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Effect of Over-The-Counter Drugs on DNA Analysis of Blood Ingested by Medicinal Leeches

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ABSTRACT

Painkillers, such as aspirin (acetylsalicylic acid), are used by many to alleviate the pain associated with common ailments. Although the half-life for aspirin is only 2-5 hours it is possible that there may still be sufficient amounts of it in the body post-mortem. Hence, if a medicinal leech, *Macrobdella Decora*, fed on the blood of a victim or a suspect who had recently ingested an analgesic, it may be possible to simultaneously obtain a drug identification and a complete DNA profile from the human blood extracted from the gut of the leech. The potential for generating a DNA profile depends on whether or not acetylsalicylic acid, ibuprofen, and caffeine inhibit the extraction and quantification of DNA due to their aromaticity.

Male human blood was spiked with 1:1 ratio of aspirin, caffeine, and ibuprofen solutions followed by DNA extraction done at 0, 24 and 48 hours. The extracted DNA was then quantified using the PowerPlex® Fusion 6 C system for autosomal study and HPLC was then used to determine the presence of the drugs in the blood samples. A standard amplified product was also examined by capillary electrophoresis and genetic analyzer 3130xl to develop a DNA profile which was used as reference for all future DNA samples which were extracted using this method as all blood used in this experiment was from the same male donor. Blood mixed with the drug was fed to medicinal leeches which were then euthanatized by freezing at 0, 24, and 48 hours after being fed the spiked blood. Human blood from their gut was extracted and analyzed for the presence of aspirin, ibuprofen, and caffeine in order to see a full DNA profile can be generated. Samples were also analyzed with an HPLC to determine the presence of the drugs in the blood samples.

The HPLC results concluded that aspirin, ibuprofen, and caffeine were present in substantial quantities in the spiked blood samples collected from the midgut of the leeches. While the leeches did provide a full DNA profile of the male donors, when the collected results were analyzed, the hypothesis that inhibition was caused by acetylsalicylic acid, Ibuprofen, and caffeine was rejected as no inhibition of DNA amplification was noted in both the spiked blood and the blood collected from the midgut of the leeches. Some samples contained higher IPC CT values, but no inhibition was noted upon quantification.

Due to this, it can be concluded that acidic and basic over-the-counter drugs containing aromatic groups can be detected in blood samples. These samples can also be used in DNA extraction, quantification, and amplification methods in order to generate a full DNA profile.

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Chapter 1 : Why Medicinal Leeches?

Back in 2001, in Tasmania, Australia, Peter Cannon and his accomplice robbed and tied a 71-year-old lady to a chair in her remote home. They stole around \$550 but while they were escaping, a leech came into contact with Cannon's leg and was able to ingest some blood. The leech fell off while the convicts escaped but was later found by investigators and taken in as evidence. Detective Johnston also later reported that the leech was the only evidence gathered from the crime scene and due to there being no bite marks on the victim or the police officers, was suspected to tie back to the criminals. The blood was extracted from the leech and a DNA profile was made. This was then matched with the criminal data base and 8 years later, Cannon was finally caught and jailed (Guardian News and Media, 2009).

Leeches are hermaphroditic annelids, capable of ingesting blood at a rate of 1ml per minute and can lead to the site of ingestion having lower coagulation rates. Saliva produced by leeches is very potent in hirudin, hyaluronidase, calin, and a histamine-like vasodilator (Conley et.al, 2023). Hirudin and calin play the largest role when it comes to feeding as both of these are involved with lower blood clot formation rates.

Hirudin is an acidic polypeptide produced in the salivary glands of leeches and is found to be the most potent inhibitor of thrombin formation (Junren, et.al, 2021). In the human body, thrombin works to activate factors V, VIII, and XI which have positive feedback with thrombin and increase production of factor XIII which is connected with a higher production of fibrin linkages and clot stabilization (Lee & Ansell, 2011). Hirudin works to inhibit the initial

production of thrombin which decreases the production of the factors and leads to lower coagulation rates.

Calin is another potent substance found in the saliva of leeches and works as a collagen-mediated platelet aggregation inhibitor (Munro et.al, 1991). Calin has very rapid effects, 1-10 minutes depending on concentration, and inhibits platelet aggregation by binding to collagen and effectively reducing collagen-platelet interactions. Lower levels of platelet aggregation then leads to lower rates of blood clot formation (Deckmyn et.al, 1995). This is the same result as hirudin but done through a different pathway, thus increasing the efficiency of clot deformation. Medicinal leeches have already been used for many years in plastic surgery and other medical procedures requiring bloodletting. The first record of a leech being used medically dates back to almost 1500 BC (Conley et.al, 2023). Today, they are being used in professional cases regarding plastic and reconstructive surgery but have also shown to have significant results with cardiovascular disease, diabetes mellitus, osteoarthritis, deep vein thrombosis, as well as certain autoimmune diseases (Sig et.al, 2017). Within plastics and reconstructive surgery specifically, leeches have played a huge role in relieving venous congestion in skin necrosis on the digits, nipples, lips, ears, and nasal tips (Hackenberger & Janis, 2019). These applications show the broad use of a medicinal leech in medical and forensics cases and how they can be utilized to both treat patients and also create DNA profiles.

Cannon's case was the first time in forensic history that a leech was used to convict a criminal and showed that leeches have the potential to be used in forensic cases requiring DNA analysis. Due to this and all the medical applications, a medicinal leech was utilized in this experiment.

Medicinal leeches have also been experimented with in a prior study in the Roy lab at Penn State University, where they were fed male human blood in order to test if it was possible to generate DNA profiles. Once the leeches were fed, they were euthanized at certain time intervals, and the blood was collected from their digestive system. DNA was then extracted from these samples, quantified, and full DNA profiles were made using the PowerPlex® Fusion 6 C system for autosomal study and the PowerPlex® Y-23 system for Y-STR analysis. This experiment resulted in successful generations of DNA profiles. Due to these results, this current experiment does not test the generation of full DNA profiles but instead explores how the involvement of drugs impacts the quantification of DNA.

Chapter 2 : The Analyzed Drugs

The drugs analyzed in this experiment are aspirin, ibuprofen, and caffeine. These three drugs were chosen as they are found to be the most ingested over-the-counter drugs in the United States. Chemical properties of all three of these drugs were also hypothesized to affect the processes of DNA extraction and quantification and thus picked to be examined. Leeches were also found to be able to ingest all three of these drugs and were thus included in the experiment.

Aspirin

Aspirin, or acetylsalicylic acid, is one of the most commonly consumed analgesics worldwide, especially in the United States. This drug is an orally administered non-steroidal anti-inflammatory agent utilized as an everyday painkiller. Aspirin works by binding to serine residues of cyclooxygenases and acetylates them. This results in a decreased production of prostaglandins, tissue inflammation, and platelet aggregation. Pain detection is thus lowered as a final result.

Acetylsalicylic acid is in the form of an odorless white crystal with a powdered texture and a slight bitter taste. It has a boiling point of 140°C and 760 mmHg and a melting point of 135°C. This drug is slightly soluble in water with a solubility constant of 0.33 grams per 100 mL of water at 25°C. An increase in solubility has a positive correlation with temperature and is optimal at 37°C (Nokhodchi et.al, 2022). When acetylsalicylic acid is placed in a high-performance liquid chromatography machine, the retention time is found to be around 3.28 minutes (Patel et.al, 2013).

Figure 1. shows the structure of acetylsalicylic acid which includes three functional groups: an aromatic carboxylic acid, a phenyl ester, and a benzene ring. Aromaticity plays a role in maintaining stability and affects the protein-protein and protein-DNA complex relationships. Aromatics compounds are unsaturated cyclic and planar molecules which contain extra stability due to π -electrons which are able to freely move through the structure. These aromatic properties also exist within proteins themselves which aids proteins forming stronger bonds with themselves or with other molecules such as DNA (Anjana et.al, 2012).

Acetylsalicylic acid was chosen to be one of the experimental drugs due to the aromaticity contained in the compound's structure. This aromaticity would lead to the drug binding to the protein-DNA complexes and increasing the stability of the structure. This increase in the strength of the bonding would lead to DNA not being able to dissociate from the proteins during DNA extraction, leading to lower quantities of DNA being present after quantification. Thus, this experiment works to find the effects of aspirin ingestion on DNA analysis, specifically extraction and quantification.

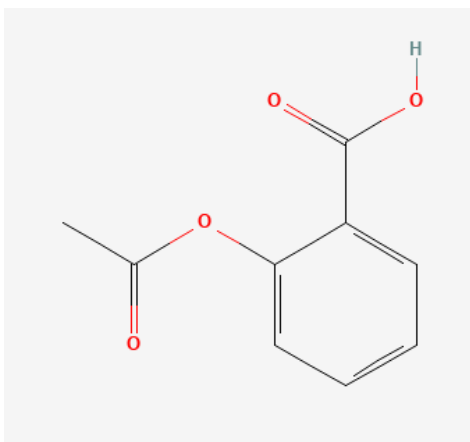


Figure 1. Chemical structure of aspirin, or acetylsalicylic acid

Ibuprofen

Ibuprofen is another common over-the-counter, non-steroidal anti-inflammatory drug (NSAID) and is researched to be the safest of all NSAIDs. This propionic acid derivative plays anti-inflammatory, antipyretic, and analgesic effects on the mammalian body by inhibiting the activities of both cyclo-oxygenase I and II. This inhibition leads to a decrease in the synthesis of prostaglandins which results in a decrease in pain reception.

Ibuprofen is a colorless crystal with a boiling point of 157°C and a melting point in the range of $75\text{-}77.5^{\circ}\text{C}$. Similarity to acetylsalicylic acid, ibuprofen has a low solubility in water with a solubility constant of 0.021 mg/mL at 25°C . When placed in a high-performance liquid chromatography machine, ibuprofen has a retention time around 4.9 minutes depending on which solutions are included as the mobile and stationary phases (Shah et.al, 2012).

Figure 2. shows the chemical structure and connectivity of an ibuprofen molecule which contains a carboxylic acid and an aromatic ring. Ibuprofen is an acid and thus acts similarly, both chemically and physiologically, to acetylsalicylic acid and thus should have similar effects on the interactions between proteins and DNA. This can also be supported by the chemical structure of ibuprofen containing an aromatic ring similar to the one found in acetylsalicylic acid's chemical structure. This ring's aromaticity increases the stability of the protein-DNA complex and would lead to a decrease in the concentration of free DNA. This decrease would be visible on the quantification data as this is the step when the sample DNA amounts are measured.

Ibuprofen's acidity, aromaticity, and prevalence in American society are the reasons this drug was included in the experiment. Being a commonly used drugs leads to the results being applicable to a larger population, thus making the results more impactful. Ibuprofen's acidity and

aromaticity being similar to acetylsalicylic acid would also lead to more solid conclusions being made about the impact of aromaticity and acidity on protein-DNA interactions and DNA analysis.

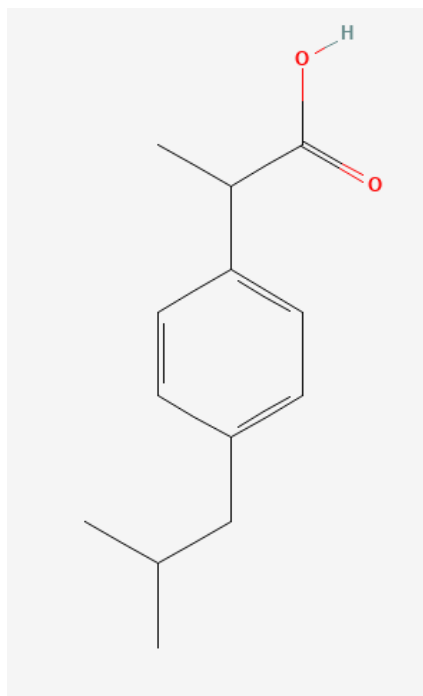


Figure 2. Chemical structure of ibuprofen

Caffeine

Caffeine is one of the most researched food components and is consumed by over 85% of the U.S. population (Mitchell et.al, 2014). This drug is a methylxanthine alkaloid originating from South America and east Asia where it is predominantly found in seeds, nuts, and leaves of some plants. Caffeine is structurally very similar to adenosine as shown in Figure 3. and Figure 4. and functions primarily as an adenosine receptor antagonist. Due to these molecules having a similar chemical structure, caffeine is able to bind to adenosine receptors located in the central

nervous system, decreasing the number of available receptors to which adenosine could bind to. This leads to adenosine-mediated actions to be downregulated. Once this downregulation takes place, the central nervous system activity is increased and leads to an individual feeling more energetic.

Caffeine also does nonselective competitive inhibition towards phosphodiesterases (PDE) which lead to an anti-inflammatory response. When these PDE molecules are inhibited from performing their normal functions, serum cyclic-AMP concentrations increase, protein kinase A is activated, and leukotriene production is stopped. These three functions in turn lead to an anti-inflammatory response, similar to the one caused by acetylsalicylic acid and ibuprofen. Caffeine is a white, odorless powder with a boiling point of 178°C and a melting point of 238°C. Unlike acetylsalicylic acid and ibuprofen, caffeine is readily soluble in water with a solubility constant of 20 g/L at room temperature (Nonappa & Kolehmainen, 2016). When placed in a high-performance liquid chromatography machine, the observed retention time for caffeine is around 4.5 minutes (Ribeiro et.al, 2019).

Figure 3. shows the chemical structure of caffeine which contains an amide and an amine, making this a basic compound. This is the main reason why caffeine is included in this study as it contains different properties than the other two drugs being tested. Caffeine in nature has a pH of around 6.55 which means it is slightly alkaline. pH range of 5 to 9 also leads to a stable protein-DNA complex, range in which caffeine is included (Fornace et.al, 1986). This compound also has aromaticity within its rings which would result in higher stability and a lower DNA concentration once quantified.

