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Quantification of Aspartame in Diet Sodas

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Quantification of Aspartame in Diet Sodas

By

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An Honors Thesis Submitted in Partial Fulfillment
of the Requirements for Graduation from the
Western Oregon University Honors Program

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ABSTRACT

Aspartame is an artificial sweetener much like high fructose corn syrup but is synthetically made compared to being derived from a naturally occurring compound. Many diet beverages and foods contain this chemical since it is about 200 times sweeter than regular table sugar and contains very few calories. Some people with a hereditary disease cannot metabolize the phenylalanine resulting from the breakdown of the aspartame. This, and potential health risks, have caused widespread controversy regarding the FDA approval of aspartame. I have used High Performance Liquid Chromatography (HPLC) to quantify the amount of aspartame present in Diet Coke and Diet Dr. Pepper. I investigated how elevated temperatures can cause aspartame to become unstable and breakdown into potentially toxic chemicals. These findings will promote the labeling of actual amounts present in each drink, similar to how caffeine is labeled.

INTRODUCTION

Aspartame is a synthetic sweetener known by the trade name Nutrasweet® that has been FDA approved since 1981.¹ It is currently sold in over one hundred companies, with more than 250 million people consuming this chemical. Public concern over aspartame's toxicity has caused this chemical to be one of the most researched compounds used in food products. This substance is a member of the aniline group and is made of two amino acids – phenylalanine and aspartic acid, as well as methyl alcohol, more commonly known as methanol or wood alcohol. Studies have been and continue to be conducted on the safety, manufacturing, and quantification of aspartame as well as the adverse effects on people who have certain allergies to phenylalanine, a compound that is broken down from aspartame in the body. Main categories that are expanded in research include the use of aspartame in place of other artificial sweeteners along with the role of this chemical in diet and/or sugar-free foods. Almost any type of chewing gum found in the United States contains aspartame; since most diet sodas contain aspartame, a new problem that has occurred is when these companies replace aspartame with saccharin or other sweeteners in their diet beverages. Before switching fully to the new artificial sweetener, they will continue to use aspartame but have bottled their product in new labelling.² This situation can cause a major concern for people who are trying to avoid phenylalanine and aspartame, and can also be problematic in a legal standing.

HISTORY

Aspartame was originally discovered in a research laboratory owned by G.D. Searle in 1965. James Schlatter was working on synthesizing a tetrapeptide to block a gastrointestinal hormone.¹ During one step of the synthesis, Mr. Schlatter was recrystallizing the aspartylphenylalanine methyl ester (aspartame) and accidentally got some of the substance on his fingers. Later on during the experiment he went to lick his finger to pick up a paper and noticed a strong sweet taste. With this accidental discovery, a new low-calorie, artificial sweetener was created. It has 150-200 times the sweetness of sucrose and does not have the bitter aftertaste that other artificial sweeteners are known for.

The Searle company owned the rights to aspartame until they were bought out by Monsanto in 1985, two years after the FDA approved aspartame for use in carbonated beverages.³ For some consumers, they are wary when they hear that Monsanto, a company known for producing a series of powerful pesticides, bought out the company that created a popular artificial sweetener. This ownership did not affect what aspartame was known for or used for in the following years. In 2000 Monsanto sold the Nutrasweet brand name to J.W. Childs Equity Partners. Production of aspartame has continued to the present with this company.⁴

Aspartame is created using two amino acids L-phenylalanine methyl ester and L-aspartic acid. These amino acids are not known to be sweet so the taste of aspartame was not something predicted. Only the LL diastereoisomer contained the sweetness; LD, DL, and DD were slightly bitter.⁵ When L-phenylalanine and L-aspartic are coupled, they create

the dipeptide methyl ester. If this is done chemically for reproduction purposes, there will be two types of aspartame present in the final product: α -aspartame and β -aspartame. Only the alpha (α) form contains the intense sweetness.¹ In all my research, I was unable to find the difference between the two types besides the sweetness. This chemical is approximately 40% aspartic acid and over half of the aspartame molecule is phenylalanine.⁵ Phenylalanine is an essential part of our daily intake since we cannot synthesize it ourselves. A small amount of phenylalanine is essential for survival but these values differ for each person. In this case, aspartame does provide an amino acid that we use in many bodily functions, however, phenylalanine can be absorbed when eating proteins including meat.

Unless it is exposed to aqueous solutions, aspartame is very stable and only breaks down when exposed to elevated temperatures.¹ According to the MSDS all potential chronic health effects (carcinogenic, mutagenic, teratogenic and developmental toxicity) are not available.⁶

There are many various ways to detect aspartame in food and drink, both qualitatively and quantitatively. Most researchers agree that identifying aspartame quantitatively is a more productive mean than just identifying aspartame that is present in the substance. The two main ways to determine quantitative and qualitative measurements is through the use of absorption spectra and Chromatography techniques using various instruments. Some are more efficient than others, and yet still others contain factors that may hinder the accuracy of the results.² High Performance Liquid Chromatography (HPLC) is the technique most commonly used and the Center for Disease Control (CDC) has used this

technique for their procedure on quantifying the aspartame. In the research section of this thesis, I will modify their procedure to fit my own analysis.

In the 1970s, various researchers from the Food and Drug Administration (FDA) estimated that the maximum consumption of aspartame to be anywhere from 22-34 mg/kg of body weight (bw) per day. So for a 140 pound person, a range of 1,397- 2,159 mg of aspartame could be consumed in a single day. Now this information is based on replacing all sucrose with aspartame in this person's diet. The actual daily consumption is less than that.¹ Currently the FDA has allotted 50 mg/kg bw per day as the Allotted Daily Intake (ADI). For a 150 lb. person, this would equate to roughly 3400 mg of aspartame in a single day.

There has been little research done on how fast aspartame breaks down in aqueous solutions. The research that has been done focuses on toxicity and possible neurological problems arising from the intake of aspartame in various mammals including humans.⁵ All of the results presented to the FDA have stemmed from the original controversy surrounding the FDA's approval of aspartame.

CONTROVERSY

Originally, the FDA approved aspartame in 1974 for dry foods. After an in depth analysis of the fifteen studies the Searle's research team conducted, there was a proposed hypothesis that aspartame could have the potential to cause brain tumors based upon rat studies.¹ For the first time in FDA history, an independent group of scientists evaluated a complaint against an FDA approved substance. A Public Board of Inquiry was created to

handle this issue. The approval was revoked by the board because of questionable practices done by the Searle's research team. Searle's company had been fighting to get FDA approval and the methods they used to present their findings were deemed inconsistent.

In January of 1981, Ronald Reagan took office and with him, Dr. Arthur Hull Hayes Jr. was selected to be the new FDA Commissioner by Donald Rumsfeld, CEO of G.D. Searle and former secretary of defense. A commissioner's panel was established after the ruling from the Public Board of Inquiry. Dr. Hayes played a key role in the passing of aspartame. Not only did he personally appoint a sixth member to the commissioner's panel to tie the vote, he also made the tie breaking decision. This was how the FDA approved the use of aspartame in dry goods in 1981.⁷ Soon after, a petition was put in to approve aspartame in the use of diet sodas. In 1983 the FDA approved aspartame for the use in carbonated beverages. In a hearing before the committee on labor and human resources, the health and safety concerns of aspartame were examined. In this discussion it was mentioned that over 3,500 complaints regarding soft drink consumption were analyzed.⁸

Aspartame breaks down into aspartic acid, phenylalanine, and methanol. From there further degradation occurs and formaldehyde and formic acid are produced. According to the Environmental Protection Agency (EPA) formaldehyde is classified as a probable carcinogen when high or long exposures occur with humans. While aspartame is one of the most thoroughly tested additives of all time, these products have been shown to cause harm to humans.⁹

One of studies first presented to the FDA during the approval process had shown that rats given large doses of aspartame had elevated cancer rates than control rats. This was brought into the public's eye in the 1970s. Another study, conducted by the G.D. Searle research team, followed behavioral patterns of neonatal monkeys that were given large doses (0-3 g aspartame/kg bodyweight). These scientists were looking for seizure susceptibility increasing from the chemical. While the monkeys showed no difference between the control group as stated in the hearing for the Labor and Human Resources Committee, nothing was stated about the physical conditions of the monkeys.⁸ According to Rich Murray, out of the seven monkeys fed aspartame, one died and two others did not finish the prescribed number of days of testing.^{3,10} While nothing this severe has appeared in humans, other symptoms that can cause problems have been reported.

H.J. Roberts, an M.D., has been monitoring people's health over the years. His focus was on hypoglycemia or low blood sugar attacks and originally believed that aspartame was a useful product to give his patients. One of his patients named Tammy began to follow his directions when he identified hypoglycemia to be her problem with strange convulsions. After putting her on a set schedule of food to stop the hypoglycemic attacks from occurring, she began to have a seizure. He was then informed that some of the food he was having her take contained aspartame in it. After being told that her grandmother had adverse reactions to aspartame, he was able to identify that aspartame was the culprit. The seizures stopped altogether after removing aspartame from her diet.⁹ H.J. Roberts has had problems finding information about these adverse side effects when consuming aspartame. From my own literary research, there are very few journals that contained

information on the hazards of aspartame. Most of these findings state that aspartame is inherently safe for human consumption and that there can be side effects in individuals who have Phenylketonuria (PKU) also known as Folling's disease. This is a genetic disorder where the human body is unable to break down phenylalanine.

According to H.J. Roberts, women outweigh men when reporting complaints about aspartame. It is unknown if this is just that more women have added complaints to the FDA or whether there are more women affected by aspartame. Another angle could be that more women use diet or sugar-free foods and beverages than men. This author adds many examples and quotes from people he has interviewed based upon the information provided by the FDA.⁹

One of the largest complaints to the FDA when consuming aspartame-laden food and beverages is the onset of headaches. Many of the manufacturers of Nutrasweet® have pointed to scientific studies that discredit this complaint. One of these studies was conducted by Dr. Susan S. Schiffman. They had 40 participants that had previous symptoms when exposed to aspartame. Her results found no difference between headaches occurring in patients that ingested aspartame versus those that ingested a placebo. When these findings were published, this became a topic for debate. The capsules used to provide the aspartame are only able to be 50% absorbed which can skew the resulting data and that the phenylalanine present in blood levels was not measured.¹¹

Most studies that were conducted in the early years after the FDA approval of aspartame were on animal subjects. This in itself can be a possible flaw in the study since testing did

not occur on the actual species in question. I am not stating that we should only do human testing, but some animal testing could be less reliable for human research.

The writers who are against aspartame rely heavily on consumer complaints and testimonies regarding their own reactions with aspartame. On the other side, B. A. Magnuson who has critically evaluated this artificial sweetener according to the FDA's maximum ADI and human subjects for testing. Overall he found that 90% of people do not have any adverse reactions towards aspartame-laden products.¹ The FDA also reports that individuals can have adverse reactions to any compound and since the majority of people do not have such reactions, that this compound is safe for human consumption.¹²

The common theme occurring in these cases is that the individual did consume large amounts of aspartame before their symptoms began. Once they stopped their intake, the symptoms either disappeared or gradually left the person. Since there are roughly 1 in 15,000 people who have the genetic disease PKU, these cases may be related to the disease.⁹ In the seizure example stated previously, it was found out that the young girl did have phenylalanine allergies in her family history and this was the apparent cause of her reaction. Not all of the examples given for these reactions contained information on the patient's family history. In general, most of the examples of problems with aspartame were caused by an abnormally large consumption of food and beverages containing the aspartame. This could be in the range of 5+ diet drinks, and additional food items that also contained aspartame per day. Each individual person will react differently to aspartame; some will have no reaction, some will have severe reactions, and others may have slight issues.

It is easy to find many articles and books written on the problems that aspartame has caused, however, there are others that have conclusions where aspartame is not inherently bad for human consumption. The people most easily susceptible to the aspartame reactions are ones who have PKU. These people not only need labels on items that contain phenylalanine, but they should also have the amounts present in the item as well. Currently, the amount of aspartame present in pharmaceuticals is labelled. I believe that this should become a requirement for food and beverage labels as well so that consumers will be able to identify just how much aspartame they are consuming in a day.

ASPARTAME AND HEAT PROCEDURE I :

Introduction:

This experiment is used to determine the concentrations of aspartame present in two major diet sodas: Diet Pepsi and Diet Dr. Pepper while being exposed to elevated and room temperatures.

Theory:

According to multiple sources, aspartame breaks down into three chemicals when exposed to high temperatures: aspartic acid, methyl alcohol, and phenylalanine. This experiment will serve to test this hypothesis and the results will identify just how much breakdown occurs.

