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CHEM 1255  
10/9/20

I Put That Stuff on Everything, Except My Eyes – Determining the Amount of Capsaicin in Various Hot Sauces

I submit this lab report as an original document. I assert that all ideas and discussion of data contained herein is my own work, unless otherwise referenced.

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## Introduction

## Data

<b>Table 1</b> – Preparation of standard solutions, detailing amounts of capsaicin and caffeine in acetonitrile					
Sample	Total Volume (mL)	C Capsaicin (mg/L)	V Standard Capsaicin Solution (mL)	C Caffeine (mg/L)	m Caffeine (mg)
1	25	1	0.125	10	0.25
2	25	10	1.25	10	0.25
3	25	25	3.125	10	0.25
4	25	25	3.125	20	0.50
5	25	50	6.25	10	0.25
6	25	100	12.5	10	0.25

<b>Table 2</b> – Masses of Hot Sauce Samples, and Volumes of Solvents Used for Samples.				
Sample	Mass of Sample (g)	V Standard Caffeine Solution (mL)	V Acetonitrile (mL)	V Total (mL)
Tabasco	16.2087	2	98	100
Sriracha	15.145	2	98	100
Frank's	16.4088	2	98	100

## Results

For the sake of organizations, the screenshots of the chromatograms will be located at the end of the report.

By looking at the chromatographs for the standard samples, four major peaks are found in a majority of them. For HPLC, the order in which chemicals will come over is in order of the dipole moment. Of the four major chemicals in the experiment, (caffeine, capsaicin, hydrocapsaicin, and nordihydrocapsaicin), caffeine is the most polar, and polarity decreases in order from nordihydrocapsacin to capsaicin to hydrocapsaicin. The peaks will be marked on the first chromatograph to show all four peaks. The peaks will also share a similar shape for each chromatograph, as well as lie on a similar x-axis value. If necessary, decreasing the concentration of caffeine in the samples would increase the resolution of the capsaicin peaks.

The table below details all of the peak areas for each peak of interest. If a peak is not visible, the area is labeled as 0. The “25 (Caffeine Spike)” sample is not included in future data points, as it is only really used for the purpose of more easily determining which peak belongs to caffeine.

<b>Table 3</b> – Data from chromatograph details areas for the peaks of interest for each sample.					
Sample	Capsaicin Concentration (mg/L)	Area Caffeine Peak (μVs)	Area Nordihydrocapsaicin (μVs)	Area Capsaicin (μVs)	Area Hydrocapsaicin (μVs)
1	1	1367445.93	0	44184.58	0
2	10	1372273.85	40819.07	500739.11	194647.11
3	25	1390352.93	115839.36	1329033.92	632256.99
5	50	1379285.92	252428.28	2806503.68	1329841.12
6	100	1377112.15	520079.23	5616809.16	2628123.55
4	25 (Caffeine Spike)	3331579.11	119111.12	1338144.32	635916.52
Tabasco	UNKNOWN	2042372.70	152486.57	2270655.48	968152.99
Sriracha	UNKNOWN	1333847.25	77334.79	523115.20	305609.33

Frank's	UNKNOWN	1384152.06	128496.47	672668.06	142574.49
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To continue with the data results data, a calibration curve will be made, to compare the peak areas for each capsaicin variety to its respective concentration. However, since caffeine is also present in the samples, its own peak areas need to be accounted for as well. To do this, the area ratios comparing the peak areas of each capsaicin variety to the peak areas of caffeine need to be considered. This is calculated by the simple equation show below.

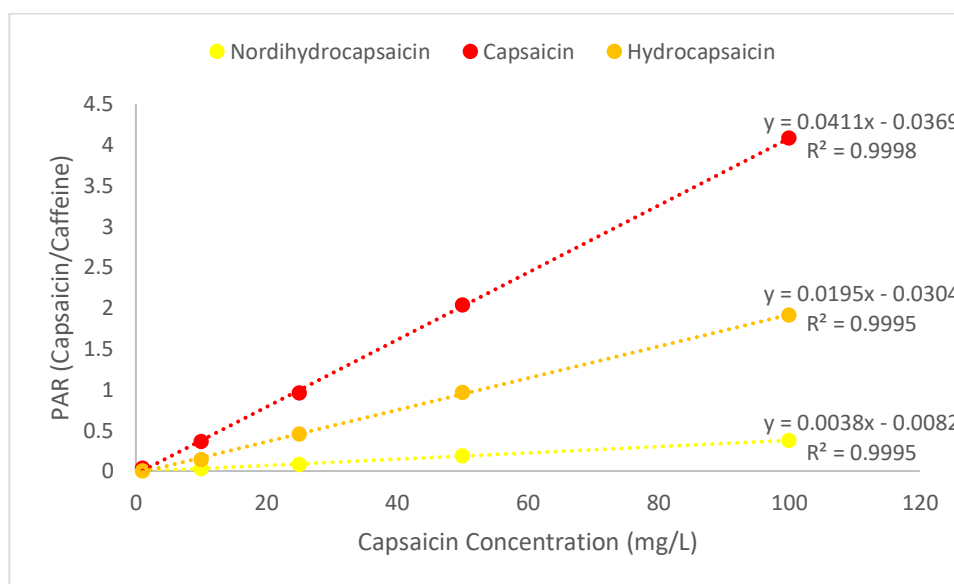
$$PAR_{\frac{x}{caff}} = \frac{PA_x}{PA_{caff}}$$

$$PAR_{\frac{Capsa}{caff}_1} = \frac{PA_{Capsa_1}}{PA_{caff_1}} = \frac{44184.58 \mu V \cdot s}{1367445.93 \mu V \cdot s} = 0.03231176$$