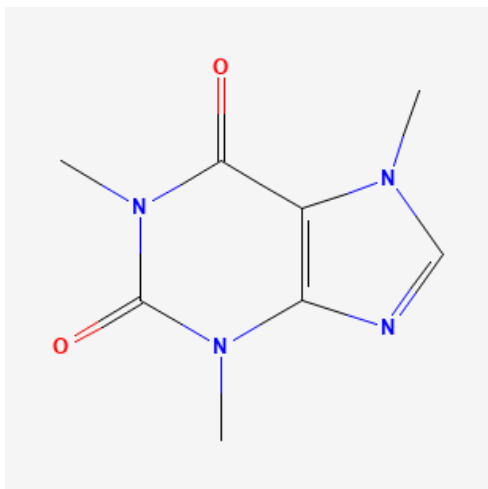


Figure 3. Chemical structure of caffeine

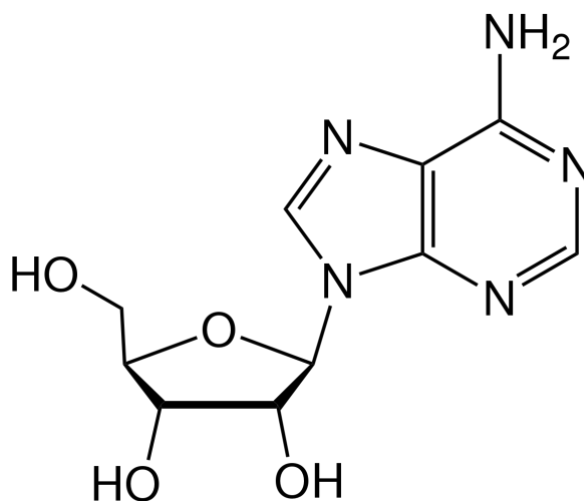


Figure 4. Chemical structure of adenosine

All of the drugs utilized in this experiment are chosen due to them all containing aromaticity within their chemical structures which would cause a protein to dissociate from DNA at a lower rate. This would lead to lower quantification results and thus an incomplete DNA profile. So, the main goal of this experiment is to figure out if quantification is disrupted when

these three drugs are mixed with human male blood and go through the digestive processes of medicinal leeches.

Chapter 3 : Blood Spiking and Feeding

Medicinal leeches were transported from Michigan, USA, and upon arrival, were placed in containers with fresh, cold water. They were left at room temperature for 10-15 minutes to let them adjust to the new water and then were stored in a refrigerator to mimic the cold of their natural habitat. These leeches were stored in the fridge for no more than one week before being incorporated in the experiment. The leeches were fed and euthanized at certain time increments which were kept consistent throughout the experiment.

Blood Spiking Methods

Blood was collected from male donors and stored in a freezer and thawed in small amounts when needed. Each sample run was done with 30 leeches and each leech was fed 0.5 mL of blood. For each sample, 15 mL of blood was thawed, and 15 mg of powdered drug was measured and combined with the blood. Due to the drugs having low solubility levels, the drug was powdered as fine as possible and when it was mixed in the blood, the solution was inverted multiple times to ensure as much mixture as possible. Blood was placed in the tubes immediately after inversion so that the drug particles did not settle to the bottom of the solution.

Leech Feeding Methods

Leeches were taken out of the refrigerator and left in room temperature for 10 minutes to have them acclimate to the new temperature. While the leeches were being acclimated, blood was spiked and feeding tubes and petri dishes were labeled.

Each 50 mL tube was filled with 0.5 mL of acetylsalicylic acid spiked blood. Leeches were then taken out of the water, dried and placed individually in each of the tubes. Once all the leeches were in the feeding tubes, the tubes were capped and placed on their side so that the leech doesn't drown in the blood and has more space to move around. They were left to feed for 30 minutes in order to give the leeches time to adjust to their new environment and feed as much of the sample blood as possible. Once the 30 minutes were over, the leeches were released back into a cold freshwater. Each was then rinsed of the blood, patted dry, and placed into their respective petri dishes. Leeches in the group T-0 (time zero hours after feeding) were euthanized by freezing immediately. Leeches in T-24 (time 24 hours after feeding) were left in the petri dishes in the room for 48 hours and then euthanized via freezing. Leeches in the final T-48 (time 48 hours after feeding) group were left in the petri dishes in the room for 48 hours and then euthanized via freezing. These leeches were only thawed once they were ready to be dissected. Aspirin and caffeine were both tested at T-0, T-24, and T-48 due to them having very different chemical properties while ibuprofen was only tested at T-0.

Chapter 4 : DNA Extraction and Quantification

This chapter first explores the usages of DNA extraction and quantification and the relevance of these methods to this experiment. The methods through which these were carried are then listed in separate sub-chapters below.

DNA Extraction and Quantification Background

DNA extraction is a purification method in which white blood cells are first separated from interstellar fluid. These cells are then lysed in order to expose the DNA followed by chemical and enzymatic methods to separate the DNA from all other cellular components. Once DNA has been extracted, its shape, size, and function when interacting with other components can be examined (Gupta, 2019). There are three different DNA extraction techniques but the one utilized in this experiment is the inorganic method with a proteinase K-based treatment. This method was chosen due to proteinase K's broad-ranged endolytic properties which work best for digesting proteins by cleaving peptide bonds located on the carboxylic end of aromatic, aliphatic, and hydrophobic amino acids. Proteinase K is also stable over a large pH range from 4.0 to 12.5 and works optimally from 50°C to 55°C (ThermoFisher Scientific).

DNA Extraction Methods

Leeches have a slippery exterior coat which makes dissections difficult. In order to reduce these difficulties, they were only thawed in batches of 10. Once the leech was thawed

enough that the skin was pierceable, a large cut was made from the pharynx to the middle of the crop region (shown in Figure 5.), making sure not to slice the leech into two pieces. The slice was made to expose the inside of the leech, after which the opening was exposed more by moving the two sides of the skin aside using blunt tweezers.

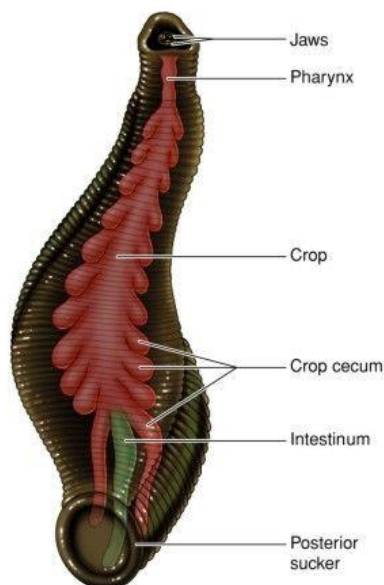


Figure 5. Anatomy of a leech

Once the opening was widened, a sterile swab was used to pick up blood by moving the swab once from the pharynx to the mid-crop and then back up to the pharynx. This method was kept consistent with all leech samples in order to standardize the blood collection. About 5 mm of the tip of the swab was cut with a sterile blade and placed in a labeled 2 mL tube.

EZ1 DNA Investigator Handbook was used for the rest of the extraction protocol. From this handbook, “Blood or Saliva Stains” unit was used. According to the handbook, 290 μL of Buffer G2 and 10 μL of Proteinase K was added to the tubes with the leech sample swabs. These tubes were then vortexed and incubated at 56 $^{\circ}\text{C}$ and 900 rpm for 30 minutes. After these 30 minutes,

the extraction was completed in a EZ1 Advanced Qiagen DNA extraction machine similar to the one shown in Figure 6.

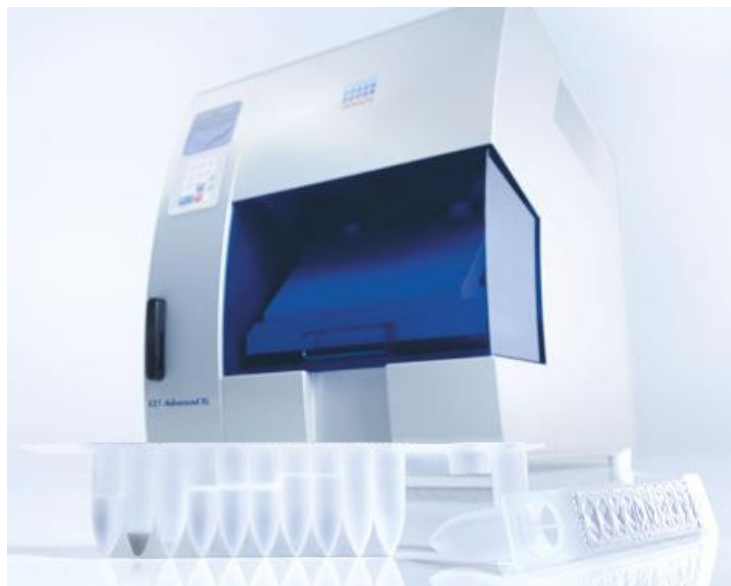


Figure 6. EZ1 Advanced Qiagen DNA extraction machine

The machine was set up using the following steps:

Table 1. Step-by-step process for setting up the EZ1 worktable

Step 1: Loading the first row	Load the first row with 1.5 mL elution tubes
Step 2: Loading the second row	Load the second row with the tip holders containing the filter-tips
Step 3: Loading the third row	Leave this row empty
Step 4: Loading the fourth row	Load the fourth row with the 2 mL samples tubes (tubes containing the incubated samples collected from the leeches)

Step 5: Loading the reagent cartridges	Take the reagent cartridges out of packed and shake them as to mix the solutions and then load them into the cartridge rack
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Once the EZI worktable is set up, it should look something like the image shown in Figure 7.



Figure 7. A fully set up EZI Advanced Qiagen worktable

The samples were run on the following settings:

Table 2. Step-by-step process of using the EZI Advanced Qiagen DNA extraction machine for "Trace Protocol"

Step 1: Starting the machine	Press "Start"
Step 2: Selecting protocol	Press "Trace Protocol"

Step 3: Selecting elution medium	Press “TE”
Step 4: Selecting volume	Press “50 μL ”
Step 5: Starting the run	Close the lid and start the process

This process took about 16 minutes for 6 samples and each step is shown in Figure 8. Once all the samples had been extracted, they were stored in the freezer and thawed when they were ready to be quantified.

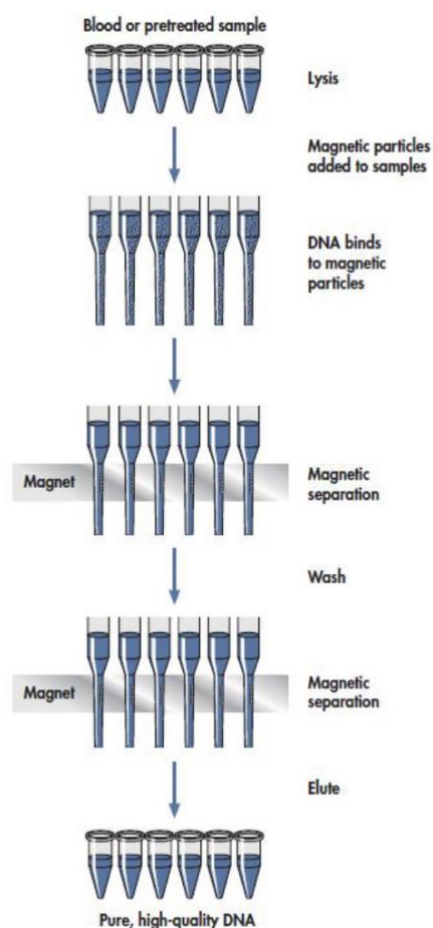


Figure 8. EZ1 Advanced Qiagen DNA extraction procedure

DNA Quantification Methods

The extracted samples were thawed slowly by placing them in the refrigerator 30 minutes before they were used. During this a master mix and the standards were prepared using “Quantification of Human DNA Using Quantifiler™ HP and Trio DNA kits and ABI 7500 Real Time PCR System” protocol.

Standard 1 was made using the following steps:

Table 3. Steps of making DNA quantification standard 1

Step 1	Thaw the Quantifiler THP DNA Standard and then vortex for 3-5 seconds
Step 2	In a sterile 2 mL tube, add 10 μ L of the 100 ng/ μ L stock solution and 10 μ L of the Quantifiler THP DNA dilution buffer
Step 3	Mix the solution in the tube thoroughly

Standards 2-5 were made using Standard 1 according to the following steps:

Table 4. Making DNA quantification standards 2-5

Making Standard 2	In a 2 mL, add 10 μ L of Standard 1 and 90 μ L of Quantifiler THP DNA dilution buffer and vortex solution to mix thoroughly
Making Standard 3	In a 2 mL, add 10 μ L of Standard 2 and 90 μ L of Quantifiler THP DNA dilution buffer and vortex solution to mix thoroughly
Making Standard 4	In a 2 mL, add 10 μ L of Standard 3 and 90 μ L of Quantifiler THP DNA dilution buffer and vortex solution to mix thoroughly
Making Standard 5	In a 2 mL, add 10 μ L of Standard 4 and 90 μ L of Quantifiler THP DNA dilution buffer and vortex solution to mix thoroughly

A1 50 ng	A2 50 ng	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 5.0 ng	B2 5.0 ng	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 0.50 ng	C2 0.50 ng	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 0.050 ng	D2 0.050 ng	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 0.005 ng	E2 0.005 ng	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Figure 9. Quantifiler HP 96-Well Grid for quantification

Once the standards were made, the master mix for the samples was also prepared. Each quantification plate has 96-wells, as shown in Figure 9., and due to there being 5 standards and each sample being run twice, 43 samples could be run at a time. The number of samples run

varied each time, so the master mix was only made for the number of samples in that run plus 5 extra samples. This extra solution was made in case more master mix was needed. The calculation was done based on Figure 10. where 8 μL of Quantifiler Trio Primer Mix and 10 μL of Quantifiler THP PCR Reaction Mix were added for each sample. These solutions were combined in one tube, vortexed to ensure mixture, and 18 μL were dispensed into each well of the Quantifiler HP tray. 2 μL of each sample, standard, and control was then added to their corresponding well, making sure to have a replicate for each of them. Once the plate was filled, it was sealed with the Optical Adhesive Cover and a visual inspection was done to ensure no bubbles were in the wells. If there were bubbles, the plate was very gently tapped.

Components	20μL reaction	# of reactions	Final volume (μL)
Quantifiler Trio Primer Mix	8.0 μL		
Quantifiler THP PCR Reaction Mix	10.0 μL		
Total volume of Reaction mix/sample	Dispense 18.0 μL		
Maximum DNA Volume	2.0 μL		

Figure 10. Quantifiler HP quantification master mix calculation box

The ABI 7500 Real Time PCR instrument was turned on and an experiment was created. In this all the samples were assigned to the correct wells using a fully filled out Figure 9. Once the machine had preheated and the experiment had been created, the plate was placed inside, and

the machine was then started with the conditions shown in Table 5. When the run was completed, the results were collected and analyzed via Excel.