A simple calibration curve is constructed using known concentrations of aspartame in water. The overall area is taken from each of the aspartame peaks of bottle and can

samples for both control and test. These numbers are then used in the calibration curve equation to calculate a concentration.

According to a source¹³, roughly 125 mg and 123 mg of aspartame is present in 8 fluid ounces of diet Coke and diet Dr. Pepper respectively. From my calculations, the concentrations should be roughly 1.79×10^{-3} mol/L and 1.77×10^{-3} mol/L for each of the samples. This will be the concentration I will be looking for when testing occurs. If the concentration is less for the heated samples then I will assume that the high temperatures degraded the aspartame samples; if the concentrations are higher, more tests will need to be run before any action can be taken to let the Coke Company know their figures are off regarding the amount of aspartame present in their diet soft drinks.

A procedure provided by the CDC was used to create the procedure for the quantification of aspartame. The CDC's procedure called for a flow rate of 1-3 L/min. This was changed to 2 mL/min.¹⁴

Equipment:

Hitachi L-4250 UV-VIS Detector HPLC

Hitachi L-6200 Intelligent Pump

Thermo Scientific Heratherm OGS100 General Lab Oven

Parafilm

(2) 1 L Erlenmeyer flasks

(1) 1 L volumetric flask

(8) 150 mL beakers

(2) 50 mL graduated cylinders

(1) funnel

Metal spatulas

Rubber stoppers

Stir bar

pH meter

Vacuum

Corks

Reagents:

1. Aspartame

2. Water, deionized

3. Acetonitrile

4. Methanol

5. 1-Heptanesulfonic acid sodium salt (Reagent for Ion-Pair Chromatography)

6. Monobasic potassium phosphate

7. Phosphoric acid

Procedure:

Each of the 8, 150 mL beakers was labelled according to what sample they contained (diet Coke can, diet Coke bottle, diet Dr. Pepper can, and diet Dr. Pepper bottle). All diet soda containers were decarbonated before testing began. Two beakers were filled with 100 mL of diet Coke from a can and covered with Parafilm. This was repeated with diet Coke from a bottle, and diet Dr. Pepper from a can and a bottle. One of each sample was placed into a Heratherm OGS100 General Lab Oven set at 50 °C. The other four samples were left at room temperature. Samples were subjected to these temperatures for 37 days.

To create Eluent A: 2.062 g of 1-Heptanesulfonic acid sodium salt and 0.453 g monobasic potassium phosphate were added to 1 L of deionized water. The pH was originally 4.6 but was adjusted to 2.6 using diluted phosphoric acid.

To create Eluent B: 2.063 g of 1-Heptanesulfonic acid sodium salt to 1 L of a 3:2 (v/v) acetonitrile-water solution. The pH was adjusted to 3.1 using diluted phosphoric acid.

Because of the various chemicals being run through the HPLC, the system was purged using HPLC-grade methanol for 30 minutes before any experimentation began. Then I repurged the system using the Eluent A and B before standards were run. The mobile phase consisted of 60/40 Eluent A to Eluent B. Since only one of our solvent tubes is working properly, I had to mix the two eluents together to create the mobile phase.

The mobile phase was degassed before being used and a flow rate of 2.00 ml/min was used. To make sure the concentrations were not too high or too low 1.7×10^{-4} M of aspartame was tested as a standard. 10 μ L of standard was injected into the system. I let the system run for ten minutes and no variation from zero occurred. Instead a higher concentration of aspartame (0.005 M) was made. This again provided no results so this procedure was scrapped altogether and a new procedure that has been used in a previous chemistry lab was used.

Calculations:

I found the amount of aspartame present in 8 fluid ounces of both diet Coke and diet Dr. Pepper and used this data to calculate the estimated concentration of aspartame in a 12 oz can and a 16.9 oz bottle of diet soda.

Diet Coke	
8 fl. oz.	125 mg
12 fl. oz. can	187.50 mg
16.9 fl. oz. bottle	264.06 mg
Concentration (M)	$1.79 \cdot 10^{-3}$
Diet Dr. Pepper	
8 fl. oz.	123 mg
12 fl. oz. can	184.5 mg
16.9 fl. oz. bottle	259.84 mg
Concentration (M)	$1.77 \cdot 10^{-3}$

Table 1

Given these values, I was able to calculate a rough estimate on the concentration of aspartame in both Diet Coke and Diet Dr. Pepper: $1.79 \cdot 10^{-4}$ mol/L and $1.77 \cdot 10^{-4}$ mol/L respectively.

Results

As you can see from the screenshots captured, there was no logical data collected. Originally data corresponding to retention time and voltage was being collected and the peak area and retention time would be used to identify aspartame. The data being collected not match with the provided aspartame peak so therefore the data was inconclusive. Eventually continuous runs provided no results whatsoever as shown in the last two screenshots. This was deemed a failure and another procedure was used to complete the experiment.

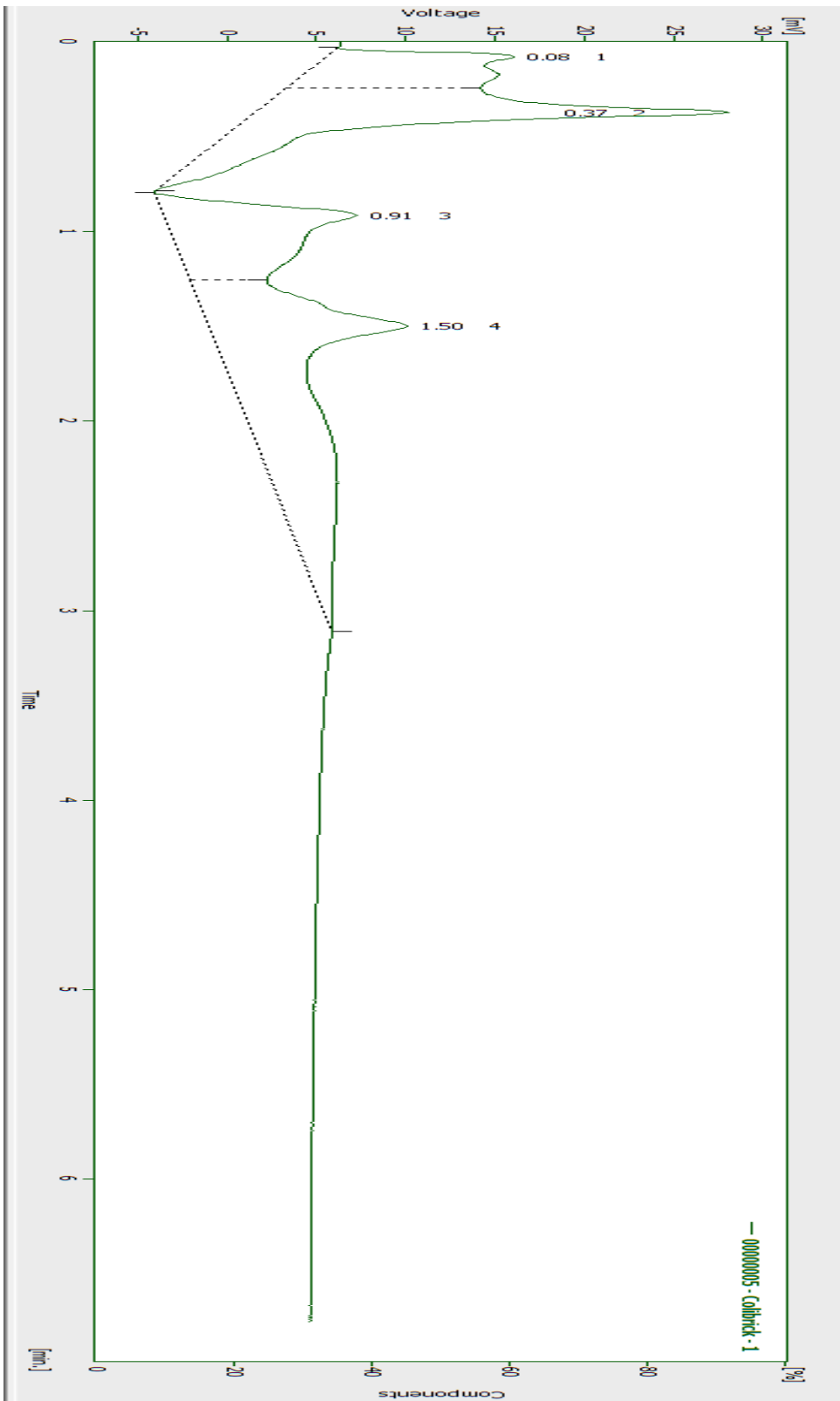


Figure 1:

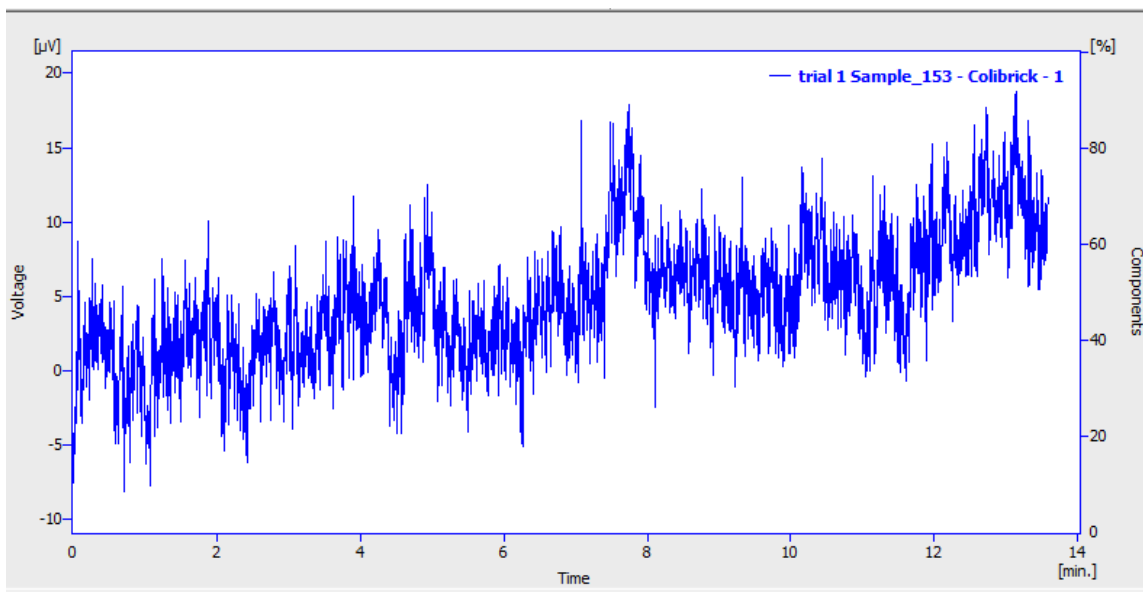


Figure 2

This is the graph of 0.005 M of aspartame. Instead of nice distinct peaks, what looks like a seismograph was instead recorded.



Figure 3

This screenshot shows that after 20 minutes of running the 0.005 M aspartame solution through the HPLC, there were no peaks and no data was collected.

POSSIBLE ERRORS

Age of monobasic potassium phosphate

Having to mix Eluent A and B to create the mobile phase

Age of aspartame

Wrong procedure used for the HPLC accessible

ASPARTAME AND HEAT PROCEDURE II:

Theory:

The theory is identical to the previous procedure. This is a new procedure to be used in the experiment.

Equipment:

Hitachi L-4250 UV-VIS Detector HPLC

Hitachi L-6200 Intelligent Pump

Thermo Scientific Heratherm OGS100 General Lab Oven

Parafilm

Stir bars

10 mL beakers

0.45 μm ptfе filters

syringe

Procedure

Before any samples or standards were run, the HPLC was set to have a flow rate of 1.5 mL/min and the detector wavelength was set to 254 nm.¹⁵ A mobile phase of 80/20 1 M acetic acid and methanol was made and degassed before being used to purge the HPLC

column. 0.200 g of aspartame was added to 100 mL the 80/20 mobile phase. 10 μ L of this solution was injected into the HPLC and the peak was recorded as Standard 1. Four standards were created as shown in Table 3 and 10 μ L of each standard was injected into the HPLC. Two tests were run for each sample and the corresponding areas were averaged to create a calibration curve.

Each of the 8, 150 mL beakers was labelled according to what sample they contained (diet Coke can, diet Coke bottle, diet Dr. Pepper can, and diet Dr. Pepper bottle). All diet soda containers were decarbonated before testing began. Two beakers were filled with 100 mL of diet Coke from a can and covered with Parafilm. This was repeated with diet Coke from a bottle, and diet Dr. Pepper from a can and a bottle. One of each sample was placed into a Heratherm OGS100 General Lab Oven set at 50 °C. The other four samples were left at room temperature. Samples were subjected to these temperatures for 37 days.