The equation is repeated for each capsaicin variety for each sample, except for the “25 (Caffeine Spike)” sample, as it is not relevant for the rest of the experimental calculations. The table below shows all the calculated peak area ratio values.

Table 4 – Ratio of Area Peaks for Capsaicin variety and Caffeine			
Sample	PAR Nordihydrocapsaicin/Caffeine	PAR Capsaicin/Caffeine	PAR Hydrocapsaicin/Caffeine
1	0	0.03231176	0
2	0.02974557	0.36489736	0.14184276
3	0.083316514	0.955896802	0.45474568
5	0.183013744	2.03475120	0.964151885
6	0.377659314	4.078686808	1.90843102
Tabasco	0.074661481	1.111773321	0.47403346
Sriracha	0.057978745	0.392185237	0.22911869
Frank's	0.092834071	0.485978441	0.10300493

Using the peak area ratios for the standard samples, along with the concentrations of capsaicin for the samples, calibration curves can be made for each of the types of capsaicin used, comparing the concentrations of total capsaicin in the sample vs the peak area ratios.



**Figure 1** – Calibration curves for each of the capsaicin varieties used in the experiment. Each set of data points and the associated trendline corresponds to a single variety of capsaicin.

With the peak area ratios for each of the capsaicin varieties for the unknown samples, the respective concentrations of the capsaicin varieties can be determined. This is done by the following calculation:

$$x = \frac{y - b}{m}$$

$$x_{NordiTabasco} = \frac{0.074661481 - (-0.0081949)}{0.0038425} = 21.563 \frac{\text{mg}}{\text{L}}$$

For the equation,  $x$  is the capsaicin concentration,  $y$  is the peak area ratio,  $m$  is the slope of the trendline, and  $b$  is the y-intercept of the trendline. The table below shows the concentration of each capsaicin type in each sample.

<b>Table 5</b> – Concentrations of each capsaicin type present in each sample of hot sauce.			
Sample	Nordihydrocapsaicin Concentration (mg/L)	Capsaicin Concentration (mg/L)	Hydrocapsaicin Concentration (mg/L)
Tabasco	21.563	27.925	25.910
Sriracha	17.221	10.431	13.330
Frank's	26.292	12.712	6.8522

In order to determine the Scoville Units for each hot sauce, the mass fraction of each capsaicin variety must be determined. The first step to do this is to convert the concentration of the capsaicin varieties to the mass of the capsaicin varieties. The calculation below shows the conversion. Keep in mind, the volume value comes from the amount of solvent that the capsaicin extract is dissolved in.

$$m = C \cdot V$$

$$m = 21.563 \frac{\text{mg}}{\text{L}} \cdot 0.1 \text{ L} \cdot \left( \frac{1 \text{ g}}{1000 \text{ mg}} \right) = 0.0021563 \text{ g}$$

The table below shows all of the masses calculated.

<b>Table 6</b> – Mass of each capsaicin variety present in each sample of hot sauce.			
Sample	Nordihydrocapsaicin Mass (g)	Capsaicin Mass (g)	Hydrocapsaicin Mass (g)
Tabasco	0.0021563	0.0027925	0.0025910
Sriracha	0.0017221	0.0010431	0.0013330
Frank's	0.0026292	0.0012712	0.00068522

The mass fraction is calculated by dividing the mass of the certain capsaicin variety by the mass of the raw hot sauce sample weighed out before the capsaicin was extracted, which is shown in **Table 1**. After each mass fraction is found, the Scoville Value can be found. Both of the equations are shown below, and **Table 7** shows all of the values for both the mass fractions and the Scoville Units.

$$m_{frac} = \frac{m_x}{m_{sample}}$$

$$m_{fracNordiTabasco} = \frac{0.0021563 \text{ g}}{16.2087 \text{ g}} = 0.00013303 \frac{\text{g}}{\text{g}}$$

$$\text{Scoville Value} = \left( \left( \frac{\text{g}}{\text{g}} \text{ Nordi} \right) + (9.3 \cdot 10^6) \right) + \left( \left( \frac{\text{g}}{\text{g}} \text{ Capsa} \right) + (16.1 \cdot 10^6) \right) + \left( \left( \frac{\text{g}}{\text{g}} \text{ Hydro} \right) + (16.1 \cdot 10^6) \right)$$

$$\text{Scoville Value}_{\text{Tabasco}} = ((0.0021563) + (9.3 \cdot 10^6)) + ((0.0027925) + (16.1 \cdot 10^6)) + ((0.0025910) + (16.1 \cdot 10^6)) = 6585$$