Table 5. Quantification run conditions

Holding Stage	40 cycles
95 °C – 2 minutes	95 °C – 9 seconds 60 °C – 30 seconds

Chapter 5 : DNA Quantification Results and Discussion

The above methods were used to collect DNA quantification data which is presented and discussed in the sections below. Only relevant data such as average IPC values and average degradation indexes are included in this section. The full raw data collected is included in Appendix A.

DNA Quantification Results

Table 6. Aspirin-spiked blood sample IPC quantification data from the initial trial run (* indicates significant inhibition)

Sample Name	Average IPC
0-1	27.21923733
0-2	27.74623966
0-3	27.06947994
0-4	27.48677158
0-5	27.51368904
0-6	27.30472469
0-7	27.44040203
0-8	27.02887821
0-9	27.21430588
0-10	27.94680023

24-1	27.06463242
24-2	27.27473831
24-3	27.501894
24-4	27.36557388
24-1A	26.91938686
24-2A	27.29633904
24-3A	27.60630417
24-4A	27.45830441
24-5A	27.32394028
24-6A	27.43318844
24-7A	27.3813858
48-1	26.99870777
48-2	27.0643959
48-3	26.98895645
48-4	27.28777885
48-5	27.25132561
48-1A	27.37907219
48-2A	27.35474491
48-3A	27.43780708
48-4A	27.44556427
48-5A	27.42054462
48-6A	27.04376984

48-7A	26.99229527
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Table 7. Average IPC values and average degradation indexes from the quantification run of the aspirin-spiked blood samples (* indicates significant inhibition; yellow color indicates minimal degradation; red color indicates significant degradation)

Sample Name	Average IPC	Average Degradation Index
A0-1	27.87601566	1.447730005
A0-2	27.80043507	0
A0-3	27.61615086	2.057689488
A0-4	27.76895046	1.12996763
A0-5	27.47022629	0
A0-6	27.46147823	0
A0-7	27.89834023	7.063656807
A0-8	27.9593811	1.359570444
A0-9	27.95260239	0.3693977
A0-10	28.12411594	2.085341871
A24-1	27.58293819	11.17954159
A24-2	27.48438931	1.368434787
A24-3	27.64392662	5.73986274
A24-4	27.58668804	0
A24-5	27.56357574	1.548322439
A24-6	27.72467995	0
A24-7	27.59915447	0.367065877

A24-8	27.61594296	1.927954078
A24-9	27.86945343	0
A24-10	27.83436108	0
A48-1	27.61225891	0.198289454
A48-2	27.63763332	0
A48-3	27.57582378	0.24030906
A48-4	27.75728703	0.545864642
A48-5	27.57991123	0
A48-6	27.51363182	0
A48-7	27.64478588	0
A48-8	27.79010963	1.142269611
A48-9	27.77605152	0
A48-10	28.03542519	0.879790902
AC-0	27.88647556	0
AC-24	27.96064949	0
AC-48	27.82104111	0

Table 8. Average IPC values and average degradation indexes from the quantification run of the caffeine-spiked blood samples (* indicates significant inhibition; yellow color indicates minimal degradation; red color indicates significant degradation)

Sample Name	Average IPC	Average Degradation Index
C-0	27.48264504	0

c0-1	27.61891937	1.444998026
c0-2	27.613451	3.769563437
c0-3	27.67155933	0.86971873
c0-4	27.4549036	0
c0-5	27.39621258	0.449547291
c0-6	27.28897953	0.605801284
c0-7	27.27782536	0
c0-8	27.17391586	0
c0-9	27.2412138	0
c0-10	27.6183033	1.361111403
C0-11	27.2932911	0.316821918
C0-12	27.16704274	2.185259283
C0-13	27.37767792	1.194548726
C0-14	27.37359524	3.161760599
C0-15	27.29577446	0.255196408
C0-16	27.20186043	0.691430211
C0-17	27.21308136	1.461704075
C0-18	27.40546131	0.46065478
C0-19	27.37711716	0.490740329
C0-20	27.63064671	6.382223607
C-24	27.42663956	2.949854792

c24-1	27.60407257	1.367633462
c24-2	27.15197849	0
c24-3	27.18062305	0
c24-4	27.24485302	6.500954151
c24-5	27.2463398	0.447957784
c24-6	27.25692272	0
c24-7	27.31355095	0
c24-8	27.50665855	0
c24-9	27.3917675	0
c24-10	27.14553738	0
C24-11	27.41052056	0
C24-12	27.15728092	1.015209645
C24-13	27.27070618	1.972224713
C24-14	27.29874611	1.310667873
C24-15	27.30875015	1.933830381
C24-16	27.08104039	4.827686787
C24-17*	28.75631333	0.335185975
C24-18	27.23292542	7.032006979
C24-19	27.40136337	5.827890277
C24-20	27.60740662	0
C-48	27.39333058	60.5217781

c48-1	27.24161625	0.519940197
c48-2	27.22785378	0
c48-3	27.02076721	1.538692713
c48-4	27.20912838	16.96112728
c48-5	27.26152706	0
c48-6	27.40782833	0
c48-7	27.50984764	0
c48-8	27.33559132	0
c48-9	27.23354435	2.682406425
c48-10	27.38799572	0
C48-11	27.09008313	0.993457198
C48-12	27.25204468	9.624954822
C48-13	27.30217648	0
C48-14	27.05351353	6.50963831
C48-15	27.1152916	1.099655748
C48-16	27.12341881	0.295887649
C48-17	27.03197194	1.216160655
C48-18	27.29778767	1888.101319
C48-19	27.17133904	0
C48-20	27.45858383	173.0477333
CC-0	27.6375246	5.41300118

CC-24	27.70071125	5.579100728
CC-48	27.70755196	0.984420776

Table 9. Average IPC values and average degradation indexes from the quantification run of the ibuprofen-spiked blood samples (* indicates significant inhibition; yellow color indicates minimal degradation; red color indicates significant degradation)

Sample Name	Average IPC	Average Degradation Index
I0-1	27.64480305	1.104658783
I0-2	27.13258267	6.439905405
I0-3	27.32074738	1.279590011
I0-4	27.36186123	0.749319196
I0-5	27.42525768	2.206230462
I0-6	27.30208969	1.106194616
I0-7	27.54652882	0.280181796
I0-8	27.69085884	7.598958015
I0-9	27.50951386	0.48180835
I0-10	27.97016335	0.107360058

DNA Quantification Discussion

Table 6 shows the average IPC values of aspirin sample from the trial run. This run was done in to figure out if quantification would even work with the presence of drugs in the samples. These samples were also not from dissections and were just blood mixed with aspirin. No leeches had been involved in this part due to this run only being a trial. All of the IPC values in this table were below 28.5 cycles which shows that no inhibition was present in the aspirin-spiked blood samples. With this information, the rest of the samples were run through the quantification machine using the same methods.

The average IPC values and the average degradation indexes of the aspirin-spiked blood samples collected from the leeches are included in Table 7. None of these samples had an IPC value greater than 28.5 cycles, which indicates that no inhibition was present and further DNA analysis can be done. Sample A24-1 was colored red due to the average degradation index of this sample being greater than 10, which indicates that this sample had significant degradation of the DNA and thus cannot be used for this analysis. This is also the only sample where this amount of degradation was found and so this sample has been labeled as being an outlier. All the samples colored yellow indicate slight to moderate amount of degradation, closer to 1 being slight and closer to 10 being moderate. This amount of degradation is not enough to significantly suppress DNA amplification and thus these samples can be used for further DNA analysis. The samples with no color had degradation indexes below 1, which indicates that they had no degradation and are thus ideal for DNA analysis (Thermo-Fisher Scientific).

The average IPC values and average degradation indexes of the caffeine-spiked blood samples collected from the dissected leeches are shown in Table 8. Only sample C24-17 had an

average IPC value greater than 28.5 cycles which indicates presence of inhibition. According to “Quantifiler HP for Quantification of Extracted DNA on the Applied Biosystems 7500 Real Time PCR System” handbook from Penn State University, this elevation in the IPC value can also be due to an increase in the amount of DNA present in the sample. When DNA concentration exceeds 5 ng/μL the IPC value will also start to exceed over 28.5. Due to this external factor, sample C24-17 was indicated as an outlier. Samples C-48, C48-4, C48-18, and C48-4 were colored red due to their degradation indexes being higher than 10. This indicated that there was significant DNA degradation in these samples and cannot be used for further analysis. Samples colored yellow had a degradation index between 1 and 10 which indicates slight to moderate degradation, but it is not enough to suppress further DNA analysis. Samples left colorless had degradation indexes below 1 and thus did not show any DNA degradation.

Table 9. includes the average IPC values and average degradation indexes of the ibuprofen-spiked blood samples collected from the midgut of leeches. All the samples had IPC values below 28.5 cycles which indicated that no inhibition was present in any of the ibuprofen samples. The samples colored yellow had slight to moderate DNA degradation but not enough to cause issues when amplified. The samples left colorless did not show any degradation due to their degradation indexes being below 1. All of the ibuprofen samples can be successfully amplified.

Chapter 6 : High-Performance Liquid Chromatography (HPLC) Methods

The blood samples collected from the leeches were also prepared and run through a high-performance liquid chromatography machine in order to figure out the composition of the blood. This process was also used to understand the concentrations of the compounds present in the blood.

HPLC Background

High-performance liquid chromatography (HPLC) can be used to identify chemical structures of drugs dissolved in a solution. This machine can also be utilized to give quantitative results regarding the concentration of these identified drugs in the solution. HPLC also surpasses other forms of chromatography in this regard due to the application of a liquid mobile phase along with transformable polarity of this mobile phase depending on the characteristics of the solution being examined. The stationary phase can also be modified in any way necessary, again depending on the experimental solution (Nikolin et.al, 2004). This experiment utilizes HPLC to figure out if the blood extracted from the leeches contains the experimental drugs or not and also to get quantitative data regarding the drug's concentration. This step was important as this gives a further insight of the digestive mechanisms of the leeches and how this affects the concentration of the drugs. Another reason this is important is to figure out if there was any drug in the extracted blood samples. If there is drug present, then a conclusion can be made regarding the effects of drugs on the extraction and quantification of DNA.

Preparing Standards

1 mg of each drug was weighed and added to 100 ml of Acetonitrile. This was used to make the 1000 ppm solution which was then used to make the 500 ppm, 250 ppm, 100 ppm, 50 ppm, 25 ppm, 10 ppm, 5 ppm, 1 ppm, and 0.5 ppm dilutions. Calculations for these dilutions were done using the $M_1V_1=M_2V_2$ equation. From these, 25 ppm was used as Standard 1, 50 ppm was used as Standard 2, 100 ppm was used as Standard 3, 250 ppm used for Standard 4, and 500 ppm was used for Standard 5. Once these dilutions were made, 1.5 ml of each solution was added to corresponding HPLC vials. An image of these vials is provided in Figure 11. These were then placed in the HPLC and run with an isocratic mobile phase composition of water:methanol 60:40 at 0.2 mL/min. The column used was column C18, 2.1 mm ID x 150 mm L x 5 μ L particles. The wavelength used was 190 nm. Data was then collected and analyzed using Excel.

These standards were used in an external calibration which was performed to quantify the three drugs in the standards and get information regarding their retention time.



Figure 11. 12 mm x 32 mm HPLC vial used for all of the HPLC runs

Collecting and Running HPLC Samples

The blood samples which were run through the HPLC were collected at the same as at the DNA extraction samples. The leech was dissected, and a sterile swab was used to swab from the pharynx to the middle of the crop and then back up to the pharynx. The tip of this swab was then cut off and placed in a labeled 2 mL tube and frozen until ready to be used for the HPLC methods.

The sample tubes were thawed for about an hour in order to let all components become liquid. 1.5 mL ACN was added to each of the tubes along with a drop of methanol. This was left in the refrigerator overnight to soak. Once all the solution had been soaking for about 24 hours, 1 mL of each sample was transferred into their corresponding HPLC vials using the inorganic

membrane filter shown in Figure 12. and placed into the machine. The samples were then run with an isocratic mobile phase composition of water:methanol 60:40 at 0.2 mL/min. The column used was column C18, 2.1 mm ID x 150 mm L x 5 μ L particles. The wavelength used was 190 nm. Each run was done for 9 minutes. The standards indicated in Table 10. were first run for 15 minutes in order to figure out the time at which all compounds have moved through the column. From this, 9 minutes was decided upon as the three drugs of interest had been detected by this time. 9 minutes also included the retention times of the three drugs indicated in prior research (Ribeiro et.al, 2019) (Shah et.al, 2012) (Patel et.al, 2013).

Once all the samples had been completed, graphs were produced, and each detected peak was analyzed and matched with the peaks found for the three drugs.

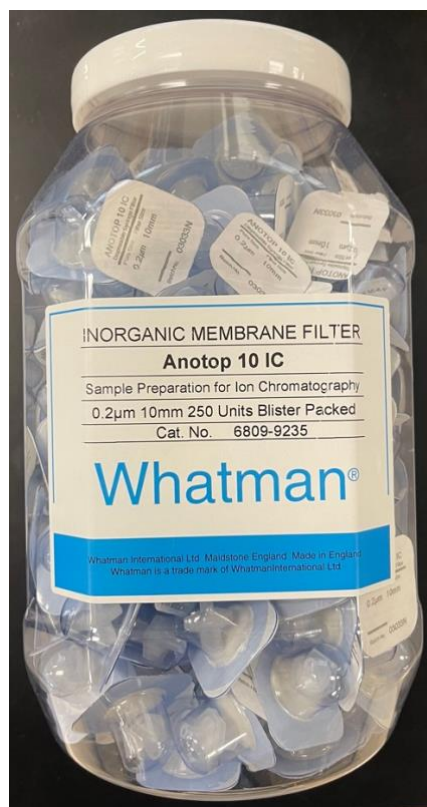


Figure 12. 0.2 μ L, 10 mm Anotop 10 IC inorganic membrane filters from Whatman

Chapter 7 : High-Performance Liquid Chromatography (HPLC) Results and Discussion

The above methods were used to collect HPLC data which has been presented and discussed in the sections below. This section only highlights the important peaks related to the three drugs of interest. All other peaks were kept out of the data included in this section. This full HPLC data can be accessed through Appendix B.