Roughly 6 mL of each of the 8 samples were filtered through 0.45 μ m PTFE-membrane filters before 10 μ L of solution was injected into the HPLC. Following the procedure, the aspartame peak should appear around 5-7 minutes if the mobile phase pH is 3-5.

Key Abbreviations

DCC – Diet Coke Can

DCB – Diet Coke Bottle

DDPC – Diet Dr. Pepper Can

DDPB – Diet Dr. Pepper Bottle

DCCH – Diet Coke Can Heat

DCBH – Diet Coke Bottle Heat

DDPCH – Diet Dr. Pepper Can Heat

DDPBH - Diet Dr. Pepper Bottle Heat

Calculations

Calculating standard molarity:

$$\frac{1}{\left(\frac{294.31 \frac{g}{mol}}{0.200 g}\right) * (0.100 L)} = 6.80 * 10^{-3} \text{ mol/L}$$

Calculating sample concentration using calibration curve:

$$y = 22788x$$

Sample DCC peak area = 26.51 mV*s

$$26.51 = 22788x$$

$$x = 0.001163 \text{ mol/L}$$

Calculating mg aspartame/can

$$= 1.163 * 10^{-3} \left(\frac{\text{mol}}{\text{L}} \right) * 294.31 \left(\frac{\text{g}}{\text{mol}} \right) * 1000 \left(\frac{\text{mg}}{\text{g}} \right) * 0.029574 \left(\frac{\text{L}}{\text{fl.oz.}} \right) * 12 \left(\frac{\text{fl.oz.}}{\text{can}} \right)$$

$$= 121.47 \text{ mg/can}$$

Sample DCCH peak area = 4.66 mV*s

$$4.66 = 22788x$$

$$x = 0.000204 \text{ mol/L}$$

Calculating mg aspartame/can heat

$$= 2.04 * 10^{-4} \left(\frac{\text{mol}}{\text{L}} \right) * 294.31 \left(\frac{\text{g}}{\text{mol}} \right) * 1000 \left(\frac{\text{mg}}{\text{g}} \right) * 0.029574 \left(\frac{\text{L}}{\text{fl.oz.}} \right) * 12 \left(\frac{\text{fl.oz.}}{\text{can}} \right)$$

$$= 21.31 \text{ mg/can}$$

Sample DDPB peak area = 38.54 mV*s

$$38.54 = 22788x$$

$$x = 0.001691 \text{ mol/L}$$

Calculating mg aspartame/bottle heat

$$= 1.691 * 10^{-3} \left(\frac{\text{mol}}{\text{L}} \right) * 294.31 \left(\frac{\text{g}}{\text{mol}} \right) * 1000 \left(\frac{\text{mg}}{\text{g}} \right) * 0.029574 \left(\frac{\text{L}}{\text{fl.oz.}} \right) * 16.9 \left(\frac{\text{fl.oz.}}{\text{bottle}} \right)$$
$$= 248.74 \text{ mg/bottle}$$

Sample DDPBH peak area = 6.1 mV*s

$$6.1 = 22788x$$

$$x = 0.000268 \text{ mol/L}$$

Calculating mg aspartame/bottle heat

$$= 2.68 * 10^{-4} \left(\frac{\text{mol}}{\text{L}} \right) * 294.31 \left(\frac{\text{g}}{\text{mol}} \right) * 1000 \left(\frac{\text{mg}}{\text{g}} \right) * 0.029574 \left(\frac{\text{L}}{\text{fl.oz.}} \right) * 16.9 \left(\frac{\text{fl.oz.}}{\text{bottle}} \right)$$
$$= 39.42 \text{ mg/bottle}$$

Results

Standard	Retention time (min)	Concentration (M)	Area (mV*s)	Avg. Area (mV*s)
1	9.55	6.8×10^{-3}	154.141	158.447
	9.03		162.753	
2	7.71	5.0×10^{-4}	21.365	16.863
	7.81		12.361	
3	7.31	5.0×10^{-3}	57.152	109.098
	6.74		161.044	
4	7.64	1.0×10^{-3}	21.055	20.514
	7.62		19.973	

Table 2

Samples	Retention Time (min)	Area (mV*s)	Concentration (M)	Concentration (mg/can or mg/bottle)
DCC	7.62	26.51	0.001163	121.47
DCB	7.59	29.71	0.001304	191.81
DDPC	7.58	25.92	0.001137	118.76
DDPB	7.44	38.54	0.001691	248.74
DCCH	7.80	4.66	0.000204	21.31
DCBH	7.80	3.72	0.000163	23.98
DDPCH	6.80	184.13	0.008080	843.93
DDPBH	7.78	6.1	0.000268	39.42

Table 3

Standard Results

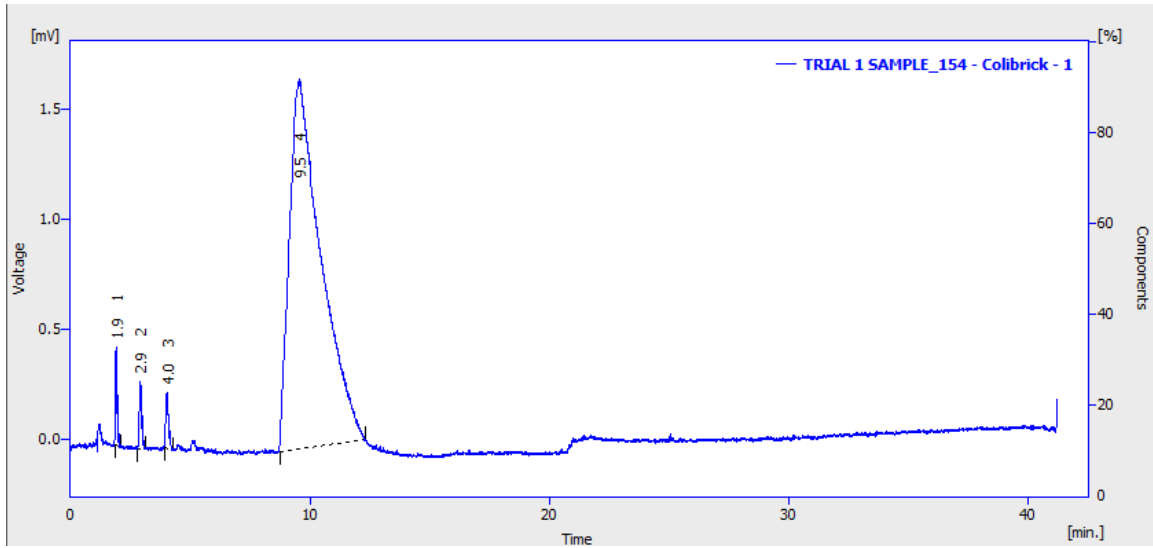


Figure 4: Standard 1 Run 1 Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_154 - Colibrick - 1)							
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.908	2.058	0.446	1.3	16.6	0.08	
2	2.900	1.911	0.307	1.2	11.4	0.10	
3	4.040	1.997	0.253	1.2	9.4	0.12	
4	9.548	154.141	1.680	96.3	62.5	1.44	
	Total	160.107	2.686	100.0	100.0		

Figure 5: Standard 1 Run 1 Result Table

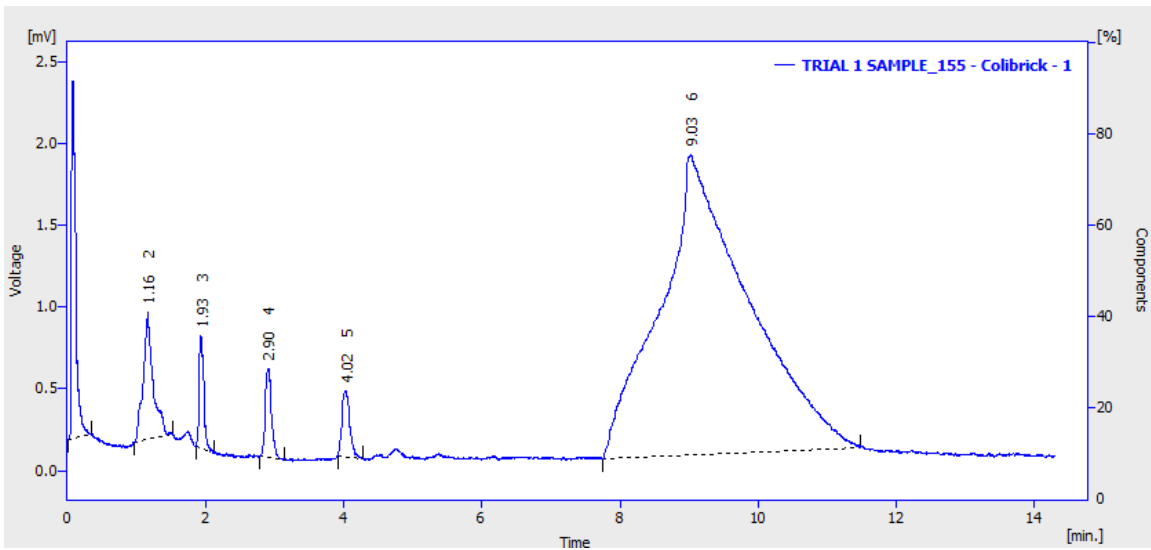


Figure 6: Chromatogram of Standard 1 Run 2

Result Table (Uncal - TRIAL 1 SAMPLE_155 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.076	8.950	2.188	4.7	34.0	0.06	
2	1.164	7.654	0.782	4.0	12.1	0.12	
3	1.932	3.197	0.688	1.7	10.7	0.08	
4	2.904	3.421	0.542	1.8	8.4	0.10	
5	4.024	3.091	0.403	1.6	6.3	0.12	
6	9.028	162.753	1.835	86.1	28.5	1.28	
	Total	189.065	6.439	100.0	100.0		

Figure 7: Standard 1 Run 2 Result Table

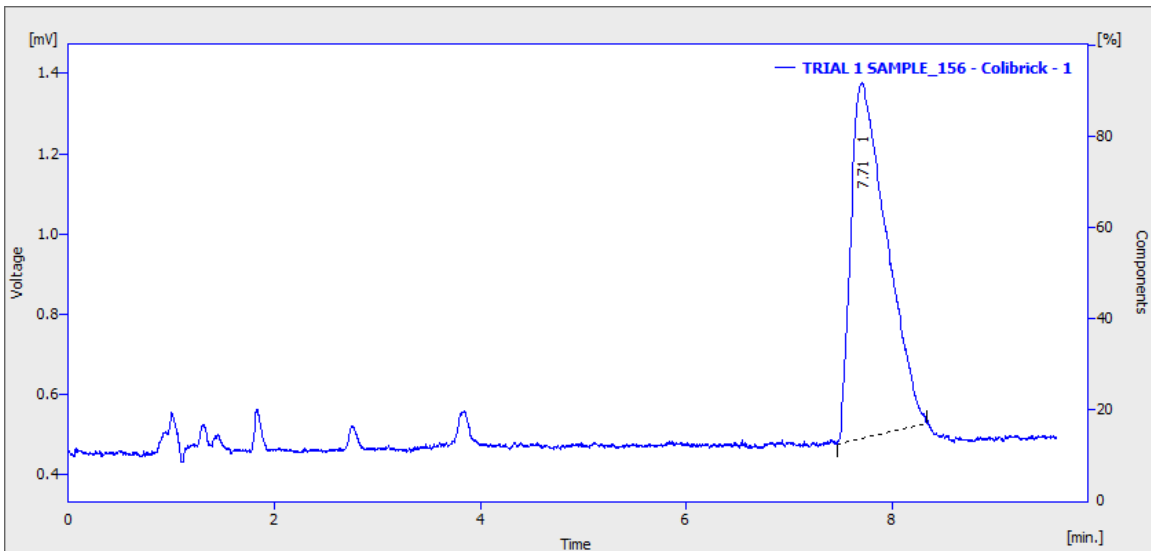


Figure 8: Standard 2 Run 1 Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_156 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.712	21.365	0.886	100.0	100.0	0.40	
	Total	21.365	0.886	100.0	100.0		