<b>Table 7</b> – Mass fraction of each capsaicin variety present in each hot sauce, and calculated Scoville values.				
Sample	Nordihydrocapsaicin Mass Fraction (g/g)	Capsaicin Mass Fraction (g/g)	Hydrocapsaicin Mass Fraction (g/g)	Scoville Units
Tabasco	0.00013303	0.00017228	0.00015985	6585
Sriracha	0.00011371	0.000068877	0.000088016	3583
Frank's	0.00016023	0.000077468	0.000041759	3410

## Discussion

The data received from the experiment revealed that Tabasco sauce has a higher Scoville Heat Value than both Sriracha sauce and Frank's Red Hot sauce. **Table 7** details the Scoville Heat Values received. The experimental calculations also show that the Scoville Heat Value is correlative to the concentrations of each capsaicin variety present in the hot sauces.

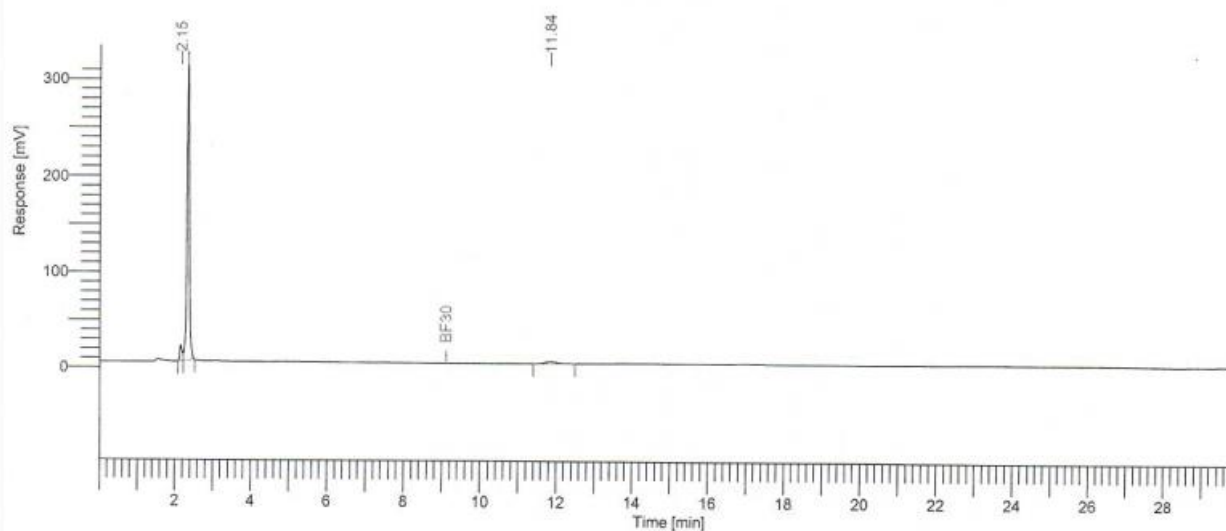
The experiment done shows the importance of HPLC as well as its ease of use and reliability. As long as the samples are purified, the chromatograms received from the HPLC should show distinct peaks for each of the chemicals present with minimal noise. HPLC works by comparing the polarity of the mobile phase and the compounds dissolved in it. Chemicals with polarities more similar to the solvent will elute first, and chemicals that are different will come over later. This was proven in the experiment, as caffeine and acetonitrile showed peaks first, considering that they are similar in both polarity and atom composition. The large sizes of the capsaicin molecules make them much more non-polar, which in turn makes them elute later. They come over in order of size, as the size corresponds to the polarity. Nordihydrocapsaicin comes over first because it is the smallest capsaicin variety, lacking one carbon compared to the other two varieties. Dihydrocapsaicin comes over last because it has two extra hydrogen atoms compared to capsaicin. Overall, it is most likely that HPLC is the most convenient instrument to use in this case.

The experiment was far from perfect when it comes to the procedure. A lot of possible error could occur in the procedure, and lead to resulting data not being as accurate as it should be. It is safe to say that a majority of the error comes from the extraction of capsaicin from the hot sauce samples. As is the case with most other organic extractions, the extraction procedure is not 100% reliable to receive a 100% yield. Multiple factors can lower the yield from the point that the hot sauce is weighed out. For one, the viscosity of the hot sauce prevented from making sure all the hot sauce sample was transferred to the flask to be boiled. Likewise, the filtration step could also have contributed to the source of the error. Even though the filtrate was rinsed multiple times with acetonitrile, it is possible that a quantity of capsaicin was trapped in the solids caught by the filter, and therefore diminished the amount of the capsaicin that would be used in the run through the HPLC. Of course, there are plenty of other errors that could have caused a shift in the data, such as the lack of precision with gathering amounts of solvent or solid, but it is most likely that the most error came from the extraction step. Also, the calibration curves did not lead to significant amounts of error, as each calibration curve has an  $R^2$  values of over 0.999.

