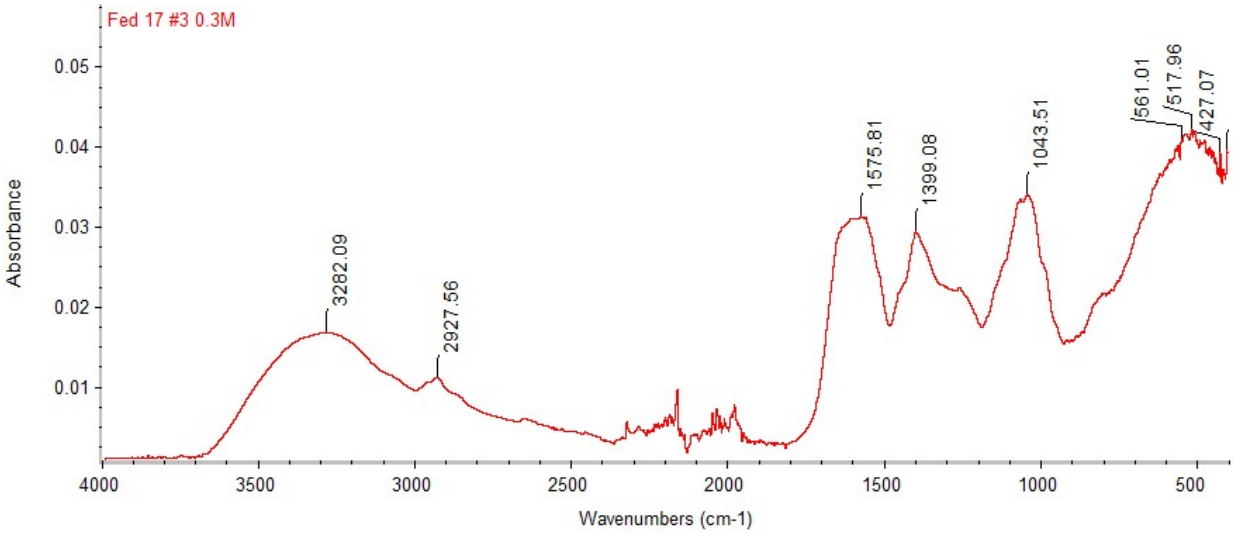


Peak at 3289.48 cm^{-1} is O-H stretching from carbohydrate. Peak at 1041.02 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2927.37 cm^{-1} and 1604.86 cm^{-1} are O-H and C=O stretching from uronic acids.

Fedora 17 #3



Peak at 3290.18 cm^{-1} is O-H stretching from carbohydrate. Peak at 1039.46 cm^{-1} is C-O stretching from carbohydrate. Peak at 1606.71 cm^{-1} is C=O stretching from uronic acids.



Peak at 3282.09 cm^{-1} is O-H stretching from carbohydrate. Peak at 1043.51 cm^{-1} is C-O stretching from carbohydrate. Peaks at 2927.56 cm^{-1} and 1575.81 cm^{-1} are O-H and C=O stretching from uronic acids.