Figure 9: Standard 2 Run 1 Result Table

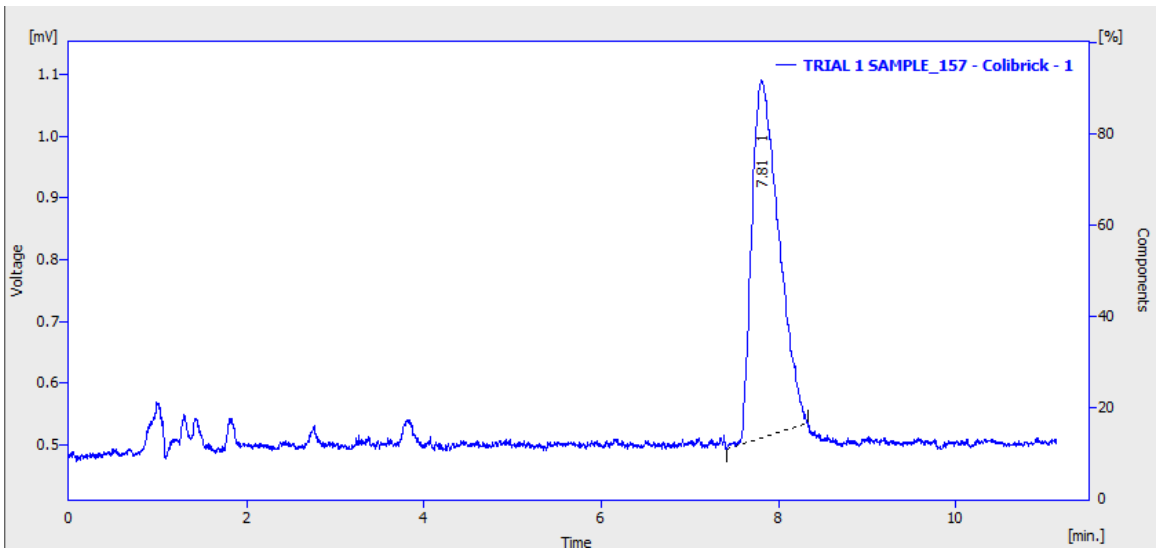


Figure 10: Standard 2 Run 2Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_157 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.808	12.361	0.581	100.0	100.0	0.35	
	Total	12.361	0.581	100.0	100.0		

Figure 11: Standard 2 Run 2 Result Table

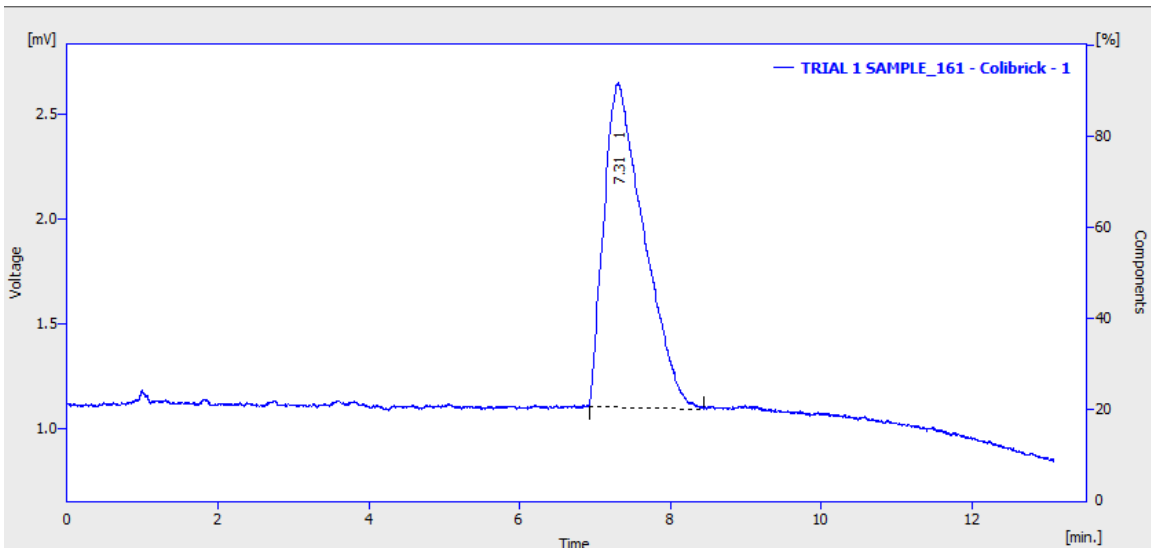


Figure 12: Standard 3 Run 1 Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_161 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.312	57.152	1.546	100.0	100.0	0.59	
	Total	57.152	1.546	100.0	100.0		

Figure 13: Standard 3 Run 1 Result Table

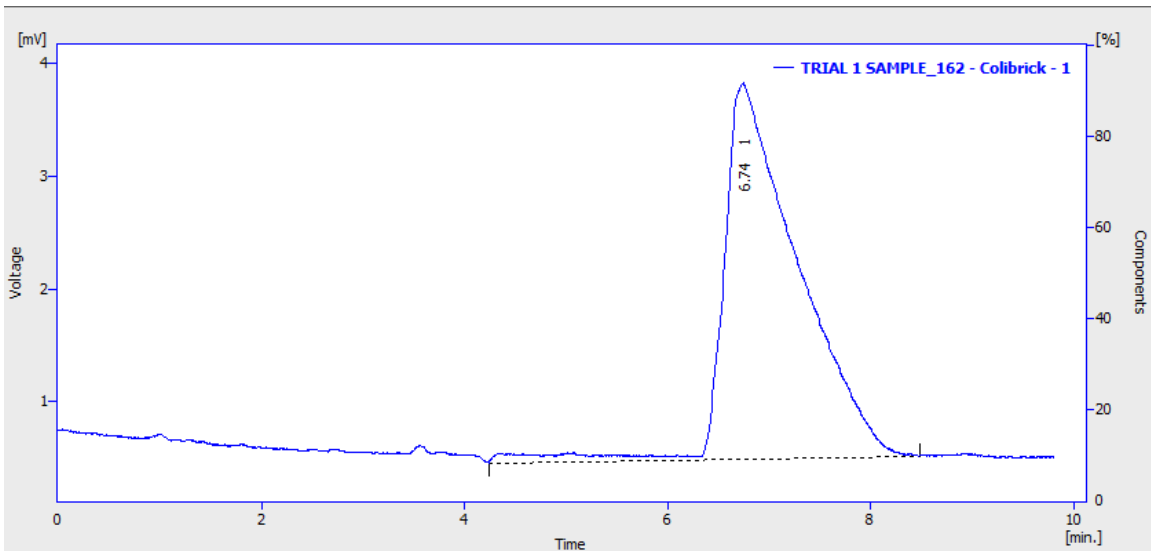


Figure 14: Standard 3 Run 2 Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_162 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.744	168.902	3.324	100.0	100.0	0.76	
	Total	168.902	3.324	100.0	100.0		

Figure 15: Standard 3 Run 2 Result Table

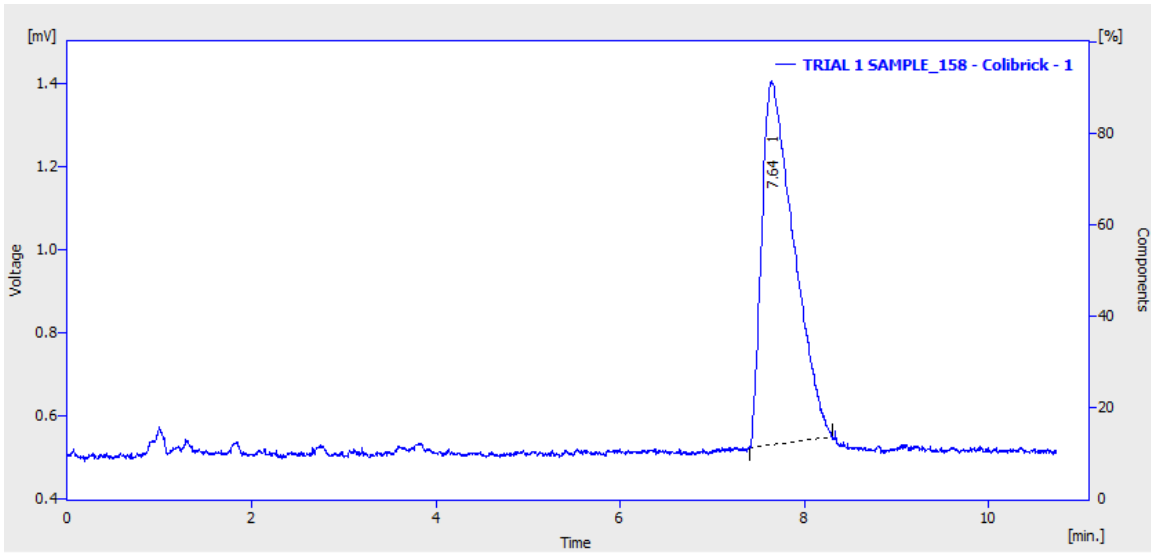


Figure 16: Standard 4 Run 1 Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_158 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.644	21.055	0.877	100.0	100.0	0.39	
	Total	21.055	0.877	100.0	100.0		

Figure 17: Standard 4 Run 1 Result Table

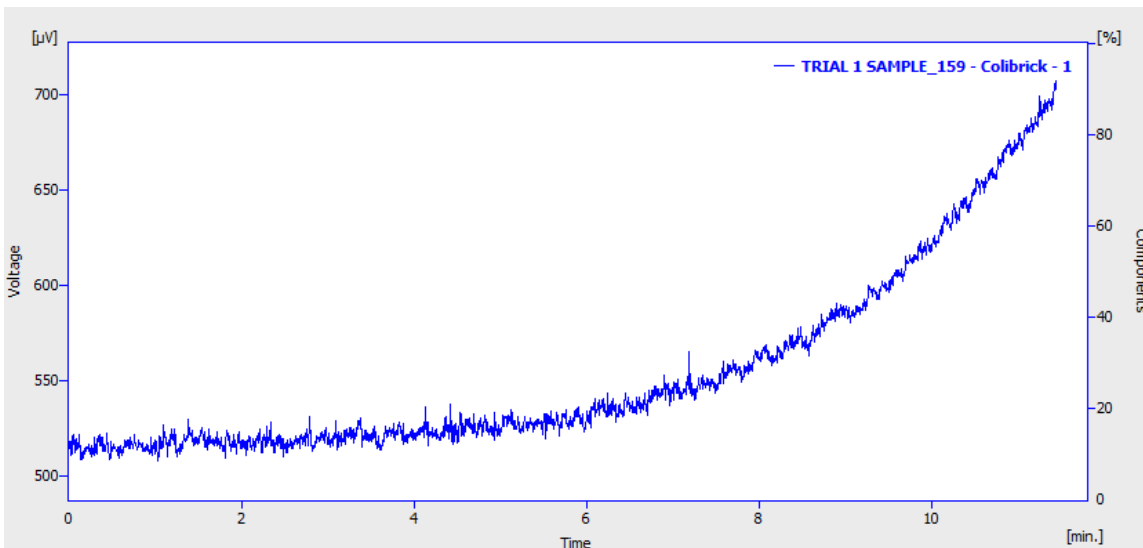


Figure 18: Standard 4 Run 2 Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_159 - Colibrick - 1)							
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
No peak to report							

Figure 19: Standard 4 Run 2 Result Table

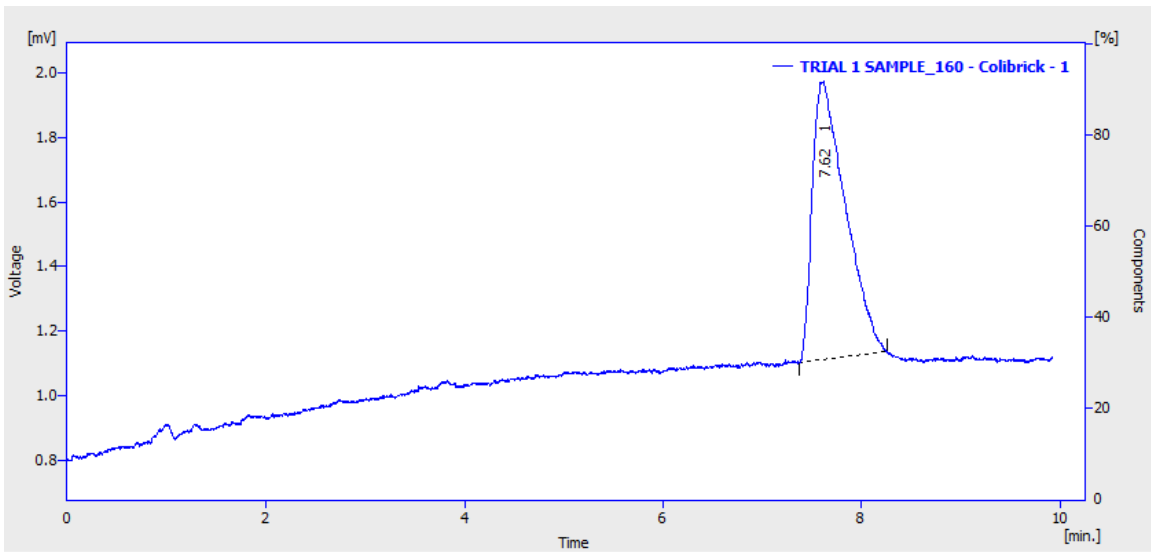


Figure 20: Standard 4 Run 3 Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_160 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.620	19.973	0.862	100.0	100.0	0.38	
	Total	19.973	0.862	100.0	100.0		