## Chromatograms

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Sample Name	: 1		
Instrument Name	: HPLC200	Data Acquisition Time	: 9/16/2020 2:14:38 PM
Rack/Vial	: 1/91	Channel	: A
Sample Amount	: 1.000000	Operator	: analytical
Cycle	: 2	Dilution Factor	: 1.000000

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Sequence File : C:\Sequences\HPLC Pepper Lab\_Rerick(Fall2020)\_Standards.seq

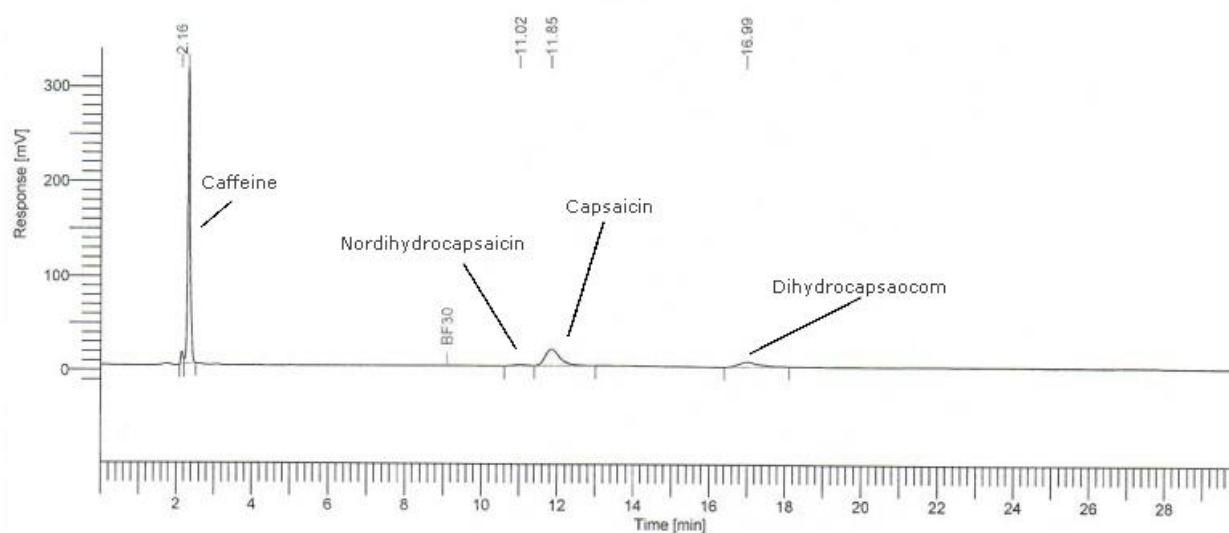


### HPLC Pepper Lab

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [uV]
1		2.155	74427.44	5.01	16630.89
2		2.336	1367445.93	92.02	308321.42
3		11.837	44184.58	2.97	1702.64
			1486057.95	100.00	326654.96

Software Version	: 6.3.4.0700	Date	: 9/16/2020 3:16:33 PM
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Sample Name	: 10	Data Acquisition Time	: 9/16/2020 2:46:18 PM
Instrument Name	: HPLC200	Channel	: A
Rack/Vial	: 1/92	Operator	: analytical
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 3		

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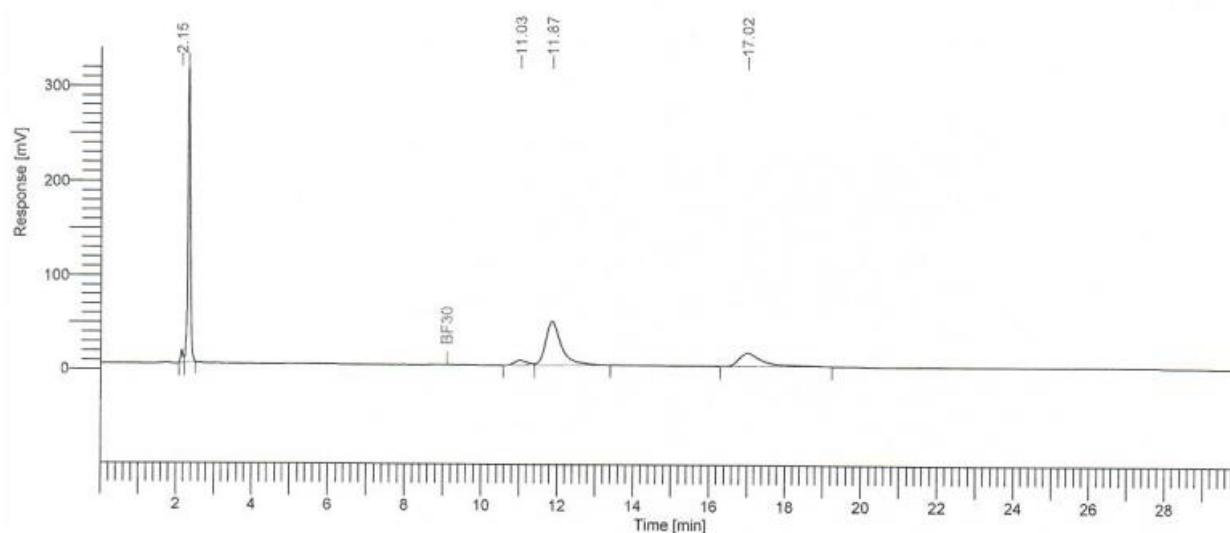