HPLC Results

Standard Results

Table 10. Aspirin, caffeine, and ibuprofen HPLC data from the 5 standard samples

Standard 1	Retention Time (mins)	Area	Height	Area %	Height %
Aspirin	3.497	1134038	96839	1.97	2.253
Caffeine	4.165	5592934	699974	9.715	16.286
Ibuprofen	5.034	4045603	287618	7.027	6.692

Standard 2	Retention Time (mins)	Area	Height	Area %	Height %
Aspirin	3.497	1134038	96839	1.97	2.253
Caffeine	4.165	5592934	699974	9.715	16.286
Ibuprofen	5.034	4045603	287618	7.027	6.692

Standard 3	Retention Time (mins)	Area	Height	Area %	Height %
Aspirin	3.302	106758	49686	0.952	2.569
Caffeine	4.131	1817074	553494	16.199	28.622
Ibuprofen	5.013	1352210	217590	12.055	11.252

Standard 4	Retention Time (mins)	Area	Height	Area %	Height %
Aspirin	3.246	204538	95598	1.383	3.155
Caffeine	4.108	2381943	861219	16.111	28.421
Ibuprofen	5.002	1582101	356970	10.701	11.78

Standard 5	Retention Time (mins)	Area	Height	Area %	Height %
Aspirin	3.217	494067	225832	3.089	6.196
Caffeine	4.098	2009463	775894	12.563	21.287
Ibuprofen	5	1969947	671861	12.316	18.433

These standards show the retention time for the three drugs as well as the areas, heights, area percents, and height percents of the aspirin, caffeine, and ibuprofen.

Leech Sample Results

Table 11. Aspirin data from the HPLC runs of the aspirin-spiked blood samples

Aspirin Samples	Retention Time (mins)	Area	Height	Area %	Height %

0-1	3.372	2277350	83452	4.146	2.208
0-6	3.374	740810	75315	1.443	2.311
0-10	3.372	736330	77189	1.419	1.773
A0-6	3.375	875838	103856	1.408	1.622
A0-8	3.344	1988618	328595	3.597	6.474
24-1	3.384	754518	80131	1.451	1.972
24-6	3.392	2534819	187222	4.87	4.124
24-8	3.388	990450	107103	1.908	2.253
A24-1	3.344	2079905	144676	3.81	3.13
A24-3	3.358	1353905	88102	2.447	1.718
48-5	3.386	2156778	330807	3.896	5.611
A48-1	3.349	815817	99514	1.499	2.259
AC-48	3.362	1022345	118235	1.799	2.313

Table 11. includes the identified aspirin peaks for all the aspirin-spiked blood samples. All samples had identifiable peaks.

Table 12. Caffeine data from the HPLC runs of the caffeine-spiked blood samples

Caffeine Samples	Retention Time (mins)	Area	Height	Area %	Height %
CC-0	3.937	13576657	783638	13.841	9.823
C0-9	4.041	12050989	1487481	13.965	18.838

C0-19	4.04	11902442	1274327	13.422	14.527
C24-16	4.038	10822347	1242702	13.02	15.765
C24-18	4.038	11926737	1310782	14.117	16.313
CC-24	4.049	1849401	242318	3.372	5.049
C48-4	4.04	10611005	1483623	11.868	16.137
C48-6	4.04	12094784	1441446	13.701	15.858
C48-12	4.035	8158589	1354875	7.892	10.906
C48-18	4.037	9094739	1263911	10.286	15.223
C48-20	4.041	12305535	1309043	13.771	15.494

Table 11. includes the identified caffeine peaks for all the caffeine-spiked blood samples. All samples had identifiable peaks.

Table 13. Ibuprofen data from the HPLC runs of the ibuprofen-spiked blood samples

Ibuprofen Samples	Retention Time (mins)	Area	Height	Area %	Height %
10-1	5.044	1242990	306516	1.536	3.812
10-3	5.042	1350314	292285	1.589	3.253
10-7	5.038	1086919	296697	1.423	4.308
10-8	5.041	1149047	278636	1.557	3.905

Table 11. includes the identified ibuprofen peaks for all the ibuprofen-spiked blood samples. All samples had identifiable peaks.

HPLC Discussion

The data collected from the standards was used as a reference for the retention times of the three different drugs. Papers analyzed in the background also gave insight into the retention times which were also used to identify the peaks in the HPLC graphs such as the ones shown in Figure 13. (Ribeiro et.al, 2019) (Shah et.al, 2012) (Patel et.al, 2013).

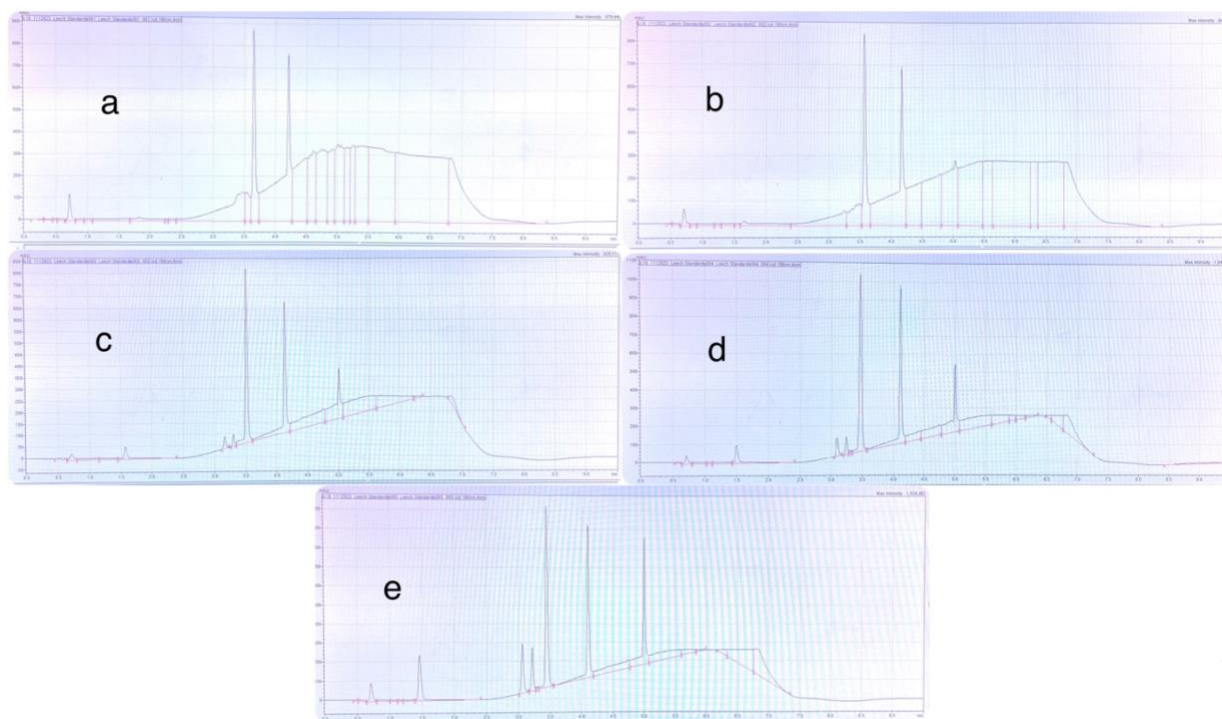


Figure 13. HPLC graphs of (a) Standard 1, (b) Standard 2, (c) Standard 3, (d) Standard 4, and (e) Standard 5

The standards provided with an average retention time of 3.352 minutes for aspirin. This shows that when the HPLC was run, the aspirin was detected at 3.352 minutes into the run. When the aspirin samples extracted from the leeches were run through the HPLC, they gave

similar results such as Figure 13. from which the peak at around 3.352 minutes was analyzed and labeled as the aspirin peak. If there was no peak present around this time, then it could be concluded that no aspirin was present in the sample. Table 11. shows the aspirin samples run through the HPLC. Each of these samples had a peak near 3.352 minutes, and thus it was concluded that aspirin was present in the blood sample after being extracted from the leech at all three time intervals.

The standards showed an average retention time of 4.133 minutes for caffeine. The HPLC data collected the blood samples containing caffeine were all analyzed for peaks near 4.133 minutes. Table 12. shows this analysis and provided that all the samples had a peak which could be identified as caffeine. This shows that caffeine was present in the samples after being digested by the leeches.

From the standards, it was found that ibuprofen has an average retention time of 5.017 minutes. Due to this, the HPLC results for the ibuprofen spiked blood samples was analyzed for peaks near 5.017 minutes. All of the samples showed significant peaks near this time and were thus labeled as being for this compound. Table 13. analyzes each of these samples and shows the retention time of ibuprofen in each of the samples.

Each of the blood samples were diluted in ACN before being placed in the HPLC. Due to this each HPLC run had a very large peak at 3.5 minutes (Ramesh et.al, 2014). This ACN peak was prominent in each of the graphs and was one of the highest peaks present due to most of the solution being ACN.

The peak area, height, area percent and height percent were also analyzed to see how large the peaks were. There was no conclusion made regarding the size of the peaks and their significance when compared to each other due to each leech having a different digestive system.

Each leech has different metabolite concentrations acting in the body and thus digest compounds at different rates. If the concentration of the three drugs was found to be low, then two conclusions can be made. Either the leech drank less amounts of blood when compared to others or the leech's digestive system was able to break down the drug at a faster rate. Due to these factors, only the presence of the peaks was analyzed and not the concentration itself.

Chapter 8 : Future Advancements and Experiment Limitation

If I were to perform this experiment again, I would make the following changes to better the experiment.

1. Increase the number of replicates for the HPLC samples. Due to time and resource restraints, replicates of each sample were not performed. If the number of replicates is increased, the results can be analyzed to a higher accuracy.
2. Euthanizing the leeches are more time levels. In this experiment, the leeches were euthanized at 0, 24, and 48 hours which can be increased to 0, 12, 24, 48, 36, and 72 hours. Leeches were able to survive in the petri dish for at least 72 hours so if this experiment is to be continued, Euthanization can take place up to hour 72 after feeding.
3. Use different concentrations of drugs. In this experiment, only one concentration of drug was tested due to time restraints but if this experiment is to be continued, more concentrations should be tested.
4. Using different types of drugs. Due to this being an undergraduate experiment, nothing other than over-the-counter drugs were utilized but if these methods are to be done in the forensics field, then more drugs should be tested. There are many ingestible drugs which victims and culprits can take and thus those should also be studied in order to see if successful DNA extraction and quantification methods can be performed with blood spiked with the said drugs.

Chapter 9 : Conclusion

The leeches were found to be alive even after 72 hours after they were fed, which showed that they would be able to withstand the stress which they are going to be put under in the experiment. This was an important part of this experiment as this let helped us figure out the feeding and Euthanization time increments. This was also done in room temperature which was another important aspect as this gave us insight regarding what conditions to store the leeches in while they digested the spiked blood.

The HPLC standards showed that the machine was able to detect presence of aspirin, caffeine, and ibuprofen at any concentration higher than 20ppm without any issues. Any concentrations below this became harder to detect due to disruptions. Due to this, all concentrations that should be analyzed in any further experiments, should be above this limit. In this experiment we used a 1:1 drug:blood ratio which is a high enough that of present, it should be detected. The HPLC standards also showed that when an isocratic mobile phase composition of water:methanol 60:40 at 0.2 mL/min and a column C18, 2.1 mm ID x 150 mm L x 5 μ L particles, is used, aspirin has a retention time in between 3.2 to 3.5 minutes, caffeine had a retention time in between 3.9 to 4.2 minutes, and ibuprofen had a retention time in between 5 and 5.1 minutes. With this, all the samples which were run through HPLC showed the presence of the drugs, indicating that the drug was still present in the sample after being extracted from the digestive system of the leech.

HPLC results showed that blood samples spiked with aspirin, ibuprofen, and caffeine can be successfully run through an HPLC. Peaks of the drugs can also be identified which can confirm their presence in the sample.

Due to most of the quantification samples, other than 5, showing none to moderate DNA degradation as well as having IPC values below 28.5 cycles, it can be concluded that having acidic and basic over-the-counter drugs does not hinder DNA analysis. Successful extraction and amplification can be performed with these drug-spiked blood samples and amplification can also be performed.

In conclusion, leeches are a great source to forensic science as they can be used to extract coagulated blood from a victim. This blood can then be tested in order to figure out if the person had any drugs in their system. Further DNA analysis can also be done with this sample in order to create a full DNA profile.