Figure 21: Standard 4 Run 3 Result Table

Sample Results

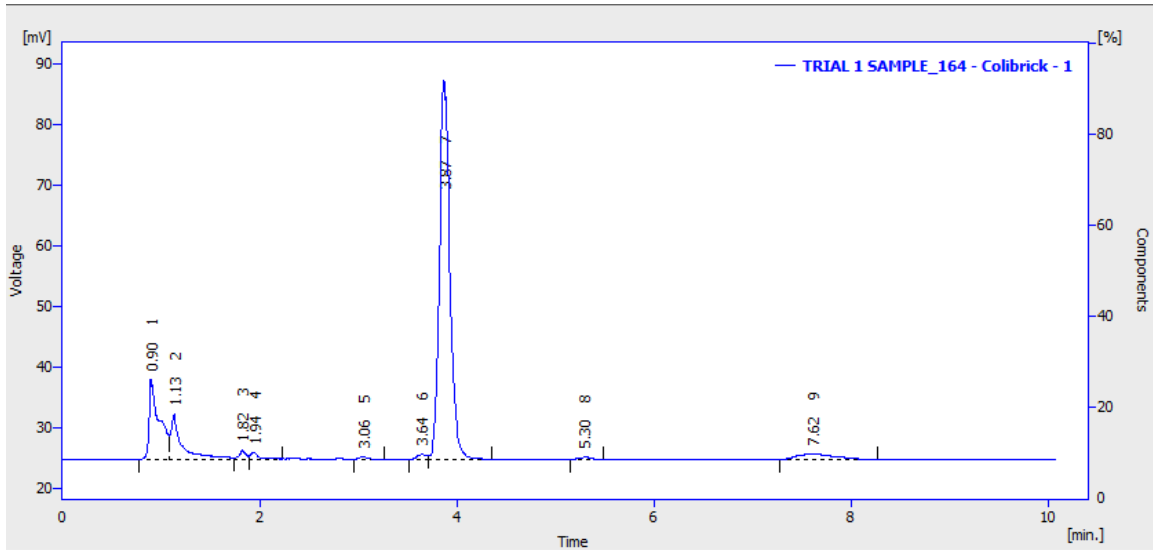


Figure 22: Diet Coke Can Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_164 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.900	98.804	13.303	14.7	15.1	0.08	
2	1.128	61.703	7.426	9.2	8.4	0.09	
3	1.824	6.805	1.444	1.0	1.6	0.07	
4	1.940	5.786	1.040	0.9	1.2	0.08	
5	3.056	2.493	0.431	0.4	0.5	0.09	
6	3.644	5.403	0.781	0.8	0.9	0.13	
7	3.872	461.767	62.246	68.7	70.8	0.12	
8	5.296	3.128	0.348	0.5	0.4	0.14	
9	7.616	26.514	0.937	3.9	1.1	0.47	
	Total	672.404	87.957	100.0	100.0		

Figure 23: Diet Coke Can Result Table

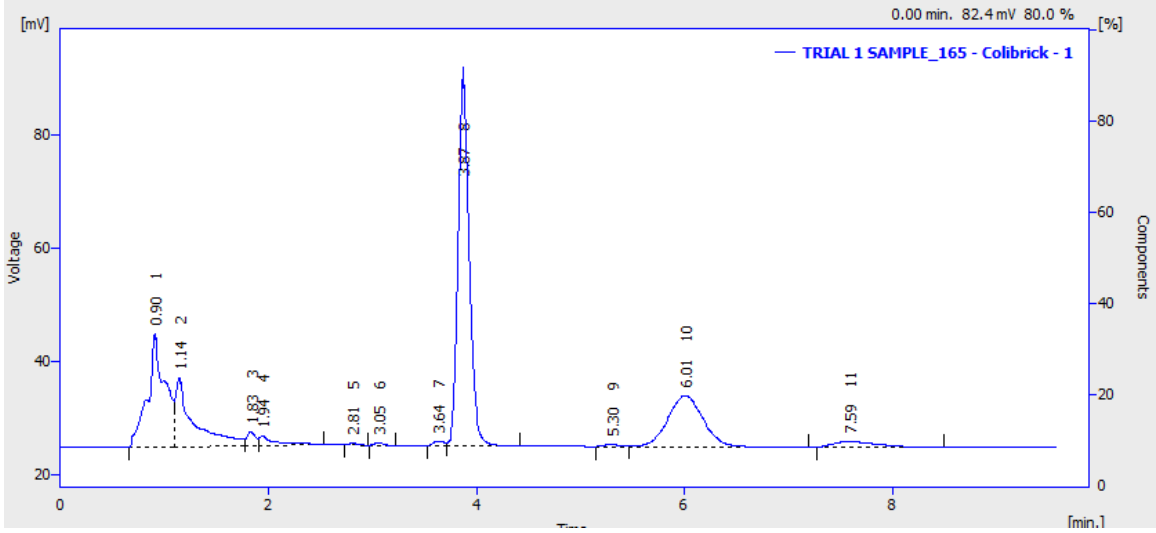


Figure 24: Diet Coke Bottle Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_165 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.900	234.061	20.128	20.2	17.5	0.18	
2	1.140	147.472	12.157	12.7	10.6	0.11	
3	1.832	12.944	2.435	1.1	2.1	0.10	
4	1.944	16.495	1.618	1.4	1.4	0.11	
5	2.812	1.578	0.277	0.1	0.2	0.09	
6	3.052	2.597	0.448	0.2	0.4	0.09	
7	3.636	6.112	0.830	0.5	0.7	0.14	
8	3.872	493.754	66.713	42.5	58.0	0.12	
9	5.304	3.708	0.394	0.3	0.3	0.15	
10	6.008	212.694	9.011	18.3	7.8	0.36	
11	7.588	29.710	1.006	2.6	0.9	0.47	
	Total	1161.125	115.018	100.0	100.0		

Figure 25: Diet Coke Bottle Result Table

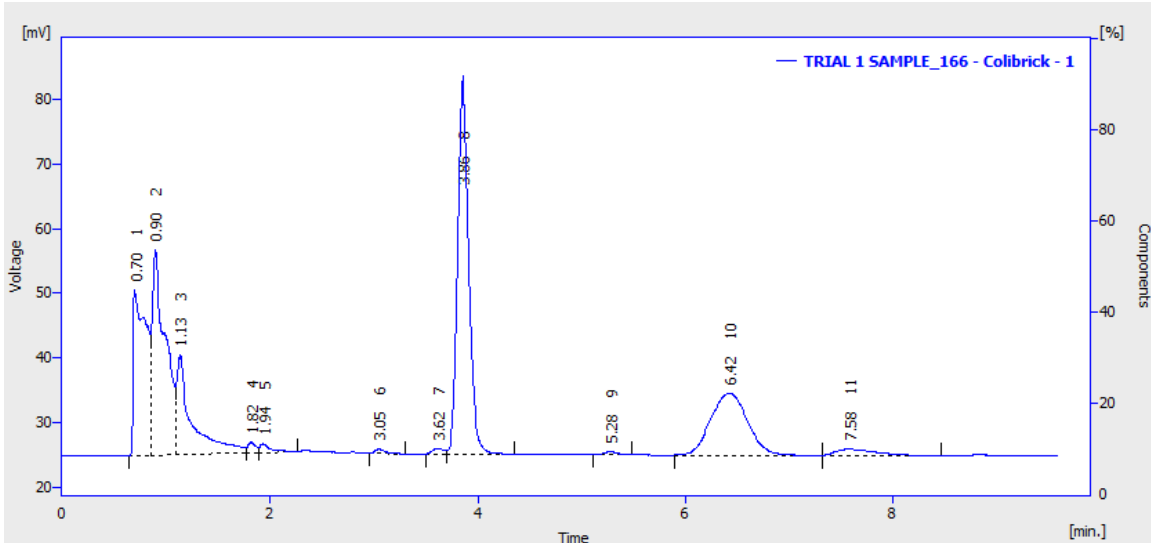


Figure 26: Diet Dr. Pepper Can Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_166 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.700	224.767	25.622	16.2	17.5	0.18	
2	0.896	285.609	31.713	20.5	21.7	0.18	
3	1.132	154.998	15.398	11.1	10.5	0.10	
4	1.820	8.568	1.551	0.6	1.1	0.12	
5	1.940	8.897	1.268	0.6	0.9	0.10	
6	3.052	3.839	0.628	0.3	0.4	0.10	
7	3.620	6.215	0.871	0.4	0.6	0.14	
8	3.856	432.374	58.332	31.1	39.8	0.12	
9	5.280	4.104	0.438	0.3	0.3	0.15	
10	6.424	234.974	9.600	16.9	6.6	0.40	
11	7.576	25.922	0.970	1.9	0.7	0.42	
	Total	1390.267	146.391	100.0	100.0		

Figure 27: Diet Dr. Pepper Can Result Table

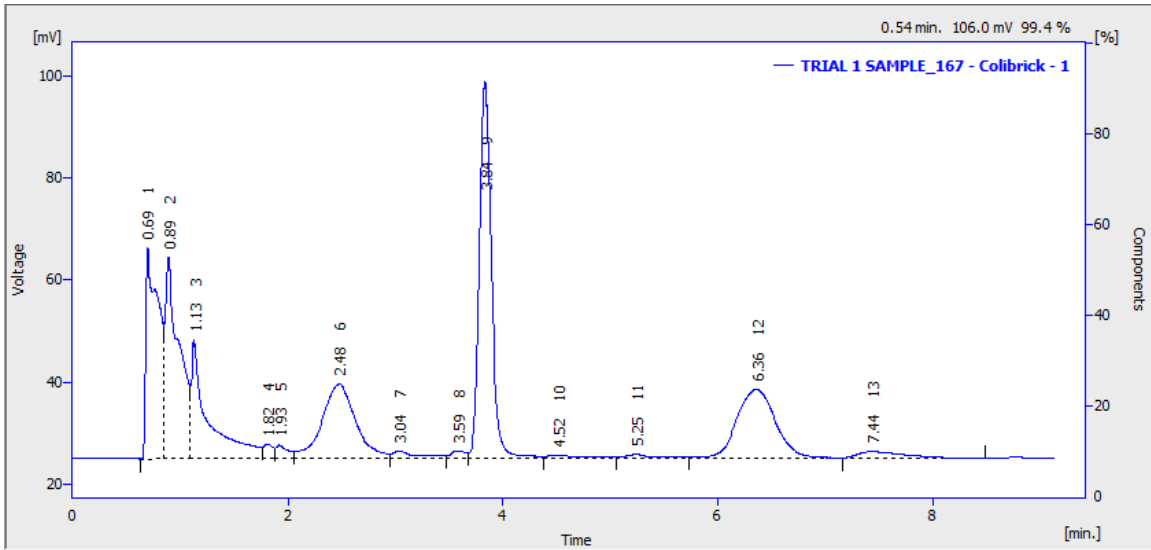


Figure 28: Diet Dr. Pepper Bottle Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_167 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.692	344.396	41.371	14.7	19.0	0.17	
2	0.888	368.004	39.510	15.7	18.1	0.18	
3	1.132	260.180	23.465	11.1	10.8	0.10	
4	1.816	18.026	2.901	0.8	1.3	0.12	
5	1.928	21.915	2.695	0.9	1.2	0.18	
6	2.476	319.380	14.650	13.6	6.7	0.32	
7	3.040	26.269	1.556	1.1	0.7	0.19	
8	3.586	13.769	1.522	0.6	0.7	0.16	
9	3.836	565.959	74.188	24.1	34.0	0.12	
10	4.520	16.957	0.662	0.7	0.3	0.43	
11	5.248	15.692	0.770	0.7	0.4	0.22	
12	6.364	339.515	13.623	14.5	6.2	0.39	
13	7.436	38.536	1.280	1.6	0.6	0.48	
	Total	2348.598	218.192	100.0	100.0		