### HPLC Pepper Lab

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [uV]
1		2.156	59155.94	2.73	13753.32
2		2.337	1372273.85	63.31	313097.18
3		11.019	40819.07	1.88	1788.38
4		11.845	500739.11	23.10	17899.57
5		16.989	194647.11	8.98	5153.81
			2167635.09	100.00	351692.26

Software Version	: 6.3.4.0700	Date	: 9/16/2020 3:48:11 PM
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Sample Name	: 25		
Instrument Name	: HPLC200	Data Acquisition Time	: 9/16/2020 3:17:58 PM
Rack/Vial	: 1/93	Channel	: A
Sample Amount	: 1.000000	Operator	: analytical
Cycle	: 4	Dilution Factor	: 1.000000

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Sequence File : C:\Sequences\HPLC Pepper Lab\_Rerick(Fall2020)\_Standards.seq



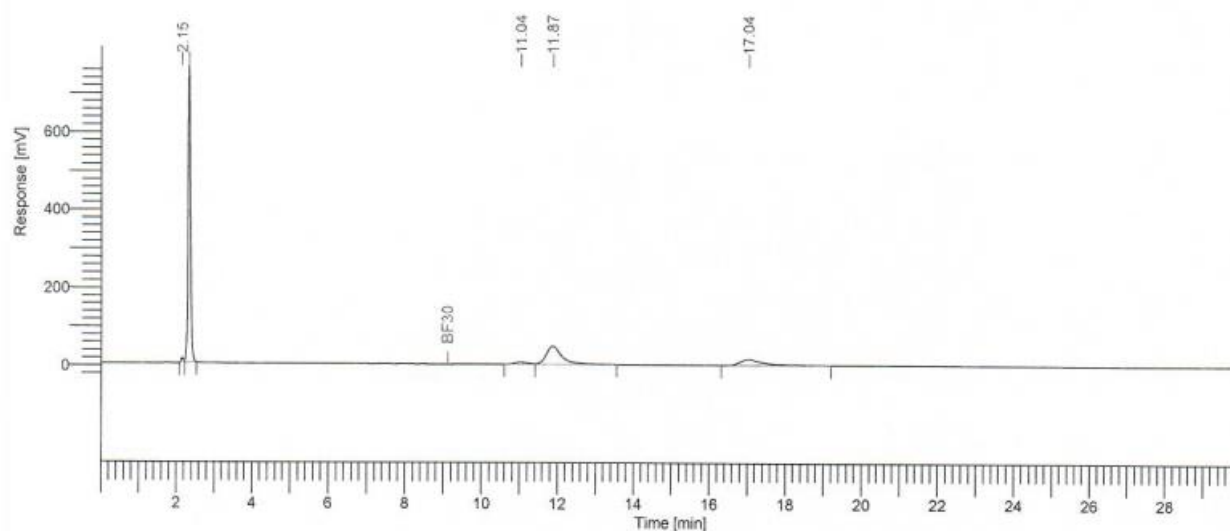
## HPLC Pepper Lab

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [uV]
1		2.155	58594.45	1.66	13539.45
2		2.337	1390352.93	39.43	313610.74
3		11.034	115839.36	3.29	4898.77
4		11.871	1329033.92	37.69	46446.13
5		17.016	632256.99	17.93	14165.16
			3526077.65	100.00	392660.25



Software Version	: 6.3.4.0700	Date	: 9/16/2020 4:19:53 PM
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Sample Name	: 25 spike	Data Acquisition Time	: 9/16/2020 3:49:39 PM
Instrument Name	: HPLC200	Channel	: A
Rack/Vial	: 1/94	Operator	: analytical
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 5		

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Sequence File : C:\Sequences\HPLC Pepper Lab\_Rerick(Fall2020)\_Standards.seq