Appendix A : DNA Quantification Raw Data

Table 14. Raw quantification data for aspirin-spiked blood samples

Sample Name	Target Name	C _T	C _T Mean	Quantity	Quantity Mean	Degradation Index	Degradation Index Mean
A0-1	T.IPC	28.0417 7856	28.041 77856				
A0-1	T.Large Autosomal	37.5775 4135	37.577 54135	0.0023 79166	0.00237 9166	1.9184942 25	1.918494225
A0-1	T.Small Autosomal	38.4944 8013	38.494 48013	0.0045 64417	0.00456 4417	1.9184942 25	1.918494225
A0-1	T.Y	Undetermined					
A0-2	T.IPC	27.8869 2856	27.886 92856				
A0-2	T.Large Autosomal	38.0528 9841	38.052 89841	0.0016 63561	0.00166 3561		
A0-2	T.Small Autosomal	Undetermined					
A0-2	T.Y	Undetermined					
A0-3	T.IPC	27.6266 7465	27.626 67465				
A0-3	T.Large Autosomal	36.9928 4744	36.992 84744	0.0036 94462	0.00369 4462	1.0701669 45	1.070166945
A0-3	T.Small Autosomal	38.6682 2815	38.668 22815	0.0039 53691	0.00395 3691	1.0701669 45	1.070166945
A0-3	T.Y	Undetermined					
A0-4	T.IPC	27.8548 6221	27.854 86221				
A0-4	T.Large Autosomal	33.7224 9985	33.722 49985	0.0433 08355	0.04330 8355	1.4246333 84	1.424633384
A0-4	T.Small Autosomal	35.3447 2656	35.344 72656	0.0616 9853	0.06169 853	1.4246333 84	1.424633384
A0-4	T.Y	35.4640 5411	35.464 05411	0.0527 55989	0.05275 5989		
A0-5	T.IPC	27.4938 736	27.493 8736				
A0-5	T.Large Autosomal	Undetermined					
A0-5	T.Small Autosomal	Undetermined					

A0-5	T.Y	38.2502 8229	38.250 28229	0.0064 63361	0.00646 3361		
A0-6	T.IPC	27.5292 6064	27.529 26064				
A0-6	T.Large Autosomal	Undeter mined					
A0-6	T.Small Autosomal	Undeter mined					
A0-6	T.Y	Undeter mined					
A0-7	T.IPC	27.9236 5456	27.923 65456				
A0-7	T.Large Autosomal	35.9838 4094	35.983 84094	0.0078 95472	0.00789 5472	2.7598066 33	2.759806633
A0-7	T.Small Autosomal	36.6036 8729	36.603 68729	0.0217 89975	0.02178 9975	2.7598066 33	2.759806633
A0-7	T.Y	35.8824 234	35.882 4234	0.0384 90839	0.03849 0839		
A0-8	T.IPC	28.1830 5969	28.183 05969				
A0-8	T.Large Autosomal	29.8427 2766	29.842 72766	0.8031 61085	0.80316 1085	1.4330725 67	1.433072567
A0-8	T.Small Autosomal	31.8053 0548	31.805 30548	1.1509 88102	1.15098 8102	1.4330725 67	1.433072567
A0-8	T.Y	30.8568 2869	30.856 82869	1.6983 20866	1.69832 0866		
A0-9	T.IPC	27.9818 2297	27.981 82297				
A0-9	T.Large Autosomal	35.9554 9393	35.955 49393	0.0080 65741	0.00806 5741	0.7387954	0.7387954
A0-9	T.Small Autosomal	38.1720 0089	38.172 00089	0.0059 58932	0.00595 8932	0.7387954	0.7387954
A0-9	T.Y	38.0902 0233	38.090 20233	0.0072 91984	0.00729 1984		
A0-10	T.IPC	28.0336 8568	28.033 68568				
A0-10	T.Large Autosomal	38.9746 666	38.974 6666	0.0008 31244	0.00083 1244	2.3294167 52	2.329416752
A0-10	T.Small Autosomal	39.5317 1539	39.531 71539	0.0019 36313	0.00193 6313	2.3294167 52	2.329416752
A0-10	T.Y	37.2243 3853	37.224 33853	0.0140 02511	0.01400 2511		
Trio Standard 2	T.IPC	28.0515 8234	28.050 04311				

Trio Standard 2	T.Large Autosomal	27.0166 5306	27.546 44966	5			
Trio Standard 2	T.Small Autosomal	29.3566 5512	30.046 07201	5			
Trio Standard 2	T.Y	28.8317 6613	29.451 67923	5			
Trio Standard 2	T.IPC	28.0485 0388	28.050 04311				
Trio Standard 2	T.Large Autosomal	28.0762 4626	27.546 44966	5			
Trio Standard 2	T.Small Autosomal	30.7354 8889	30.046 07201	5			
Trio Standard 2	T.Y	30.0715 9424	29.451 67923	5			
A0-1R	T.IPC	27.7102 5276	27.710 25276				
A0-1R	T.Large Autosomal	36.7449 0738	36.744 90738	0.0044 52445	0.00445 2445	0.9769657 85	0.976965785
A0-1R	T.Small Autosomal	38.5527 1149	38.552 71149	0.0043 49886	0.00434 9886	0.9769657 85	0.976965785
A0-1R	T.Y	37.7625 0839	37.762 50839	0.0093 3439	0.00933 439		
A0-2R	T.IPC	27.7139 4157	27.713 94157				
A0-2R	T.Large Autosomal	Undeter mined					
A0-2R	T.Small Autosomal	Undeter mined					
A0-2R	T.Y	38.1755 6381	38.175 56381	0.0068 37708	0.00683 7708		
A0-3R	T.IPC	27.6056 2706	27.605 62706				
A0-3R	T.Large Autosomal	37.7495 9183	37.749 59183	0.0020 90184	0.00209 0184	3.0452120 3	3.04521203
A0-3R	T.Small Autosomal	38.0922 5082	38.092 25082	0.0063 65052	0.00636 5052	3.0452120 3	3.04521203
A0-3R	T.Y	Undeter mined					

A0-4R	T.IPC	27.6830 3871	27.683 03871				
A0-4R	T.Large Autosomal	33.0685 2341	33.068 52341	0.0708 50901	0.07085 0901	0.8353018 76	0.835301876
A0-4R	T.Small Autosomal	35.3950 9964	35.395 09964	0.0591 81891	0.05918 1891	0.8353018 76	0.835301876
A0-4R	T.Y	36.3138 1607	36.313 81607	0.0278 0872	0.02780 872		
A0-5R	T.IPC	27.4465 7898	27.446 57898				
A0-5R	T.Large Autosomal	Undeter mined					
A0-5R	T.Small Autosomal	39.3940 9256	39.394 09256	0.0021 69641	0.00216 9641		
A0-5R	T.Y	Undeter mined					
A0-6R	T.IPC	27.3936 9583	27.393 69583				
A0-6R	T.Large Autosomal	Undeter mined					
A0-6R	T.Small Autosomal	Undeter mined					
A0-6R	T.Y	Undeter mined					
A0-7R	T.IPC	27.8730 2589	27.873 02589				
A0-7R	T.Large Autosomal	39.0025 9781	39.002 59781	0.0008 13951	0.00081 3951	11.367506 98	11.36750698
A0-7R	T.Small Autosomal	37.6397 6288	37.639 76288	0.0092 52594	0.00925 2594	11.367506 98	11.36750698
A0-7R	T.Y	36.4658 6609	36.465 86609	0.0247 9828	0.02479 828		
A0-8R	T.IPC	27.7357 0251	27.735 70251				
A0-8R	T.Large Autosomal	29.6077 4612	29.607 74612	0.9585 48546	0.95854 8546	1.2860683 2	1.28606832
A0-8R	T.Small Autosomal	31.7222 8622	31.722 28622	1.2327 5888	1.23275 888	1.2860683 2	1.28606832
A0-8R	T.Y	30.9025 1732	30.902 51732	1.6408 45895	1.64084 5895		
A0-9R	T.IPC	27.9233 8181	27.923 38181				
A0-9R	T.Large Autosomal	39.5369 8349	39.536 98349	0.0005 44398	0.00054 4398		

A0-9R	T.Small Autosomal	Undeter mined					
A0-9R	T.Y	38.4612 8464	38.461 28464	0.0055 13233	0.00551 3233		
A0-10R	T.IPC	28.2145 462	28.214 5462				
A0-10R	T.Large Autosomal	38.9445 0378	38.944 50378	0.0008 50331	0.00085 0331	1.8412669 9	1.84126699
A0-10R	T.Small Autosomal	39.7887 0773	39.788 70773	0.0015 65687	0.00156 5687	1.8412669 9	1.84126699
A0-10R	T.Y	38.2151 0696	38.215 10696	0.0066 36969	0.00663 6969		
Trio Standard 3	T.IPC	27.8665 1802	27.779 93011				
Trio Standard 3	T.Large Autosomal	31.3367 5575	30.943 39371	0.5			
Trio Standard 3	T.Small Autosomal	33.6303 4058	33.392 34924	0.5			
Trio Standard 3	T.Y	33.0891 0751	32.978 46985	0.5			
Trio Standard 3	T.IPC	27.6933 4412	27.779 93011				
Trio Standard 3	T.Large Autosomal	30.5500 3357	30.943 39371	0.5			
Trio Standard 3	T.Small Autosomal	33.1543 5791	33.392 34924	0.5			
Trio Standard 3	T.Y	32.8678 3218	32.978 46985	0.5			
A24-1	T.IPC	27.4627 6855	27.462 76855				
A24-1	T.Large Autosomal	31.2877 2736	31.287 72736	0.2706 80547	0.27068 0547	11.888751 03	11.88875103
A24-1	T.Small Autosomal	30.5616 5123	30.561 65123	3.2180 53579	3.21805 3579	11.888751 03	11.88875103
A24-1	T.Y	29.8653 8315	29.865 38315	3.5849 03717	3.58490 3717		

A24-2	T.IPC	27.7710 3043	27.771 03043				
A24-2	T.Large Autosomal	38.4289 0549	38.428 90549	0.0012 53512	0.00125 3512	1.8882098 2	1.88820982
A24-2	T.Small Autosomal	39.2888 3743	39.288 83743	0.0023 66893	0.00236 6893	1.8882098 2	1.88820982
A24-2	T.Y	37.2807 2357	37.280 72357	0.0134 20031	0.01342 0031		
A24-3	T.IPC	27.6471 2524	27.647 12524				
A24-3	T.Large Autosomal	38.1893 8065	38.189 38065	0.0015 01153	0.00150 1153	1.0093933 34	1.009393334
A24-3	T.Small Autosomal	39.8283 1192	39.828 31192	0.0015 15254	0.00151 5254	1.0093933 34	1.009393334
A24-3	T.Y	Undeter mined					
A24-4	T.IPC	27.6213 4361	27.621 34361				
A24-4	T.Large Autosomal	Undeter mined					
A24-4	T.Small Autosomal	Undeter mined					
A24-4	T.Y	Undeter mined					
A24-5	T.IPC	27.4795 8565	27.479 58565				
A24-5	T.Large Autosomal	38.3313 4842	38.331 34842	0.0013 49019	0.00134 9019	3.0966448 78	3.096644878
A24-5	T.Small Autosomal	38.6016 4261	38.601 64261	0.0041 77434	0.00417 7434	3.0966448 78	3.096644878
A24-5	T.Y	Undeter mined					
A24-6	T.IPC	27.7031 7459	27.703 17459				
A24-6	T.Large Autosomal	Undeter mined					
A24-6	T.Small Autosomal	39.5316 5054	39.531 65054	0.0019 36417	0.00193 6417		
A24-6	T.Y	Undeter mined					
A24-7	T.IPC	27.4544 7731	27.454 47731				
A24-7	T.Large Autosomal	Undeter mined					

A24-7	T.Small Autosomal	37.7242 3935	37.724 23935	0.0086 28455	0.00862 8455		
A24-7	T.Y	Undeter mined					
A24-8	T.IPC	27.5060 5965	27.506 05965				
A24-8	T.Large Autosomal	35.4455 9479	35.445 59479	0.0118 39177	0.01183 9177	2.2932457 92	2.293245792
A24-8	T.Small Autosomal	36.3376 5793	36.337 65793	0.0271 50143	0.02715 0143	2.2932457 92	2.293245792
A24-8	T.Y	38.9360 733	38.936 0733	0.0038 55035	0.00385 5035		
A24-9	T.IPC	27.7836 0367	27.783 60367				
A24-9	T.Large Autosomal	Undeter mined					
A24-9	T.Small Autosomal	Undeter mined					
A24-9	T.Y	Undeter mined					
A24-10	T.IPC	27.7558 67	27.755 867				
A24-10	T.Large Autosomal	Undeter mined					
A24-10	T.Small Autosomal	Undeter mined					
A24-10	T.Y	Undeter mined					
Trio Standard 4	T.IPC	28.3435 0014	27.963 97018				
Trio Standard 4	T.Large Autosomal	32.5915 5655	32.588 69171	0.0500 00001			
Trio Standard 4	T.Small Autosomal	35.8485 2219	35.370 98312	0.0500 00001			
Trio Standard 4	T.Y	35.3632 431	34.770 38956	0.0500 00001			
Trio Standard 4	T.IPC	27.5844 4023	27.963 97018				

Trio Standard 4	T.Large Autosomal	32.5858 2306	32.588 69171	0.0500 00001			
Trio Standard 4	T.Small Autosomal	34.8934 4406	35.370 98312	0.0500 00001			
Trio Standard 4	T.Y	34.1775 3601	34.770 38956	0.0500 00001			
A24-1R	T.IPC	27.7031 0783	27.703 10783				
A24-1R	T.Large Autosomal	31.0345 4971	31.034 54971	0.3275 0392	0.32750 392	10.470332 15	10.47033215
A24-1R	T.Small Autosomal	30.4848 2513	30.484 82513	3.4290 74764	3.42907 4764	10.470332 15	10.47033215
A24-1R	T.Y	29.8027 7824	29.802 77824	3.7580 74522	3.75807 4522		
A24-2R	T.IPC	27.1977 4818	27.197 74818				
A24-2R	T.Large Autosomal	37.9224 5865	37.922 45865	0.0018 35174	0.00183 5174	0.8486597 54	0.848659754
A24-2R	T.Small Autosomal	39.7950 9735	39.795 09735	0.0015 57438	0.00155 7438	0.8486597 54	0.848659754
A24-2R	T.Y	Undeter mined					
A24-3R	T.IPC	27.6407 28	27.640 728				
A24-3R	T.Large Autosomal	36.9860 6491	36.986 06491	0.0037 1337	0.00371 337		
A24-3R	T.Small Autosomal	Undeter mined					
A24-3R	T.Y	Undeter mined					
A24-4R	T.IPC	27.5520 3247	27.552 03247				
A24-4R	T.Large Autosomal	38.1339 2258	38.133 92258	0.0015 6514	0.00156 514		
A24-4R	T.Small Autosomal	Undeter mined					
A24-4R	T.Y	Undeter mined					
A24-5R	T.IPC	27.6475 6584	27.647 56584				
A24-5R	T.Large Autosomal	Undeter mined					