Figure 29: Diet Dr. Pepper Bottle Result Table

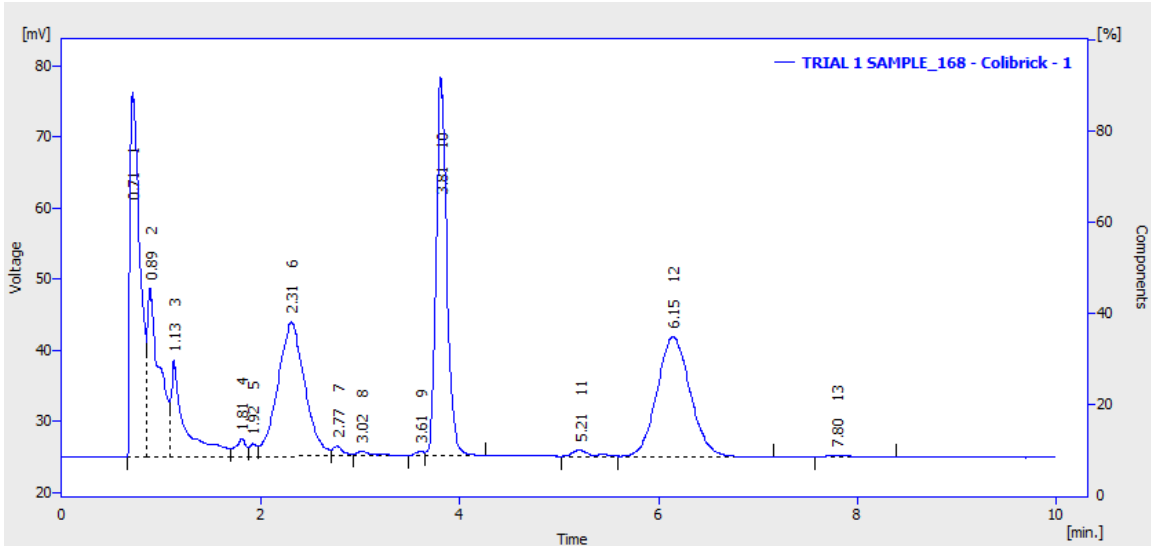


Figure 30: Diet Coke Can Heat Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_168 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.712	346.977	51.341	18.3	27.7	0.11	
2	0.888	200.194	23.762	10.6	12.8	0.16	
3	1.128	133.609	13.585	7.1	7.3	0.10	
4	1.808	18.016	2.526	1.0	1.4	0.15	
5	1.920	9.450	1.804	0.5	1.0	0.10	
6	2.308	356.036	18.894	18.8	10.2	0.29	
7	2.768	10.530	1.357	0.6	0.7	0.13	
8	3.016	7.415	0.670	0.4	0.4	0.12	
9	3.612	3.344	0.552	0.2	0.3	0.10	
10	3.812	400.194	53.077	21.1	28.6	0.12	
11	5.208	11.021	0.885	0.6	0.5	0.16	
12	6.148	392.163	16.832	20.7	9.1	0.36	
13	7.796	4.663	0.285	0.2	0.2	0.25	
	Total	1893.613	185.570	100.0	100.0		

Figure 31: Diet Coke Can Heat Result Table

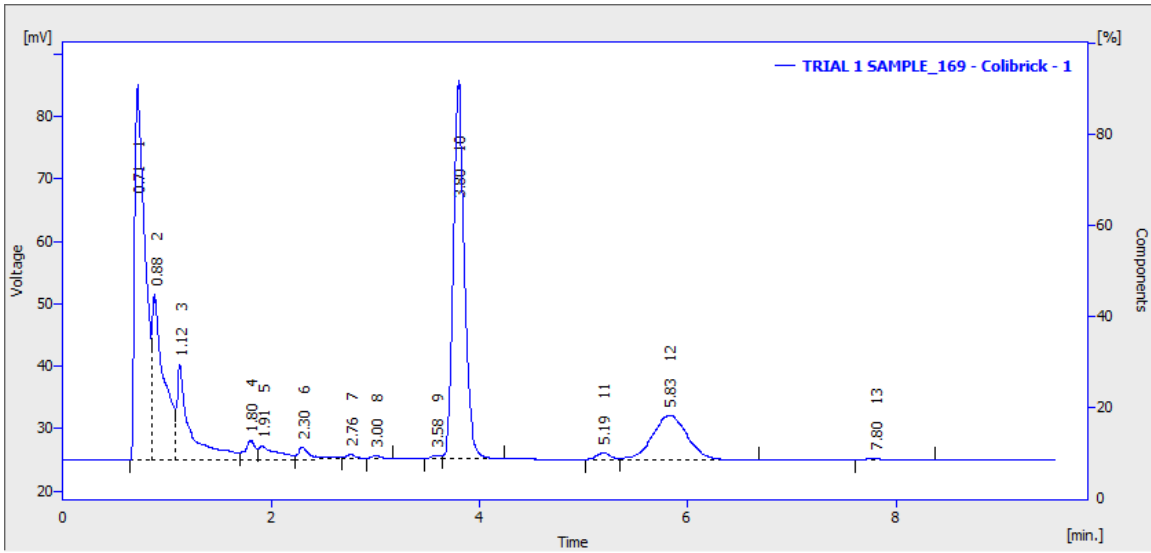


Figure 32: Diet Coke Bottle Heat Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_169 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.712	423.019	60.213	28.6	33.5	0.12	
2	0.880	212.477	26.565	14.3	14.8	0.11	
3	1.124	141.610	15.301	9.6	8.5	0.10	
4	1.800	20.818	3.148	1.4	1.7	0.11	
5	1.912	26.443	2.150	1.8	1.2	0.21	
6	2.300	16.860	1.903	1.1	1.1	0.10	
7	2.760	4.795	0.659	0.3	0.4	0.11	
8	3.004	3.043	0.491	0.2	0.3	0.10	
9	3.584	3.507	0.573	0.2	0.3	0.11	
10	3.796	447.725	60.478	30.2	33.6	0.12	
11	5.192	10.049	1.057	0.7	0.6	0.15	
12	5.828	166.738	7.121	11.3	4.0	0.36	
13	7.800	3.718	0.231	0.3	0.1	0.24	
	Total	1480.803	179.891	100.0	100.0		

Figure 33: Diet Coke Bottle Heat Result Table

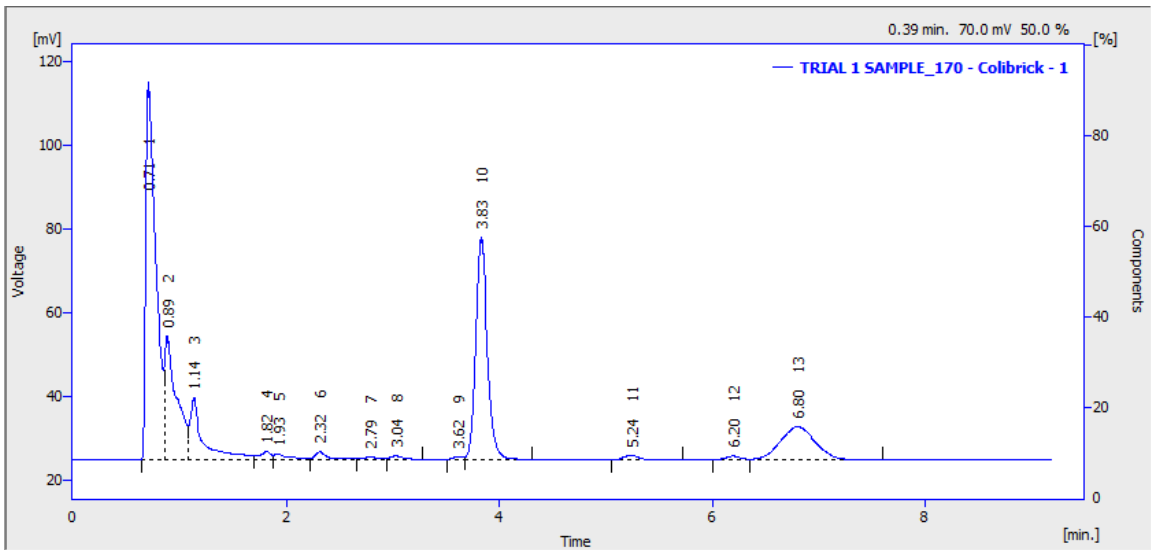


Figure 34: Diet Dr. Pepper Can Heat Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_170 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.712	565.573	90.114	35.4	44.0	0.10	
2	0.888	234.687	29.783	14.7	14.5	0.12	
3	1.136	132.798	14.952	8.3	7.3	0.10	
4	1.816	14.811	2.046	0.9	1.0	0.16	
5	1.928	14.439	1.455	0.9	0.7	0.11	
6	2.320	15.525	1.823	1.0	0.9	0.10	
7	2.788	6.045	0.610	0.4	0.3	0.16	
8	3.036	5.959	0.777	0.4	0.4	0.12	
9	3.616	3.437	0.564	0.2	0.3	0.11	
10	3.832	397.306	52.941	24.9	25.8	0.12	
11	5.240	11.817	1.044	0.7	0.5	0.15	
12	6.196	9.185	0.907	0.6	0.4	0.16	
13	6.800	184.130	7.916	11.5	3.9	0.36	
	Total	1595.713	204.931	100.0	100.0		

Figure 35: Diet Dr. Pepper Can Heat Result Table

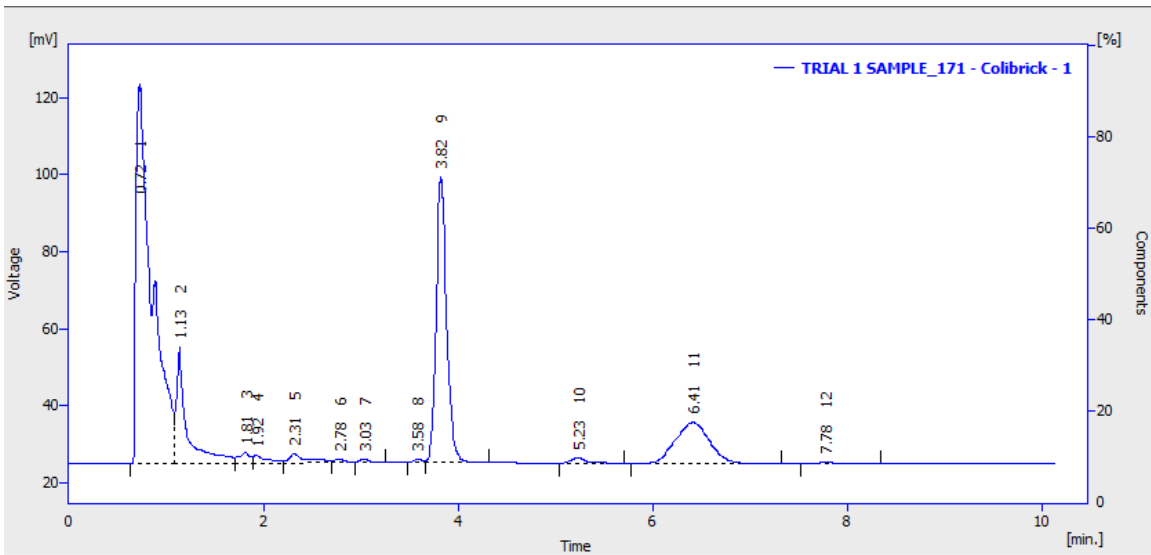


Figure 36: Diet Dr. Pepper Bottle Heat Chromatogram

Result Table (Uncal - TRIAL 1 SAMPLE_171 - Colibrick - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	0.724	1130.756	98.738	49.3	43.8	0.14	
2	1.132	228.466	30.250	10.0	13.4	0.08	
3	1.812	21.232	2.748	0.9	1.2	0.18	
4	1.924	20.982	2.098	0.9	0.9	0.11	
5	2.312	31.029	2.521	1.4	1.1	0.11	
6	2.780	7.318	0.893	0.3	0.4	0.14	
7	3.028	5.819	0.898	0.3	0.4	0.10	
8	3.580	4.800	0.732	0.2	0.3	0.12	
9	3.820	556.881	73.989	24.3	32.8	0.12	
10	5.232	16.786	1.447	0.7	0.6	0.15	
11	6.412	263.649	10.691	11.5	4.7	0.40	
12	7.776	6.095	0.361	0.3	0.2	0.26	
	Total	2293.814	225.367	100.0	100.0		

Figure 37: Diet Dr. Pepper Bottle Heat Result Table

Discussion

Retention time was kept as close as possible since it is necessary to identify compounds being extracted. Standard 1 had the longest retention time of 9 minutes while all others

stayed between 6.5 - 8 minutes. When the soda samples were run, the peaks found in this time range were also identified as aspartame and the peak area was used to calculate aspartame concentration.