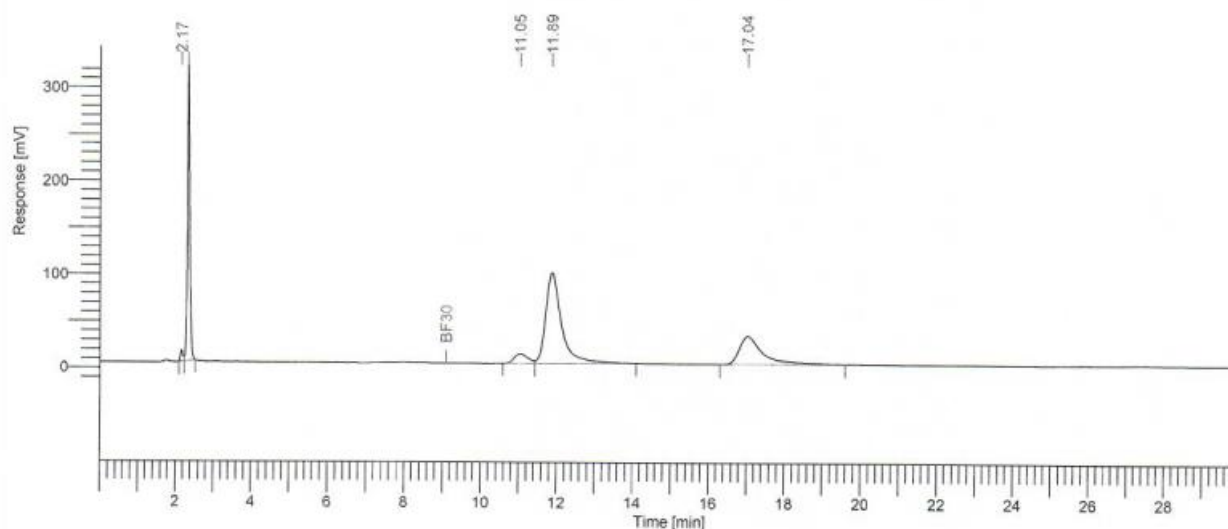


## HPLC Pepper Lab

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [uV]
1		2.153	57440.67	1.05	13721.52
2		2.333	3331579.11	60.77	763405.19
3		11.037	119111.12	2.17	4960.71
4		11.875	1338144.32	24.41	46578.94
5		17.039	635916.52	11.60	14125.01
			5482191.73	100.00	842791.38

Software Version	: 6.3.4.0700	Date	: 9/16/2020 4:51:33 PM
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Sample Name	: 50		
Instrument Name	: HPLC200	Data Acquisition Time	: 9/16/2020 4:21:18 PM
Rack/Vial	: 1/95	Channel	: A
Sample Amount	: 1.000000	Operator	: analytical
Cycle	: 6	Dilution Factor	: 1.000000

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Sequence File : C:\Sequences\HPLC Pepper Lab\_Rerick(Fall2020)\_Standards.seq

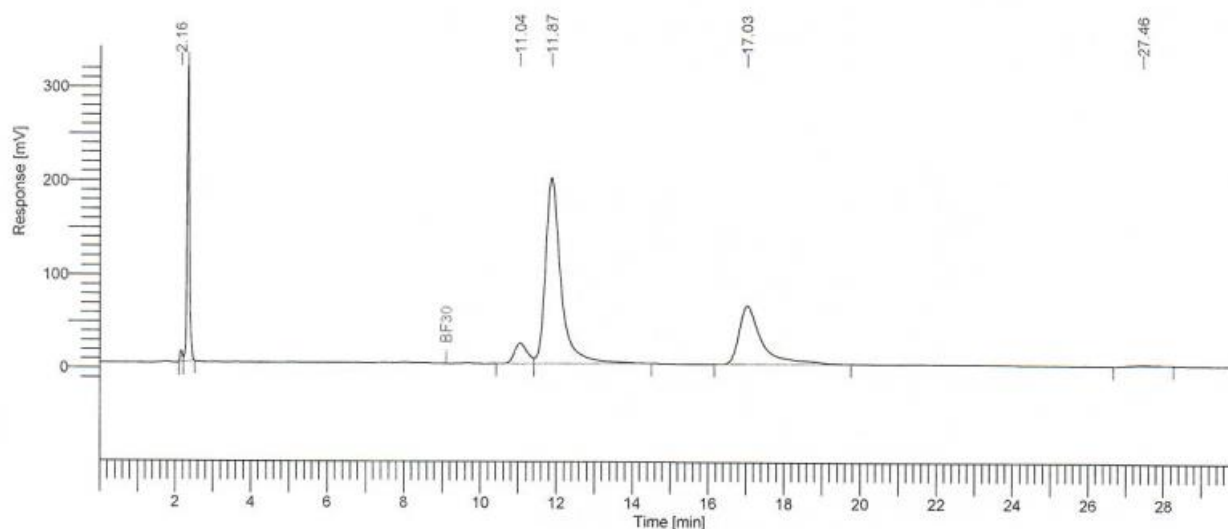


### HPLC Pepper Lab

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [uV]
1		2.167	53103.35	0.91	12268.84
2		2.348	1379285.92	23.69	316424.70
3		11.045	252428.28	4.34	10509.73
4		11.886	2806503.68	48.21	96700.60
5		17.043	1329841.12	22.84	30132.11
			5821162.35	100.00	466035.98

Software Version	: 6.3.4.0700	Date	: 9/16/2020 5:23:13 PM
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Sample Name	: 100	Data Acquisition Time	: 9/16/2020 4:52:59 PM
Instrument Name	: HPLC200	Channel	: A
Rack/Vial	: 1/96	Operator	: analytical
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 7		