A24-5R	T.Small Autosomal	Undeter mined					
A24-5R	T.Y	Undeter mined					
A24-6R	T.IPC	27.7461 853	27.746 1853				
A24-6R	T.Large Autosomal	Undeter mined					
A24-6R	T.Small Autosomal	38.3940 6967	38.394 06967	0.0049 59486	0.00495 9486		
A24-6R	T.Y	39.0137 7487	39.013 77487	0.0036 35799	0.00363 5799		
A24-7R	T.IPC	27.7438 3163	27.743 83163				
A24-7R	T.Large Autosomal	36.4830 246	36.483 0246	0.0054 22549	0.00542 2549	0.7341317 53	0.734131753
A24-7R	T.Small Autosomal	38.6599 4263	38.659 94263	0.0039 80866	0.00398 0866	0.7341317 53	0.734131753
A24-7R	T.Y	Undeter mined					
A24-8R	T.IPC	27.7258 2626	27.725 82626				
A24-8R	T.Large Autosomal	35.0145 7214	35.014 57214	0.0163 76287	0.01637 6287	1.5626623 63	1.562662363
A24-8R	T.Small Autosomal	36.4092 1402	36.409 21402	0.0255 90606	0.02559 0606	1.5626623 63	1.562662363
A24-8R	T.Y	35.2358 1696	35.235 81696	0.0626 56201	0.06265 6201		
A24-9R	T.IPC	27.9553 0319	27.955 30319				
A24-9R	T.Large Autosomal	Undeter mined					
A24-9R	T.Small Autosomal	Undeter mined					
A24-9R	T.Y	Undeter mined					
A24-10R	T.IPC	27.9128 5515	27.912 85515				
A24-10R	T.Large Autosomal	Undeter mined					
A24-10R	T.Small Autosomal	Undeter mined					
A24-10R	T.Y	Undeter mined					

Trio Standard 5	T.IPC	28.0457 6683	27.667 77802				
Trio Standard 5	T.Large Autosomal	37.6585 7697	37.029 36554	0.005			
Trio Standard 5	T.Small Autosomal	38.1392 5171	38.139 25171	0.005			
Trio Standard 5	T.Y	39.2258 4915	39.225 84915	0.005			
Trio Standard 5	T.IPC	27.2897 9111	27.667 77802				
Trio Standard 5	T.Large Autosomal	36.4001 5411	37.029 36554	0.005			
Trio Standard 5	T.Small Autosomal	Undeter mined	38.139 25171	0.005			
Trio Standard 5	T.Y	Undeter mined	39.225 84915	0.005			
A48-1	T.IPC	27.4576 2062	27.457 62062				
A48-1	T.Large Autosomal	Undeter mined					
A48-1	T.Small Autosomal	37.7068 5959	37.706 85959	0.0087 53325	0.00875 3325		
A48-1	T.Y	37.4921 6461	37.492 16461	0.0114 43474	0.01144 3474		
A48-2	T.IPC	27.7487 6022	27.748 76022				
A48-2	T.Large Autosomal	Undeter mined					
A48-2	T.Small Autosomal	Undeter mined					
A48-2	T.Y	38.0138 0157	38.013 80157	0.0077 2411	0.00772 411		
A48-3	T.IPC	27.4709 7015	27.470 97015				
A48-3	T.Large Autosomal	Undeter mined					

A48-3	T.Small Autosomal	Undeter mined					
A48-3	T.Y	Undeter mined					
A48-4	T.IPC	27.6155 2429	27.615 52429				
A48-4	T.Large Autosomal	38.2084 6176	38.208 46176	0.0014 79748	0.00147 9748	1.0917292 83	1.091729283
A48-4	T.Small Autosomal	39.7508 3542	39.750 83542	0.0016 15484	0.00161 5484	1.0917292 83	1.091729283
A48-4	T.Y	Undeter mined					
A48-5	T.IPC	27.6045 7039	27.604 57039				
A48-5	T.Large Autosomal	Undeter mined					
A48-5	T.Small Autosomal	Undeter mined					
A48-5	T.Y	Undeter mined					
A48-6	T.IPC	27.3471 2601	27.347 12601				
A48-6	T.Large Autosomal	Undeter mined					
A48-6	T.Small Autosomal	39.2285 6522	39.228 56522	0.0024 8782	0.00248 782		
A48-6	T.Y	Undeter mined					
A48-7	T.IPC	27.6759 491	27.675 9491				
A48-7	T.Large Autosomal	Undeter mined					
A48-7	T.Small Autosomal	39.6570 5109	39.657 05109	0.0017 45722	0.00174 5722		
A48-7	T.Y	Undeter mined					
A48-8	T.IPC	27.7643 9857	27.764 39857				
A48-8	T.Large Autosomal	37.7167 0914	37.716 70914	0.0021 42561	0.00214 2561	1.1056649 68	1.105664968
A48-8	T.Small Autosomal	39.2877 8458	39.287 78458	0.0023 68955	0.00236 8955	1.1056649 68	1.105664968
A48-8	T.Y	Undeter mined					

A48-9	T.IPC	27.8210 659	27.821 0659				
A48-9	T.Large Autosomal	Undeter mined					
A48-9	T.Small Autosomal	35.6539 6118	35.653 96118	0.0477 80097	0.04778 0097		
A48-9	T.Y	34.3850 2502	34.385 02502	0.1189 57512	0.11895 7512		
A48-10	T.IPC	28.0879 9744	28.087 99744				
A48-10	T.Large Autosomal	36.4313 2019	36.431 32019	0.0056 37737	0.00563 7737	0.9017786 38	0.901778638
A48-10	T.Small Autosomal	38.3640 7852	38.364 07852	0.0050 83991	0.00508 3991	0.9017786 38	0.901778638
A48-10	T.Y	Undeter mined					
AC-0	T.IPC	27.8511 0855	27.851 10855				
AC-0	T.Large Autosomal	Undeter mined					
AC-0	T.Small Autosomal	Undeter mined					
AC-0	T.Y	37.9356 0791	37.935 60791	0.0081 92904	0.00819 2904		
AC-0R	T.IPC	27.9218 4258	27.921 84258				
AC-0R	T.Large Autosomal	Undeter mined					
AC-0R	T.Small Autosomal	Undeter mined					
AC-0R	T.Y	Undeter mined					
A48-1R	T.IPC	27.7668 972	27.766 8972				
A48-1R	T.Large Autosomal	36.3441 9632	36.344 19632	0.0060 19828	0.00601 9828	0.3965789 08	0.396578908
A48-1R	T.Small Autosomal	39.2784 3475	39.278 43475	0.0023 87337	0.00238 7337	0.3965789 08	0.396578908
A48-1R	T.Y	38.1869 5068	38.186 95068	0.0067 79288	0.00677 9288		
A48-2R	T.IPC	27.5265 0642	27.526 50642				
A48-2R	T.Large Autosomal	Undeter mined					

A48-2R	T.Small Autosomal	Undeter mined					
A48-2R	T.Y	Undeter mined					
A48-3R	T.IPC	27.6806 7741	27.680 67741				
A48-3R	T.Large Autosomal	37.1745 1477	37.174 51477	0.0032 2231	0.00322 231	0.4806181 19	0.480618119
A48-3R	T.Small Autosomal	39.8019 0277	39.801 90277	0.0015 487	0.00154 87	0.4806181 19	0.480618119
A48-3R	T.Y	Undeter mined					
A48-4R	T.IPC	27.8990 4976	27.899 04976				
A48-4R	T.Large Autosomal	Undeter mined					
A48-4R	T.Small Autosomal	39.3652 7252	39.365 27252	0.0022 21956	0.00222 1956		
A48-4R	T.Y	Undeter mined					
A48-5R	T.IPC	27.5552 5208	27.555 25208				
A48-5R	T.Large Autosomal	Undeter mined					
A48-5R	T.Small Autosomal	Undeter mined					
A48-5R	T.Y	Undeter mined					
A48-6R	T.IPC	27.6801 3763	27.680 13763				
A48-6R	T.Large Autosomal	Undeter mined					
A48-6R	T.Small Autosomal	Undeter mined					
A48-6R	T.Y	37.9403 9917	37.940 39917	0.0081 63378	0.00816 3378		
A48-7R	T.IPC	27.6136 2267	27.613 62267				
A48-7R	T.Large Autosomal	Undeter mined					
A48-7R	T.Small Autosomal	Undeter mined					
A48-7R	T.Y	Undeter mined					

A48-8R	T.IPC	27.8158 2069	27.815 82069				
A48-8R	T.Large Autosomal	37.0421 7529	37.042 17529	0.0035 59809	0.00355 9809	1.1788742 54	1.178874254
A48-8R	T.Small Autosomal	38.5961 1511	38.596 11511	0.0041 96567	0.00419 6567	1.1788742 54	1.178874254
A48-8R	T.Y	Undeter mined					
A48-9R	T.IPC	27.7310 3714	27.731 03714				
A48-9R	T.Large Autosomal	Undeter mined					
A48-9R	T.Small Autosomal	35.1611 0229	35.161 10229	0.0718 13054	0.07181 3054		
A48-9R	T.Y	34.4421 9208	34.442 19208	0.1139 41923	0.11394 1923		
A48- 10R	T.IPC	27.9828 5294	27.982 85294				
A48- 10R	T.Large Autosomal	36.1676 2543	36.167 62543	0.0068 75465	0.00687 5465	0.8578031 66	0.857803166
A48- 10R	T.Small Autosomal	38.1844 7495	38.184 47495	0.0058 97796	0.00589 7796	0.8578031 66	0.857803166
A48- 10R	T.Y	Undeter mined					
AC-24	T.IPC	27.9796 5813	27.979 65813				
AC-24	T.Large Autosomal	Undeter mined					
AC-24	T.Small Autosomal	Undeter mined					
AC-24	T.Y	Undeter mined					
AC-24R	T.IPC	27.9416 4085	27.941 64085				
AC-24R	T.Large Autosomal	Undeter mined					
AC-24R	T.Small Autosomal	39.6656 3797	39.665 63797	0.0017 33373	0.00173 3373		
AC-24R	T.Y	Undeter mined					
NTC	T.IPC	27.6907 9399	27.579 02527				
NTC	T.Large Autosomal	37.9946 8613					

NTC	T.Small Autosomal	Undeter mined					
NTC	T.Y	38.0420 2271					
AC-48	T.IPC	28.1133 8234	28.113 38234				
AC-48	T.Large Autosomal	32.0837 7838	32.083 77838	0.1486 76261	0.14867 6261		
AC-48	T.Small Autosomal	31.5790 4243	31.579 04243	1.3877 41208	1.38774 1208		
AC-48	T.Y	30.7432 6897	30.743 26897	1.8500 47946	1.85004 7946		
AC-48R	T.IPC	27.5286 9987	27.528 69987				
AC-48R	T.Large Autosomal	31.3836 5173	31.383 65173	0.2518 26257	0.25182 6257		
AC-48R	T.Small Autosomal	31.0314 1403	31.031 41403	2.1823 74716	2.18237 4716		
AC-48R	T.Y	29.9951 8013	29.995 18013	3.2508 76665	3.25087 6665	9.3339796 07	9.333979607

Table 15. Raw quantification data for caffeine-spiked blood samples

Sample Name	Target Name	C _T	C _T Mean	Quantity	Quantity Mean	Degradation Index	Degradation Index Mean
Trio Standard 1	T.IPC	29.1269 2642	29.441 89453				
Trio Standard 1	T.Large Autosomal	19.5986 7859	19.700 31738	50			
Trio Standard 1	T.Small Autosomal	21.7005 3482	21.936 17249	50			
Trio Standard 1	T.Y	21.4122 2	21.410 2478	50			
Trio Standard 1	T.IPC	29.7568 6264	29.441 89453				
Trio Standard 1	T.Large Autosomal	19.8019 5808	19.700 31738	50			

Trio Standard 1	T.Small Autosomal	22.1718 1206	21.936 17249	50			
Trio Standard 1	T.Y	21.4082 7751	21.410 2478	50			
c0-4	T.IPC	27.5976 2764	27.597 62764				
c0-4	T.Large Autosomal	Undeter mined					
c0-4	T.Small Autosomal	38.3949 2416	38.394 92416	0.0003 14787	0.00031 4787		
c0-4	T.Y	Undeter mined					
c0-4r	T.IPC	27.3121 7957	27.312 17957				
c0-4r	T.Large Autosomal	Undeter mined					
c0-4r	T.Small Autosomal	Undeter mined					
c0-4r	T.Y	39.5804 7485	39.580 47485	8.1737 6E-05	8.17376 E-05		
c24-2	T.IPC	27.0968 0176	27.096 80176				
c24-2	T.Large Autosomal	Undeter mined					
c24-2	T.Small Autosomal	Undeter mined					
c24-2	T.Y	Undeter mined					
c24-2r	T.IPC	27.2071 5523	27.207 15523				
c24-2r	T.Large Autosomal	Undeter mined					
c24-2r	T.Small Autosomal	Undeter mined					
c24-2r	T.Y	Undeter mined					
c24-10	T.IPC	27.1487 8654	27.148 78654				
c24-10	T.Large Autosomal	38.0759 4681	38.075 94681	0.0001 20357	0.00012 0357		
c24-10	T.Small Autosomal	Undeter mined					

c24-10	T.Y	Undeter mined					
c24-10r	T.IPC	27.1422 8821	27.142 28821				
c24-10r	T.Large Autosomal	Undeter mined					
c24-10r	T.Small Autosomal	38.0402 298	38.040 2298	0.0004 07516	0.00040 7516		
c24-10r	T.Y	Undeter mined					
c48-8	T.IPC	26.9702 1675	26.970 21675				
c48-8	T.Large Autosomal	Undeter mined					
c48-8	T.Small Autosomal	Undeter mined					
c48-8	T.Y	Undeter mined					
c48-8r	T.IPC	27.7009 6588	27.700 96588				
c48-8r	T.Large Autosomal	Undeter mined					
c48-8r	T.Small Autosomal	Undeter mined					
c48-8r	T.Y	Undeter mined					
Trio Standard 2	T.IPC	28.6235 2753	28.510 07462				
Trio Standard 2	T.Large Autosomal	22.9903 2021	22.975 52109	5			
Trio Standard 2	T.Small Autosomal	25.2452 0111	25.149 63531	5			
Trio Standard 2	T.Y	24.4373 455	24.454 62036	5			
Trio Standard 2	T.IPC	28.3966 2361	28.510 07462				
Trio Standard 2	T.Large Autosomal	22.9607 2006	22.975 52109	5			