According to the manufacturer's information Diet Coke in a can should contain approximately 187.50 mg, while Diet coke in a bottle should have about 264.06 mg. In the case of Diet Dr. Pepper, a can should contain 184.50 mg and a bottle should have 259.84 mg. The results were that all soda samples had less aspartame concentrations than what was listed on the company's website. Aspartame is known to be very stable in solid form, however, aqueous solutions may cause breakdown to occur. This could be the reason why the concentrations did not match well with what each company provided.

The second part of this experiment was researching the effects of aspartame when exposed to prolonged periods of high heat. Since aspartame breaks down in higher temperatures and in aqueous solutions, I hypothesized that less aspartame would be detected in the samples exposed to 50 °C. This again was the answer that the data agreed with. Not only did the aspartame breaks down more than I originally thought it would, there were also two samples that had smaller peaks areas thus lower concentrations than the standards I created. This could be a cause for error to occur. More research should be done.

There are a few errors that did arise during testing. As you can see in Figures 18 and 19, no result table or peak resulted from running Standard 4 (0.001 M) through the HPLC. I believed this might have been human error so a third run was conducted to account for this mishap. Another error occurred when testing the Diet Dr. Pepper can exposed to

heat. The resulting peak area was 30 times that of the next highest peak area (184 vs. 6 mV*s). Retention time is to blame for this result; all other retention times were in the 7.5 range and DDPCH had a retention time of 6.8 minutes. Since retention time is crucial in this experiment, this seems to be the logical conclusion as to why the peak area of DDPCH was higher than any other peak area including the same sample that was not exposed to heat.

These results show that just 30 days of high heat exposure to diet sodas containing aspartame can cause a tremendous breakdown to occur. There are controversies surrounding aspartame and its breakdown products such as phenylalanine, methanol, and eventually formaldehyde. This experiment highlights a potential problem when diet sodas are not stored in a temperature regulated environment.

Finally I would like to use this experiment as a call to have diet soda brands label the amount of aspartame used in their drinks. It was difficult to find information on the amounts of aspartame present. I believe that with the worry of PKU sufferers and others trying to avoid aspartame, it would be a logical idea to provide the aspartame amounts on all drinks similar to how caffeine or sugar itself is labeled on the Nutrition Facts.

CONCLUSION

Aspartame, the most tested artificial sweetener in FDA history is still deemed to be safe for human consumption. Although it has had a life plagued with controversy, it still remains one of the most used sweeteners in the world. There are people who have allergies where they cannot metabolize the phenylalanine by products of aspartame

breakdown, so any food, beverage, or drug that contains aspartame must have a warning labeled printed on the packaging.

In conclusion, aspartame breaks down over time in aqueous solutions and the break down is faster when exposed to high temperatures. The experimental results coincided with these statements. When Diet Coke and Diet Dr. Pepper were exposed to 50 °C for 37 days, more than 5 times the original concentration of aspartame broke down compared to samples left at room temperature. This rapid decrease in aspartame concentration over a short period of time should continue to be tested to see whether large doses of harmful chemicals are being ingested when the sodas are consumed. Finally diet sodas should carry a label representing the amount of aspartame added to each drink to help people with PKU in order to keep track of the amount of phenylalanine they are consuming daily. Since we can track calories, fat, sugar, and caffeine we ingest in these sodas, we should also be able to track the artificial sweeteners used to sweeten our diet beverages.

LITERATURE REVIEW

In a compilation study done by B.A. Magnuson, extensive investigations were done on the use of aspartame in both human and animal trials before being available to the public for consumption. Various tests included Biochemical effects, Animal and Human studies regarding the absorption, distribution, metabolism, and elimination of aspartame and its byproducts, and neurotoxicity studies. This study compiles various results into a comprehensive look at many aspects and common complaints regarding aspartame consumption. The overall theme from this study is that more than 90% of all

subjects had no serious reactions towards the aspartame whether it is neurological or physical. A common response when reacting to aspartame was headaches; however no substantial evidence was found in any studies present in the compilation. A main focus of this study was to look at the maximum allotted daily intake (ADI) of aspartame and how plausible the chance of exceeding that level would be. From their data, it was shown that only by replacing all sugar substitutes with aspartame, having maximum allotted levels of aspartame in the foods, and that the normal intake was by the worst diabetic group being studied, that the ADI of aspartame would be exceeded by roughly 14%. This is highly unlikely and most if not all groups fall well below the daily limit.¹

A study that argues against the notion that aspartame is relatively safe for consumption is found in Aspartame (Nutrasweet®): Is it Safe? This book focuses on the few cases regarding the negative effects of aspartame on humans. Dr. H. J. Roberts is trying to emphasize the idea that even though these cases seem few and far between, there are more that are confirmed than there are approximated by the current FDA warnings and labels. He highlights multiple studies with patients of his own who had severe reactions towards aspartame. One in particular, talks of a girl who has seizures after consuming products containing aspartame. Her allergy is confirmed after finding history of phenylalanine intolerance in other family members and eliminating the substance from her diet before introducing a small amount back in. This doctor argues that the labelling of aspartame by the FDA and by food and beverage companies is skewed. He suggests that all products containing this chemical are to be labelled with the amount of aspartame as well as a suggested expiration date. The expiration date is to help remove products that have been stored at too high of temperatures or that have been on

the shelves for far too long. According to this source, the ADI was increased from 20 mg/kg to 50 mg/kg by the FDA. One kilogram is roughly 2.2 pounds. A person would have to drink 4-5 liters of diet soda in one day to reach this limit. This limit was created by being based on animal testing not on human subjects. This number identifies the maximum daily level of aspartame a person should consume. In reality the ADI is based on the lifetimes of these animals tested. What Dr. Roberts argues is that the ADI limit should be much less than it is currently because of a variance of tolerance levels associated with aspartame. Some of his patients and studies had extreme symptoms from small amounts, whereas others could ingest larger amounts and would then experience symptoms.

Dr. Roberts argues that the safety in the consumption of aspartame has been disquieted by false information from government agencies, skewed test results from testing, and overall false assurance that aspartame is harmless when ingested. These stem from his own personal experiences with individuals who have had reactions toward aspartame and its components. His views contradict guidelines from the FDA. He does not believe that the information provided by their agency is correct or viable, but is instead, a false claim based on the large studies without focusing on the small cases that arise in some individuals.⁹

In an evaluation of aspartame edited by four Monsanto scientists, studies were conducted on the effects of aspartame on normal adults and potentially sensitive persons, safety evaluation in pregnancy, tolerance in various individuals, as well as evaluations of medical complaints. This source agrees on the FDA's ADI for aspartame which is 50

mg/kg body weight. It clarifies that issues were raised involving the various topics above and that because of these concerns, they were more thoroughly investigated.

For the effects on normal adults, phenylalanine concentrations did not exceed levels associated with adverse effects even after a consumption of 200 mg/kg body weight. When consuming aspartame, the research in this study claim that consuming one large dose of aspartame in a given period causes less build up than small amounts throughout the day. Also indicated in this study is that dietary proteins can control the rise of the phenylalanine concentration in individuals. Their overall conclusion for normal adult humans is that there are no potentially adverse effects with the use of aspartame; even when consuming large doses, the phenylalanine and methanol levels in test subjects were below cautionary levels.

Sensitive persons were also tested with varying amounts of aspartame anywhere from 34 mg/kg to a maximum of 100 mg/kg. From this testing, it was shown that infants were able to metabolize the aspartame similarly to normal adults. For persons with sensitivities to phenylalanine, the same various aspartame concentrations were administered and found to be excreted at half the rate of normal adults. These results were from one large single dose of the aspartame. Other cases used multiple small doses given over a time period. What this study failed to demonstrate clearly was the reactions caused from the elevated plasma phenylalanine concentrations. The levels found present in patients were highly studied however. Adverse effects on pregnant women were also studied although no apparent consensus was reached on whether or not there were any problematic symptoms from the ingestion of aspartame. Though these studies all focused on the rises in phenylalanine concentrations, so far none have offered a procedure to test

for the aspartame concentrations found in humans, or in food or drink containing aspartame.¹⁶

Many procedures that do highlight the techniques for identification of the concentrations of aspartame list the use of a High Performance Liquid Chromatography (HPLC). The Center for Disease Control (CDC) has a method to identify the concentrations of aspartame found in liquids using HPLC. The unknown sample is measured and the absorbance is found. Standards that have known concentrations of aspartame are also measured using the HPLC and a calibration curve is created. The absorbance from the unknown sample is then fitted to the linear equation and a concentration is calculated. Based on the initial volume of the sample, an estimate of the amount of aspartame present can be determined. This procedure uses chemicals easily available to a common college chemistry lab. Two eluents will need to be mixed up and each sampling pump will need to be calibrated before sampling can occur. This source also identifies certain standard values it chose and these results can be compared to personal data.¹⁷

In a study done by Bernard F. Gibbs, HPLC was used to determine metabolites and aspartame in foods. These foods included Diet Coke, Diet Pepsi, and Diet Minute Maid as well as pudding mix and hot chocolate powder. The liquids were eluted with a water-acetonitrile solution and monitored at 210 nm. From their results, the diet drink concentrations were similar in comparison with the manufacturer's listed amounts. These concentrations were found in the millimolar or the microgram range. Some problems occurred from interferences from dyes, flavors or other sweeteners. This is a drawback when using rapid chromatographic methods.²

According to *Adverse Reactions to Food* by Therese Beaudette, Aspartame is classified as a food additive although technically sweeteners are flavors. Ms. Beaudette highlights that although there have been many questions regarding the safety; it has been the most voluntarily consumed synthetic chemical. Some of the problems reported from aspartame use include headache, dizziness, mood alterations, gastrointestinal disturbances, dermatologic symptoms and menstrual changes. These complaints were assessed by the CDC and were deemed not a major concern to the greater population although some individuals may experience these symptoms. While aspartame is considered safe, conflicting studies have been brought to attention over the major effect caused from consumption of aspartame, such as headaches. A group of studies concluded that headache incidences after aspartame ingestion were not comparatively different than headache incidences after placebo ingestion. Other studies gathered evidence that showed headache frequency increased during aspartame and stayed relatively constant with placebo.

A survey was taken to compare headache frequency between caffeine, aspartame, and alcohol. The responders indicated that aspartame caused headaches significantly more than caffeine did but less than alcohol. From this data, a conclusion was reached that labelled aspartame as a possible probable cause for headaches in some consumers. When concerns were raised about the phenylalanine component, this article just quotes what the FDA guidelines are and that some physicians have been arguing to not only put a PKU warning on products containing aspartame, but also the milligram content. From the conclusion of this article, it is apparent that no particular side of the aspartame

controversy has been taken and that both sides have ideas that may be beneficial for the general public.¹⁸

Dr. Joseph Mercola and Dr. Kendra Pearsall have published a book called *Sweet Deception* which documents the health problems related to highly refined sugar and artificially produced sweeteners. According to their research over four billion gallons of diet sodas are being sold every year. These beverages can contain one or a blend of artificial sweeteners so the actual number of diet sodas being sold that contains aspartame is not known. In 1958 an amendment requiring the premarket proof of safety was introduced by the FDA; aspartame was the first of the artificial sweeteners to be placed under that amendment. Dr. Mercola argues that large-scale human studies have yet to be conducted so the general safety of aspartame and other artificial sweeteners is only limited to the toxicological animal data that is required for FDA approval. This is one main concern found throughout all independent studies that disagree with the FDA's approval of aspartame.¹⁹

According to this book, adverse reactions to aspartame had the most reports to the FDA than all other food additives combined besides Olestra. In 1988, 80% of the food additive complaints to the FDA were about the various aspartame products. Since aspartame can be fully metabolized in the body compared to Splenda or other sweeteners, more complications can occur. In 1967 during early stage testing in the safety of aspartame, multiple red flags appeared. Some side effects recorded from animals studies included potential neurotoxicity, defects of the brain, seizures, and even death. These findings were swept under the rug by the G.D. Searle Company and instead the company produced over 100 studies that showed the safety of their product. These verdicts were

brought before the FDA in 1973, however, aspartame was not fully FDA approved until 1981. This was the first time where a food manufacturer had a criminal investigation requested by the FDA. In a crazy plot twist, the lead U.S. attorney for this investigation not only quit, but began working for G.D. Searle's law firm. From this main event, there was no indictment, however, in 1979 The Public Board of Inquiry declared that aspartame should not be approved because of the potential brain tumors found in animal test subjects.