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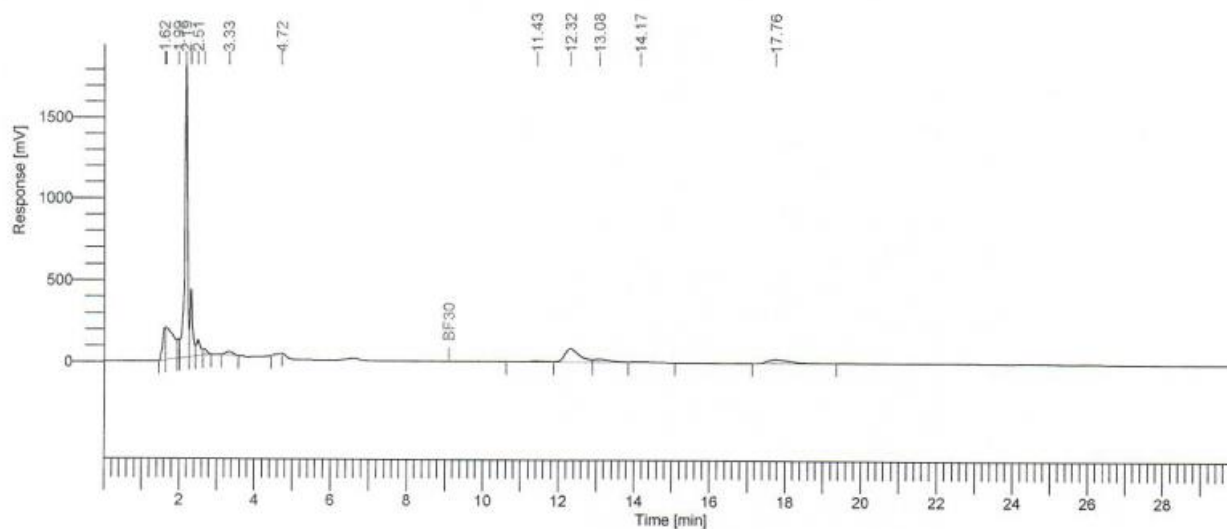
### HPLC Pepper Lab

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [uV]
1		2.156	52625.62	0.51	12100.77
2		2.337	1377112.15	13.44	316061.55
3		11.044	520079.23	5.07	21892.35
4		11.874	5616809.16	54.81	197737.46
5		17.030	2628123.55	25.64	62779.94
6		27.456	53628.97	0.52	1072.81
			10248378.69	100.00	611644.88

Software Version : 6.3.4.0700  
 Sample Name : Tabasco  
 Instrument Name : HPLC200  
 Rack/Vial : 1/63  
 Sample Amount : 1.000000  
 Cycle : 4

Date : 9/17/2020 10:23:14 AM  
 Data Acquisition Time : 9/10/2020 1:00:34 PM  
 Channel : A  
 Operator : analytical  
 Dilution Factor : 1.000000

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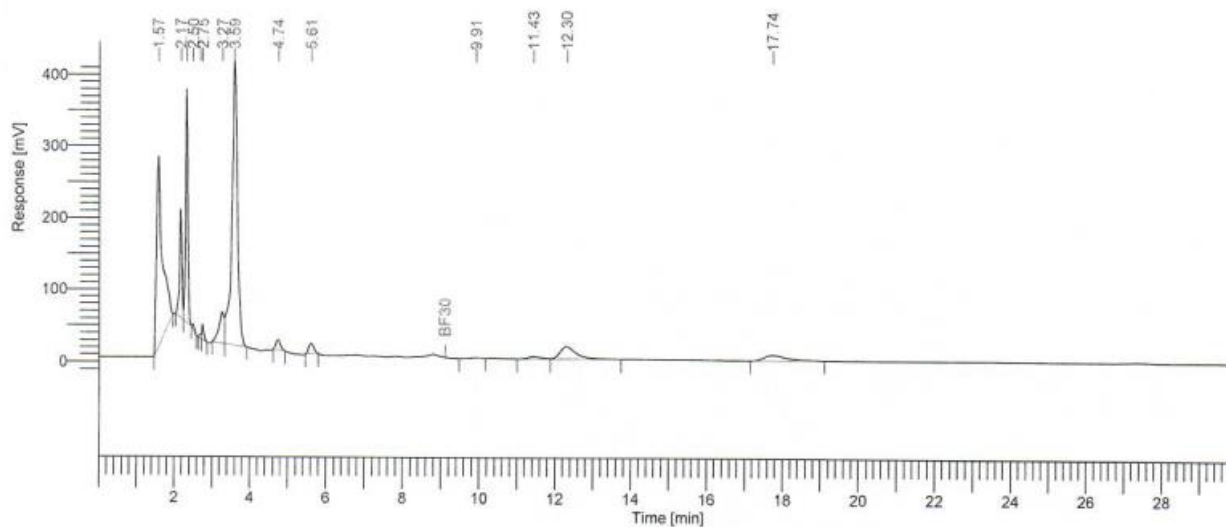
## HPLC Pepper Lab

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [μV]
1		1.618	907099.14	4.28	197650.19
2		1.663	3009204.72	14.20	196237.17
3		1.989	491042.61	2.32	119093.11
4		2.189	9251376.72	43.65	1.81e+06
5		2.321	2042372.70	9.64	412569.42
6		2.509	742122.08	3.50	97982.36
7		2.684	254081.22	1.20	35227.17
8		3.326	281768.62	1.33	20799.55
9		4.722	33003.42	0.16	753.02
10		11.428	152486.57	0.72	5366.38
11		12.318	2270655.48	10.71	81429.78
12		13.083	621606.64	2.93	19094.72
13		14.172	171588.11	0.81	4615.15
14		17.761	968152.99	4.57	24290.00
			21196561.03	100.00	3.02e+06