Trio Standard 2	T.Small Autosomal	25.0540 6761	25.149 63531	5			
Trio Standard 2	T.Y	24.4718 9522	24.454 62036	5			
c0-5	T.IPC	27.4536 2473	27.453 62473				
c0-5	T.Large Autosomal	Undeter mined					
c0-5	T.Small Autosomal	38.6300 6973	38.630 06973	0.0002 65266	0.00026 5266		
c0-5	T.Y	36.9849 205	36.984 9205	0.0005 47256	0.00054 7256		
c0-5r	T.IPC	27.3388 0043	27.338 80043				
c0-5r	T.Large Autosomal	34.2544 6701	34.254 46701	0.0017 74381	0.00177 4381	0.8990945 82	0.899094582
c0-5r	T.Small Autosomal	36.1653 1754	36.165 31754	0.0015 95336	0.00159 5336	0.8990945 82	0.899094582
c0-5r	T.Y	36.9690 3992	36.969 03992	0.0005 53659	0.00055 3659		
c24-3	T.IPC	26.8817 9588	26.881 79588				
c24-3	T.Large Autosomal	Undeter mined					
c24-3	T.Small Autosomal	Undeter mined					
c24-3	T.Y	Undeter mined					
c24-3r	T.IPC	27.4794 5023	27.479 45023				
c24-3r	T.Large Autosomal	Undeter mined					
c24-3r	T.Small Autosomal	Undeter mined					
c24-3r	T.Y	Undeter mined					
c48-1	T.IPC	27.1540 6036	27.154 06036				
c48-1	T.Large Autosomal	Undeter mined					
c48-1	T.Small Autosomal	Undeter mined					

c48-1	T.Y	Undeter mined					
c48-1r	T.IPC	27.3291 7213	27.329 17213				
c48-1r	T.Large Autosomal	37.2229 0039	37.222 90039	0.0002 19444	0.00021 9444	1.0398803 95	1.039880395
c48-1r	T.Small Autosomal	38.8368 6447	38.836 86447	0.0002 28196	0.00022 8196	1.0398803 95	1.039880395
c48-1r	T.Y	Undeter mined					
c48-9	T.IPC	27.0799 4843	27.079 94843				
c48-9	T.Large Autosomal	37.1068 306	37.106 8306	0.0002 38132	0.00023 8132	5.3648128 51	5.364812851
c48-9	T.Small Autosomal	36.4705 1239	36.470 51239	0.0012 77534	0.00127 7534	5.3648128 51	5.364812851
c48-9	T.Y	Undeter mined					
c48-9r	T.IPC	27.3871 4027	27.387 14027				
c48-9r	T.Large Autosomal	Undeter mined					
c48-9r	T.Small Autosomal	36.8096 8857	36.809 68857	0.0009 98045	0.00099 8045		
c48-9r	T.Y	35.9972 5723	35.997 25723	0.0011 28268	0.00112 8268		
Trio Standard 3	T.IPC	27.5319 7479	27.567 01279				
Trio Standard 3	T.Large Autosomal	26.2479 4769	26.234 19762	0.5			
Trio Standard 3	T.Small Autosomal	28.2266 9411	28.196 60759	0.5			
Trio Standard 3	T.Y	27.7036 3045	27.690 59372	0.5			
Trio Standard 3	T.IPC	27.6020 5078	27.567 01279				
Trio Standard 3	T.Large Autosomal	26.2204 4754	26.234 19762	0.5			

Trio Standard 3	T.Small Autosomal	28.1665 2107	28.196 60759	0.5			
Trio Standard 3	T.Y	27.6775 5508	27.690 59372	0.5			
c0-6	T.IPC	27.2944 4695	27.294 44695				
c0-6	T.Large Autosomal	Undeter mined					
c0-6	T.Small Autosomal	37.5426 5594	37.542 65594	0.0005 85383	0.00058 5383		
c0-6	T.Y	Undeter mined					
c0-6r	T.IPC	27.2835 1212	27.283 51212				
c0-6r	T.Large Autosomal	31.5846 8628	31.584 68628	0.0116 26356	0.01162 6356	1.2116025 69	1.211602569
c0-6r	T.Small Autosomal	33.1729 8889	33.172 98889	0.0140 86523	0.01408 6523	1.2116025 69	1.211602569
c0-6r	T.Y	32.8609 7717	32.860 97717	0.0112 25634	0.01122 5634		
c24-4	T.IPC	27.2565 155	27.256 5155				
c24-4	T.Large Autosomal	38.8335 3043	38.833 53043	7.0600 4E-05	7.06004 E-05	13.001908 3	13.0019083
c24-4	T.Small Autosomal	36.9246 2921	36.924 62921	0.0009 1794	0.00091 794	13.001908 3	13.0019083
c24-4	T.Y	Undeter mined					
c24-4r	T.IPC	27.2331 9054	27.233 19054				
c24-4r	T.Large Autosomal	Undeter mined					
c24-4r	T.Small Autosomal	Undeter mined					
c24-4r	T.Y	Undeter mined					
c48-2	T.IPC	27.0956 8214	27.095 68214				
c48-2	T.Large Autosomal	Undeter mined					
c48-2	T.Small Autosomal	Undeter mined					

c48-2	T.Y	Undeter mined					
c48-2r	T.IPC	27.3600 2541	27.360 02541				
c48-2r	T.Large Autosomal	Undeter mined					
c48-2r	T.Small Autosomal	38.2633 7051	38.263 37051	0.0003 46421	0.00034 6421		
c48-2r	T.Y	Undeter mined					
c48-10	T.IPC	27.4655 2849	27.465 52849				
c48-10	T.Large Autosomal	Undeter mined					
c48-10	T.Small Autosomal	39.1056 5948	39.105 65948	0.0001 87644	0.00018 7644		
c48-10	T.Y	Undeter mined					
c48-10r	T.IPC	27.3104 6295	27.310 46295				
c48-10r	T.Large Autosomal	Undeter mined					
c48-10r	T.Small Autosomal	37.2669 6396	37.266 96396	0.0007 15474	0.00071 5474		
c48-10r	T.Y	Undeter mined					
Trio Standard 4	T.IPC	27.5724 3729	27.453 46069				
Trio Standard 4	T.Large Autosomal	29.6684 3987	29.528 88489	0.0500 00001			
Trio Standard 4	T.Small Autosomal	31.2559 7572	31.473 40393	0.0500 00001			
Trio Standard 4	T.Y	30.9633 2169	30.967 64183	0.0500 00001			
Trio Standard 4	T.IPC	27.3344 841	27.453 46069				
Trio Standard 4	T.Large Autosomal	29.3893 28	29.528 88489	0.0500 00001			

Trio Standard 4	T.Small Autosomal	31.6908 3023	31.473 40393	0.0500 00001			
Trio Standard 4	T.Y	30.9719 6198	30.967 64183	0.0500 00001			
c0-7	T.IPC	27.1792 1829	27.179 21829				
c0-7	T.Large Autosomal	Undeter mined					
c0-7	T.Small Autosomal	Undeter mined					
c0-7	T.Y	Undeter mined					
c0-7r	T.IPC	27.3764 3242	27.376 43242				
c0-7r	T.Large Autosomal	Undeter mined					
c0-7r	T.Small Autosomal	Undeter mined					
c0-7r	T.Y	Undeter mined					
c24-5	T.IPC	27.2552 8145	27.255 28145				
c24-5	T.Large Autosomal	Undeter mined					
c24-5	T.Small Autosomal	Undeter mined					
c24-5	T.Y	Undeter mined					
c24-5r	T.IPC	27.2373 9815	27.237 39815				
c24-5r	T.Large Autosomal	36.3439 8651	36.343 98651	0.0004 07464	0.00040 7464	0.8959155 68	0.895915568
c24-5r	T.Small Autosomal	38.1913 9862	38.191 39862	0.0003 65053	0.00036 5053	0.8959155 68	0.895915568
c24-5r	T.Y	Undeter mined					
c48-3	T.IPC	26.9733 2954	26.973 32954				
c48-3	T.Large Autosomal	33.4445 4575	33.444 54575	0.0031 38442	0.00313 8442	3.0773854 26	3.077385426
c48-3	T.Small Autosomal	33.6914 7873	33.691 47873	0.0096 58194	0.00965 8194	3.0773854 26	3.077385426

c48-3	T.Y	34.4128 685	34.412 8685	0.0036 01481	0.00360 1481		
c48-3r	T.IPC	27.0682 0488	27.068 20488				
c48-3r	T.Large Autosomal	Undeter mined					
c48-3r	T.Small Autosomal	37.9867 9352	37.986 79352	0.0004 23679	0.00042 3679		
c48-3r	T.Y	Undeter mined					
Trio Standard 5	T.IPC	27.8573 9326	27.704 79202				
Trio Standard 5	T.Large Autosomal	32.9347 3816	32.775 06638	0.005			
Trio Standard 5	T.Small Autosomal	34.7858 5434	34.590 9996	0.005			
Trio Standard 5	T.Y	33.7294 0826	33.870 13245	0.005			
Trio Standard 5	T.IPC	27.5521 9269	27.704 79202				
Trio Standard 5	T.Large Autosomal	32.6153 9459	32.775 06638	0.005			
Trio Standard 5	T.Small Autosomal	34.3961 4487	34.590 9996	0.005			
Trio Standard 5	T.Y	34.0108 5663	33.870 13245	0.005			
c0-8	T.IPC	27.1403 8277	27.140 38277				
c0-8	T.Large Autosomal	Undeter mined					
c0-8	T.Small Autosomal	Undeter mined					
c0-8	T.Y	Undeter mined					
c0-8r	T.IPC	27.2074 4896	27.207 44896				

c0-8r	T.Large Autosomal	Undeter mined					
c0-8r	T.Small Autosomal	Undeter mined					
c0-8r	T.Y	Undeter mined					
c24-6	T.IPC	27.2080 8601	27.208 08601				
c24-6	T.Large Autosomal	Undeter mined					
c24-6	T.Small Autosomal	Undeter mined					
c24-6	T.Y	Undeter mined					
c24-6r	T.IPC	27.3057 5943	27.305 75943				
c24-6r	T.Large Autosomal	Undeter mined					
c24-6r	T.Small Autosomal	Undeter mined					
c24-6r	T.Y	Undeter mined					
c48-4	T.IPC	27.2079 4296	27.207 94296				
c48-4	T.Large Autosomal	32.3169 6701	32.316 96701	0.0069 42538	0.00694 2538	16.599006 65	16.59900665
c48-4	T.Small Autosomal	30.2855 3963	30.285 53963	0.1152 39233	0.11523 9233	16.599006 65	16.59900665
c48-4	T.Y	29.6941 3948	29.694 13948	0.1142 17103	0.11421 7103		
c48-4r	T.IPC	27.2103 138	27.210 3138				
c48-4r	T.Large Autosomal	32.4151 535	32.415 1535	0.0064 78786	0.00647 8786	17.323247 91	17.32324791
c48-4r	T.Small Autosomal	30.3218 4601	30.321 84601	0.1122 33616	0.11223 3616	17.323247 91	17.32324791
c48-4r	T.Y	29.3728 9238	29.372 89238	0.1445 22339	0.14452 2339		
c0-1	T.IPC	27.8469 7914	27.846 97914				
c0-1	T.Large Autosomal	38.3328 9337	38.332 89337	0.0001 00438	0.00010 0438	2.8899960 52	2.889996052
c0-1	T.Small Autosomal	38.5063 4003	38.506 34003	0.0002 90265	0.00029 0265	2.8899960 52	2.889996052

c0-1	T.Y	Undeter mined					
c0-1r	T.IPC	27.3908 596	27.390 8596				
c0-1r	T.Large Autosomal	Undeter mined					
c0-1r	T.Small Autosomal	Undeter mined					
c0-1r	T.Y	38.1351 5472	38.135 15472	0.0002 35639	0.00023 5639		
c0-9	T.IPC	27.1564 4073	27.156 44073				
c0-9	T.Large Autosomal	Undeter mined					
c0-9	T.Small Autosomal	Undeter mined					
c0-9	T.Y	Undeter mined					
c0-9r	T.IPC	27.3259 8686	27.325 98686				
c0-9r	T.Large Autosomal	Undeter mined					
c0-9r	T.Small Autosomal	37.9365 8066	37.936 58066	0.0004 39451	0.00043 9451		
c0-9r	T.Y	Undeter mined					
c24-7	T.IPC	27.4555 4352	27.455 54352				
c24-7	T.Large Autosomal	Undeter mined					
c24-7	T.Small Autosomal	38.2027 1301	38.202 71301	0.0003 62059	0.00036 2059		
c24-7	T.Y	39.3871 4981	39.387 14981	9.4173 3E-05	9.41733 E-05		
c24-7r	T.IPC	27.1715 5838	27.171 55838				
c24-7r	T.Large Autosomal	Undeter mined					
c24-7r	T.Small Autosomal	Undeter mined					
c24-7r	T.Y	Undeter mined					
c48-5	T.IPC	27.1930 275	27.193 0275				

c48-5	T.Large Autosomal	Undeter mined					
c48-5	T.Small Autosomal	Undeter mined					
c48-5	T.Y	Undeter mined					
c48-5r	T.IPC	27.3300 2663	27.330 02663				
c48-5r	T.Large Autosomal	Undeter mined					
c48-5r	T.Small Autosomal	39.2493 6676	39.249 36676	0.0001 69007	0.00016 9007		
c48-5r	T.Y	Undeter mined					
c0-2	T.IPC	27.7885 17	27.788 517				
c0-2	T.Large Autosomal	35.8449 0585	35.844 90585	0.0005 79034	0.00057 9034	2.3321127 89	2.332112789
c0-2	T.Small Autosomal	36.3943 367	36.394 3367	0.0013 50372	0.00135 0372	2.3321127 89	2.332112789
c0-2	T.Y	Undeter mined					
c0-2r	T.IPC	27.4383 8501	27.438 38501				
c0-2r	T.Large Autosomal	36.5278 7399	36.527 87399	0.0003 57979	0.00035 7979	5.2070140 84	5.207014084
c0-2r	T.Small Autosomal	35.9514 9612	35.951 49612	0.0018 64003	0.00186 4003	5.2070140 84	5.207014084
c0-2r	T.Y	Undeter mined					
c0-10	T.IPC	27.3153 1906	27.315 31906				
c0-10	T.Large Autosomal	Undeter mined					
c0-10	T.Small Autosomal	Undeter mined					
c0-10	T.Y	39.7645 7214	39.764 57214	7.1425 3E-05	7.14253 E-05		
c0-10r	T.IPC	27.9212 8754	27.921 28754				
c0-10r	T.Large Autosomal	37.5019 3405	37.501 93405	0.0001 80301	0.00018 0301	2.7222228 05	2.722222805
c0-10r	T.Small Autosomal	37.7847 0612	37.784 70612	0.0004 9082	0.00049 082	2.7222228 05	2.722222805