The most horrific account in this book regarding the so-called studies performed by G. D. Searle scientists was that they knowingly falsified results. Comparing original observations and final observations received by the FDA, it was shown that there were errors and inconsistencies throughout the studies. These findings were highlighted by the Bressler Report. Other warnings also came to light when lymph nodes that swelled to extreme sizes were deemed normal swelling sizes. It was quoted that the data was obviously not normal and the amount covered up was tremendous. Although these findings did slow the aspartame approval by the FDA, this book suggests that the CEO of G. D. Searle used political power to remove people who disapproved of aspartame. Because of this, an internal panel that originally had a 3 to 2 vote on banning aspartame was given an extra member who tied the verdict. This tie was broke by the new FDA commissioner Dr. Hayes; as a side not, Dr. Hayes entered a job with the G. D. Searle Company not long after aspartame was approved by the FDA.¹⁹

After only one year of aspartame's approval in soft drinks, the FDA had received over 600 consumer complaints concerning side effects such as headaches and dizziness. The CDC reviewed part of these complaints and reported that neurological and

behavioral problems caused by aspartame needed further study before any definite conclusions could be made. The author from here on becomes a bit biased and critiques Frederick Trowbridge of the CDC use of the words “mild nature” to describe the complaints that arose at that time. Dr. Mercola believes that the CDC misidentified the actual severity of the cases.

A concern that arises when aspartame studies are brought before the FDA is where the funding is coming from. Dr. Mercola identifies that out of 166 articles from 1980 to 1985, seventy-four were financed by their industry. Of those, 100% indicated the safety of the sweetener. The other ninety-two articles were independently funded and showed that 91% of the data showed problematic health effects. Based on these results, it seems apparent that depending on which group funds the research, the results are skewed more to what the donor wants to be found. More research is needed before true conclusions can be determined.

A final argument on flaws found in the G.D. Searle’s aspartame research was the use of this chemical in powdered form, not in products. Dr. Mercola states that through this form of testing, results could become flawed or skewed. Any food that is combined with aspartame can have potential modifications.¹⁹ One such example is the breakdown of aspartame in diet sodas under high temperatures. These unstable conditions have not been properly tested and therefore consumers may have more health concerns after aspartame has reacted with such foods. Because of this distinct situation, the research I will be conducting will focus not only the quantification of aspartame in diet sodas; it will also highlight any differences when these samples are exposed to temperature levels where aspartame is known to decompose.

Overall, *Sweet Deception* highlights problems with the research conducted by G.D. Searle, the biased results that occur based on who funds the research, and the overall dangers that these investigations have not necessarily tested for adequately. Although this book does not offer a means to test for the safety of aspartame, it does outline the problems in such a way that procedures can be designed for proper testing.¹⁹

In the third edition of *Alternative Sweeteners* edited by Lyn O'Brien Nabors, the overall history, characteristics, applications, and safety of aspartame is summarized. As with all of the other resources mentioned previously, the date of discovery and the uses of this chemical are all in agreement. This book does have a slight bias towards the notion that aspartame is not inherently toxic to humans. Only two cases are highlighted that point to this idea; One is the allegations of brain tumors becoming more prevalent since the use of aspartame are false, and the other is that the internet misinforms the general public.

In the case of alleged brain tumors, *Alternative Sweeteners* argues that a report from John Olney stating the increase of brain tumors from aspartame is false. Mr. Olney assumes that only aspartame was the factor that caused the dramatic increase in brain tumors in a period of four years. Ms. Nabors highlights the data showing that brain tumors rates were increasing before aspartame was introduced to the general public and that the patients having these tumors were in old age whereas the majority of aspartame drinkers were significantly younger. The consensus from the United States, Australia/New Zealand, the United Kingdom, and the European Union is that aspartame does not cause cancer. From this information it appears that Mr. Olney may have data that is considered flawed.

Ms. Nabors also identifies that use of the internet as a way for the general public to become misinformed. In the day of the internet, any person can write about any topic whether they have the necessary knowledge or not. False allegations have arisen regarding aspartame and many other products. Although seemingly harmless, the articles more than likely do not contain any scientific evidence or citations. This misinformation on the internet has been acknowledged by the FDA and they have concluded that no reliable scientific evidence is present concerning the danger from aspartame. Overall, more than one hundred countries including the United States have deemed the artificial sweetener aspartame safe for human consumption.²⁰

According to *Politically Incorrect Nutrition* by Michael Barbee, the food industry is nothing more than propaganda that is used to make the general public believe that what they are consuming is not harmful to their health. This book states that the general public is too unwilling to consider that the FDA is not as pro health as they seem. It argues that since aspartame breaks down into methanol, aspartic acid and phenylalanine, the danger is not in the amount of aspartame taken in, but the amount of the three components. While the FDA had an ADI of 50 mg/kg body weight for aspartame, the Environmental Protection Agency (EPA) has the maximum safety limit for methanol set to 7.8 mg per day. One liter of a diet soda contains roughly 56 mg of methanol and therefore causes concerns. Another cause of worry among consumers and this book is the chance that the use of aspartame increases brain cancer rates. Whereas *Alternative Sweeteners* states that Mr. Olney had data that was not stable, *Politically Incorrect Nutrition* claims that Mr. Olney was indeed correct in his findings. The outcomes showed that between 1984 and 1985 there was a 10% increase in the incidence of brain cancer. The development of

brain cancer in animal test subjects was one of the main holdbacks for the FDA allowing aspartame to be consumed by the general public. It was shown that when aspartame broke down into diketopiperazine (DKP), a brain-tumor agent, the animals began to develop brain tumors.

Mr. Olney does agree that brain cancer rates did appear to rise in 1973, which was before the introduction of aspartame. He states that with the sudden spike of brain tumor rates in the 1984-1985 period suggests a carcinogen was introduced which would cause such a dramatic increase to occur. According to the National Soft Drink Association, aspartame cannot be a carcinogen because it never enters the bloodstream. Mr. Barbee believes that this statement is false. He does not give an argument or evidence as to why he believes that the above report is not true. To back the idea that aspartame is not necessarily as safe as it seems Mr. Barbee cites Dr. Joseph Mercola and the findings listed in many of his books. According to Dr. Mercola, 167 symptoms and/or reasons to avoid aspartame are listed by the National Institutes for Health in 1991. Other ideas and arguments of his are quoted in above paragraphs.

Mr. Barbee ideas and information agree with previous sources that state it took 16 years for aspartame to become FDA approved. He also agrees that G.D. Searle Company had problematic animal studies and that some were fraudulent. This article also points out that the FDA did induce an investigation into the validity of the tests conducted by Searle scientists. The U.S. attorney who was responsible for filing the fraud actually took a position at the company and therefore, the charges were never placed on G.D. Searle. This information agrees with previously quoted sources.

Mr. Barbee believes that aspartame is not a chemical that should be used for human consumption. He points out hazardous chemical by-products, faulty testing practices, and a lack of legitimate research as concern for the safety of this product. Most of his points are taken from the previous sources listed and very little of his own research was added to this report.²¹

In *Sweeteners and Sugar Alternatives in Food Technology* edited by Helen Mitchell, there is a section discussing the history and safety of aspartame as well as the other similar artificial sweeteners. This source is unbiased and tries to provide the information that other references above have already mentioned, however, there is no discussion on the side opposing the safety of aspartame. An interesting piece of information that had not been highlighted in previous works is that the Ajinomoto Company of Japan developed and patented most of the commercial production of aspartame, even though G.D. Searle originally discovered it. Also noted is that because of aspartame's maximum sweetness intensity, it can be used as a sole sweetener and does not have to be combined with other natural or artificial sweeteners. Comparing stabilities, aspartame is much more stable in solid form than in liquid. The pH, temperature, and time are all functions that affect the artificial sweetener. According to this source, the effect of high temperature on the degradation of aspartame is lessened when the pH of the solution is kept around 3.5-5 on a 1-14 scale. Although *Sweetness and Sugar Alternatives in Food Technology* acknowledges that various factors can affect the perceived sweetness, these factors are not listed or talked about. Theoretical calculations were performed for the level of methanol found in a 330 mL can of diet soda. This was done because aspartame's decomposition products include methanol which is toxic in certain

limits. Their results identified 18.3 mg or 0.26 mg/kg body weight of methanol in one 330 mL can. Compared to the toxic level of 200-500 mg/kg body weight, this is very slight and no cause for concern according to their theoretical calculations. Based on the information provided by Ms. Mitchell, the safety and information regarding aspartame agrees with previous sources and adds very little to unknown situations.²²

WORKS CITED

¹Magnuson, B., et. al; *Aspartame: A Safety Evaluation Based on Current Use Levels, Regulations, and Toxicological and Epidemiological Studies*; Informa Healthcare, USA

²Gibbs, B.; et. al; Simple and Rapid High-Performance Liquid Chromatographic for the Determination of Aspartame and its Metabolites in Foods. *Journal of Chromatography*.**1996**, 725, 372-377.

³Murray, R.; *How Aspartame Became Legal-The Timeline*;
<http://www.rense.com/general33/legal.htm> (accessed April 22, 2016)

⁴*Company News; Monsanto Selling Sweetener Ingredient Business*
<http://www.nytimes.com/2000/03/28/business/company-news-monsanto-selling-sweetener-ingredient-business.html> (accessed April 22, 2016)

⁵Stegink, L.D.; Filer, L.J. Jr.; *Aspartame: Physiology and Biochemistry*; Marcel Dekker, Inc., New York, 1984.

⁶*Aspartame*; MSDS <http://www.sciencelab.com/msds.php?msdsId=9922975> (accessed March 20, 2016).

⁷Gennet, R.; http://www.huffingtonpost.com/robbie-gennet/donald-rumsfeld-and-the-s_b_805581.html (accessed May 1, 2016).

⁸"*Nutrasweet*" –*Health and Safety Concerns*; Labor and Human Resources; U.S. Government Printing Office, Washington; 1988.

⁹Roberts, H.; *Aspartame (Nutrasweet) Is it Safe?*; The Charles Press: Philadelphia, PA

¹⁰Nil, A. G. *The History of Aspartame (2000 Third Year Paper)*

[https://dash.harvard.edu/bitstream/handle/1/8846759/Nil, Ashley -
The History of Aspartame.pdf?sequence=3](https://dash.harvard.edu/bitstream/handle/1/8846759/Nil,_Ashley_-_The_History_of_Aspartame.pdf?sequence=3) (accessed March 23, 2016).

¹¹Blaylock, R.L.; *Excitotoxins: The Taste that Kills*; Health Press; Santa Fe, New Mexico; 1994

¹²*Additional Information about High-Intensity Sweeteners Permitted for use in Food in the United States*

<http://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm397725.htm#Aspartame> (accessed April 10, 2016).

¹³*Amounts of Sweeteners in Popular Diet Sodas*

http://static.diabetesselfmanagement.com/pdfs/DSM0310_012.pdf (accessed March 26, 2016).

¹⁴*Aspartame* <http://www.cdc.gov/niosh/docs/2003-154/pdfs/5031.pdf> (accessed October 5, 2015).

¹⁵Bidlingmeyer, B. A.; *The Analysis of Artificial Sweeteners and Additives in Beverages by HPLC*; The Modern Student Library. **1991**, 8, A195-A196.

¹⁶*The Clinical Evaluation of a Food Additive: Assessment of Aspartame*; Tschanz, C. et. al; CRC Press: Boca Raton, FL

¹⁷Neumeister, C.; Center for Disease Control. <http://www.cdc.gov/niosh/docs/2003-154/pdfs/5031.pdf> (accessed May 6, 2015)

¹⁸Beaudette, T.; Adverse Reactions to Food Additives. In *Adverse Reactions to Food*;
The American Dietetic Association: Chicago IL

¹⁹Mercola, J.; Pearsall, K. *Sweet Deception: Why Splenda, Nutrasweet, and the FDA may
be hazardous to your health*; Nelson Books: Nashville, TN

²⁰Butchko, H.et. al. Aspartame. In *Alternative Sweeteners*; Nabors, L. Ed.; Marcel
Dekker, Inc.: New York NY

²¹Barbee, M.; *Politically Incorrect Nutrition*; Vital Health Publishing: Ridgefield, CT

²²O'Donnell, K. Aspartame and Neotame. In *Sweeteners and Sugar Alternatives in Food
Technology*; Mitchell, H., Ed.; Blackwell Publishing Ltd: Oxford, UK