Software Version : 6.3.4.0700  
 Sample Name : Sriracha  
 Instrument Name : HPLC200  
 Rack/Vial : 1/62  
 Sample Amount : 1.000000  
 Cycle : 3

Date : 9/17/2020 10:23:29 AM  
 Data Acquisition Time : 9/10/2020 12:28:58 PM  
 Channel : A  
 Operator : analytical  
 Dilution Factor : 1.000000

Result File : c:\data\instrumental\hot sauce\verick\_fall2020\sriracha.rst  
 Sequence File : C:\Sequences\HPLC Pepper Lab\_Rerick(Fall2020)\_v2.seq



## HPLC Pepper Lab

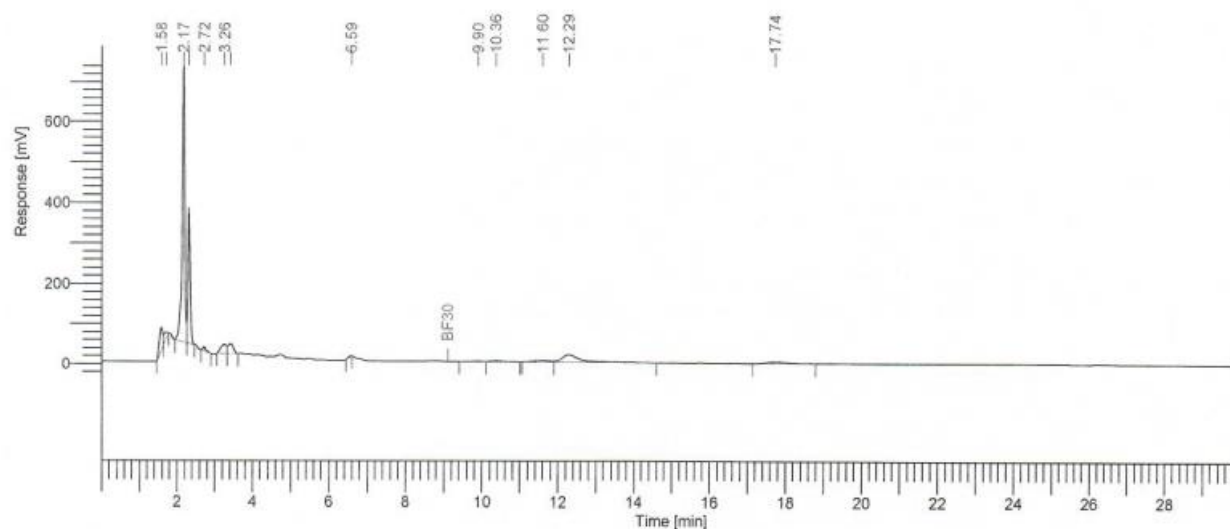
Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [uV]
1		1.573	2916288.68	27.55	266141.90
2		2.174	631438.40	5.97	150698.78
3		2.324	1333847.25	12.60	325232.84
4		2.496	29789.15	0.28	6862.57
5		2.688	15783.14	0.15	4940.05
6		2.752	74471.88	0.70	20470.94
7		3.274	403115.77	3.81	42347.72
8		3.591	4004641.03	37.83	396132.34
9		4.738	120797.10	1.14	13873.64
10		5.608	137950.02	1.30	14531.97
11		9.913	11050.06	0.10	652.39
12		11.425	77334.79	0.73	2770.72
13		12.301	523115.20	4.94	17256.49
14		17.740	305609.33	2.89	7358.34
			10585231.80	100.00	1.27e+06



Software Version : 6.3.4.0700  
 Sample Name : Franks  
 Instrument Name : HPLC200  
 Rack/Vial : 1/61  
 Sample Amount : 1.000000  
 Cycle : 2

Date : 9/17/2020 10:23:43 AM  
 Data Acquisition Time : 9/10/2020 11:57:21 AM  
 Channel : A  
 Operator : analytical  
 Dilution Factor : 1.000000

Result File : c:\data\instrumental\hot sauce\erick\_fall2020\franks\_2.rst  
 Sequence File : C:\Sequences\HPLC Pepper Lab\_Rerick(Fall2020)\_v2.seq



## HPLC Pepper Lab

Peak #	Component Name	Time [min]	Area [uV*sec]	Area [%]	Height [uV]
1		1.583	385145.52	5.81	58323.09
2		1.723	122005.25	1.84	15423.52
3		2.175	3134680.34	47.27	686771.67
4		2.318	1384152.06	20.87	334676.95
5		2.723	61798.11	0.93	11033.90
6		3.257	247270.43	3.73	23503.22
7		3.423	246451.61	3.72	24944.17
8		6.586	9479.53	0.14	980.06
9		9.900	33081.44	0.50	1716.10
10		10.363	64245.66	0.97	2795.13
11		11.596	128496.47	1.94	4146.82
12		12.289	672668.06	10.14	18385.15
13		17.738	142574.49	2.15	3555.98
			6632048.97	100.00	1.19e+06