c0-10r	T.Y	Undeter mined					
c24-8	T.IPC	27.5375 7668	27.537 57668				
c24-8	T.Large Autosomal	37.2325 6302	37.232 56302	0.0002 17956	0.00021 7956		
c24-8	T.Small Autosomal	Undeter mined					
c24-8	T.Y	Undeter mined					
c24-8r	T.IPC	27.4757 4043	27.475 74043				
c24-8r	T.Large Autosomal	Undeter mined					
c24-8r	T.Small Autosomal	39.4038 6581	39.403 86581	0.0001 5103	0.00015 103		
c24-8r	T.Y	Undeter mined					
c48-6	T.IPC	27.5186 7104	27.518 67104				
c48-6	T.Large Autosomal	Undeter mined					
c48-6	T.Small Autosomal	Undeter mined					
c48-6	T.Y	Undeter mined					
c48-6r	T.IPC	27.2969 8563	27.296 98563				
c48-6r	T.Large Autosomal	Undeter mined					
c48-6r	T.Small Autosomal	Undeter mined					
c48-6r	T.Y	Undeter mined					
c0-3	T.IPC	27.9467 0296	27.946 70296				
c0-3	T.Large Autosomal	38.7733 2306	38.773 32306	7.3657 7E-05	7.36577 E-05		
c0-3	T.Small Autosomal	Undeter mined					
c0-3	T.Y	Undeter mined					
c0-3r	T.IPC	27.3964 1571	27.396 41571				

c0-3r	T.Large Autosomal	38.0544 3954	38.054 43954	0.0001 22193	0.00012 2193	1.7394374 61	1.739437461
c0-3r	T.Small Autosomal	38.9344 5969	38.934 45969	0.0002 12547	0.00021 2547	1.7394374 61	1.739437461
c0-3r	T.Y	Undeter mined					
c24-1	T.IPC	27.8893 8332	27.889 38332				
c24-1	T.Large Autosomal	35.6614 5706	35.661 45706	0.0006 58872	0.00065 8872	2.7352669 24	2.735266924
c24-1	T.Small Autosomal	35.9978 2562	35.997 82562	0.0018 02191	0.00180 2191	2.7352669 24	2.735266924
c24-1	T.Y	Undeter mined					
c24-1r	T.IPC	27.3187 6183	27.318 76183				
c24-1r	T.Large Autosomal	Undeter mined					
c24-1r	T.Small Autosomal	Undeter mined					
c24-1r	T.Y	Undeter mined					
c24-9	T.IPC	27.3677 3872	27.367 73872				
c24-9	T.Large Autosomal	Undeter mined					
c24-9	T.Small Autosomal	Undeter mined					
c24-9	T.Y	Undeter mined					
c24-9r	T.IPC	27.4157 9628	27.415 79628				
c24-9r	T.Large Autosomal	Undeter mined					
c24-9r	T.Small Autosomal	Undeter mined					
c24-9r	T.Y	Undeter mined					
c48-7	T.IPC	27.4675 9415	27.467 59415				
c48-7	T.Large Autosomal	Undeter mined					
c48-7	T.Small Autosomal	38.4885 1395	38.488 51395	0.0002 94056	0.00029 4056		

c48-7	T.Y	Undeter mined					
c48-7r	T.IPC	27.5521 0114	27.552 10114				
c48-7r	T.Large Autosomal	Undeter mined					
c48-7r	T.Small Autosomal	Undeter mined					
c48-7r	T.Y	39.5561 7905	39.556 17905	8.3205 4E-05	8.32054 E-05		
C0-11	T.IPC	27.3323 7076	27.332 37076				
C0-11	T.Large Autosomal	36.0521 9269	36.052 19269	0.0001 54737	0.00015 4737	0.2666282 95	0.266628295
C0-11	T.Small Autosomal	38.2873 6115	38.287 36115	4.1257 2E-05	4.12572 E-05	0.2666282 95	0.266628295
C0-11	T.Y	Undeter mined					
C0-12	T.IPC	27.1862 3734	27.186 23734				
C0-12	T.Large Autosomal	34.8712 6541	34.871 26541	0.0004 49923	0.00044 9923	3.6928765 77	3.692876577
C0-12	T.Small Autosomal	34.7293 8919	34.729 38919	0.0016 6151	0.00166 151	3.6928765 77	3.692876577
C0-12	T.Y	33.9952 2018	33.995 22018	0.0025 89973	0.00258 9973		
C0-13	T.IPC	27.3643 8179	27.364 38179				
C0-13	T.Large Autosomal	36.8394 5847	36.839 45847	7.5957 5E-05	7.59575 E-05	2.3890974 52	2.389097452
C0-13	T.Small Autosomal	36.8612 8235	36.861 28235	0.0001 8147	0.00018 147	2.3890974 52	2.389097452
C0-13	T.Y	36.9778 3661	36.977 83661	0.0001 46121	0.00014 6121		
C0-14	T.IPC	27.4071 9795	27.407 19795				
C0-14	T.Large Autosomal	37.6640 4343	37.664 04343	3.6049 5E-05	3.60495 E-05	5.7582736 02	5.758273602
C0-14	T.Small Autosomal	36.7318 4967	36.731 84967	0.0002 07583	0.00020 7583	5.7582736 02	5.758273602
C0-14	T.Y	37.8059 5016	37.805 95016	6.5772 8E-05	6.57728 E-05		
C0-15	T.IPC	27.2963 0661	27.296 30661				

C0-15	T.Large Autosomal	36.5260 5057	36.526 05057	0.0001 0083	0.00010 083	0.3062469 66	0.306246966
C0-15	T.Small Autosomal	38.5663 147	38.566 3147	0.0000 30879	0.00003 0879	0.3062469 66	0.306246966
C0-15	T.Y	39.5845 5276	39.584 55276	1.1843 7E-05	1.18437 E-05		
C0-16	T.IPC	27.1553 9742	27.155 39742				
C0-16	T.Large Autosomal	34.2546 6156	34.254 66156	0.0007 85545	0.00078 5545	0.7591803 67	0.759180367
C0-16	T.Small Autosomal	35.7158 3557	35.715 83557	0.0005 96371	0.00059 6371	0.7591803 67	0.759180367
C0-16	T.Y	36.7243 9957	36.724 39957	0.0001 86554	0.00018 6554		
C0-17	T.IPC	27.2987 8044	27.298 78044				
C0-17	T.Large Autosomal	34.6417 1982	34.641 71982	0.0005 53657	0.00055 3657	0.5474704 5	0.54747045
C0-17	T.Small Autosomal	36.3673 8586	36.367 38586	0.0003 03111	0.00030 3111	0.5474704 5	0.54747045
C0-17	T.Y	38.1759 0332	38.175 90332	4.6044 4E-05	4.60444 E-05		
C0-18	T.IPC	27.4899 9214	27.489 99214				
C0-18	T.Large Autosomal	36.4165 3442	36.416 53442	0.0001 11322	0.00011 1322	0.4576925 93	0.457692593
C0-18	T.Small Autosomal	38.0841 8274	38.084 18274	5.0951 1E-05	5.09511 E-05	0.4576925 93	0.457692593
C0-18	T.Y	Undeter mined					
C0-19	T.IPC	27.3814 3539	27.381 43539				
C0-19	T.Large Autosomal	37.9567 7948	37.956 77948	2.7668 9E-05	2.76689 E-05	0.9814806 58	0.981480658
C0-19	T.Small Autosomal	38.6899 8718	38.689 98718	2.7156 5E-05	2.71565 E-05	0.9814806 58	0.981480658
C0-19	T.Y	38.4783 0963	38.478 30963	0.0000 34402	0.00003 4402		
C0-20	T.IPC	27.7723 1407	27.772 31407				
C0-20	T.Large Autosomal	36.0720 787	36.072 0787	0.0001 5198	0.00015 198	9.6941862 11	9.694186211
C0-20	T.Small Autosomal	34.8451 1566	34.845 11566	0.0014 73325	0.00147 3325	9.6941862 11	9.694186211

C0-20	T.Y	34.6116 7908	34.611 67908	0.0014 29661	0.00142 9661		
Trio Standard 4	T.IPC	27.7733 4023	27.591 78543				
Trio Standard 4	T.Large Autosomal	29.8584 3849	29.776 61896	0.0500 00001			
Trio Standard 4	T.Small Autosomal	31.7771 759	31.730 2742	0.0500 00001			
Trio Standard 4	T.Y	31.1973 7625	30.930 15289	0.0500 00001			
Trio Standard 4	T.IPC	27.4102 3254	27.591 78543				
Trio Standard 4	T.Large Autosomal	29.6947 9942	29.776 61896	0.0500 00001			
Trio Standard 4	T.Small Autosomal	31.6833 725	31.730 2742	0.0500 00001			
Trio Standard 4	T.Y	30.6629 3144	30.930 15289	0.0500 00001			
C0-11R	T.IPC	27.2542 1143	27.254 21143				
C0-11R	T.Large Autosomal	34.7936 8973	34.793 68973	0.0004 82601	0.00048 2601	0.3670155 41	0.367015541
C0-11R	T.Small Autosomal	36.8846 283	36.884 6283	0.0001 77122	0.00017 7122	0.3670155 41	0.367015541
C0-11R	T.Y	Undeter mined					
C0-12R	T.IPC	27.1478 4813	27.147 84813				
C0-12R	T.Large Autosomal	33.9881 4774	33.988 14774	0.0009 99505	0.00099 9505	0.6776419 88	0.677641988
C0-12R	T.Small Autosomal	35.5933 1512	35.593 31512	0.0006 77307	0.00067 7307	0.6776419 88	0.677641988
C0-12R	T.Y	34.1337 471	34.133 7471	0.0022 66236	0.00226 6236		
C0-13R	T.IPC	27.3909 7404	27.390 97404				

C0-13R	T.Large Autosomal	Undeter mined					
C0-13R	T.Small Autosomal	37.0456 8481	37.045 68481	0.0001 49837	0.00014 9837		
C0-13R	T.Y	35.2105 5984	35.210 55984	0.0008 02656	0.00080 2656		
C0-14R	T.IPC	27.3399 9252	27.339 99252				
C0-14R	T.Large Autosomal	37.1406 9748	37.140 69748	5.7853 1E-05	5.78531 E-05	0.5652475 95	0.565247595
C0-14R	T.Small Autosomal	38.5111 1221	38.511 11221	3.2701 3E-05	3.27013 E-05	0.5652475 95	0.565247595
C0-14R	T.Y	Undeter mined					
C0-15R	T.IPC	27.2952 4231	27.295 24231				
C0-15R	T.Large Autosomal	35.8240 4327	35.824 04327	0.0001 90173	0.00019 0173	0.2041458 49	0.204145849
C0-15R	T.Small Autosomal	38.3459 0912	38.345 90912	3.8822 9E-05	3.88229 E-05	0.2041458 49	0.204145849
C0-15R	T.Y	Undeter mined					
C0-16R	T.IPC	27.2483 2344	27.248 32344				
C0-16R	T.Large Autosomal	35.9691 4291	35.969 14291	0.0001 66799	0.00016 6799	0.6236800 55	0.623680055
C0-16R	T.Small Autosomal	37.3969 7266	37.396 97266	0.0001 04029	0.00010 4029	0.6236800 55	0.623680055
C0-16R	T.Y	36.7893 1427	36.789 31427	0.0001 75239	0.00017 5239		
C0-17R	T.IPC	27.1273 8228	27.127 38228				
C0-17R	T.Large Autosomal	36.4518 1274	36.451 81274	0.0001 07828	0.00010 7828	2.3759377	2.3759377
C0-17R	T.Small Autosomal	36.5292 8925	36.529 28925	0.0002 56193	0.00025 6193	2.3759377	2.3759377
C0-17R	T.Y	37.1323 8144	37.132 38144	0.0001 25897	0.00012 5897		
C0-18R	T.IPC	27.3209 3048	27.320 93048				
C0-18R	T.Large Autosomal	36.4920 1202	36.492 01202	0.0001 03981	0.00010 3981	0.4636169 67	0.463616967
C0-18R	T.Small Autosomal	38.1374 7787	38.137 47787	4.8207 2E-05	4.82072 E-05	0.4636169 67	0.463616967

C0-18R	T.Y	36.6732 7118	36.673 27118	0.0001 95979	0.00019 5979		
C0-19R	T.IPC	27.3727 9892	27.372 79892				
C0-19R	T.Large Autosomal	Undeter mined					
C0-19R	T.Small Autosomal	39.9779 2816	39.977 92816	7.1266 2E-06	7.12662 E-06		
C0-19R	T.Y	37.5545 7687	37.554 57687	8.3806 3E-05	8.38063 E-05		
C0-20R	T.IPC	27.4889 7934	27.488 97934				
C0-20R	T.Large Autosomal	35.2433 0521	35.243 30521	0.0003 21442	0.00032 1442	3.0702610 02	3.070261002
C0-20R	T.Small Autosomal	35.2308 8455	35.230 88455	0.0009 86911	0.00098 6911	3.0702610 02	3.070261002
C0-20R	T.Y	34.5721 55	34.572 155	0.0014 85179	0.00148 5179		
Trio Standard 5	T.IPC	27.7452 3354	27.626 48392				
Trio Standard 5	T.Large Autosomal	33.3305 3589	33.001 17493	0.005			
Trio Standard 5	T.Small Autosomal	34.3434 3338	34.227 95868	0.005			
Trio Standard 5	T.Y	33.9046 2112	34.132 45392	0.005			
Trio Standard 5	T.IPC	27.5077 3239	27.626 48392				
Trio Standard 5	T.Large Autosomal	32.6718 1015	33.001 17493	0.005			
Trio Standard 5	T.Small Autosomal	34.1124 8779	34.227 95868	0.005			
Trio Standard 5	T.Y	34.3602 8671	34.132 45392	0.005			
C24-11	T.IPC	27.3475 132	27.347 5132				

C24-11	T.Large Autosomal	Undeter mined					
C24-11	T.Small Autosomal	38.6482 7347	38.648 27347	0.0000 28359	0.00002 8359		
C24-11	T.Y	37.4913 8641	37.491 38641	8.9069 6E-05	8.90696 E-05		
C24-12	T.IPC	27.1463 7947	27.146 37947				
C24-12	T.Large Autosomal	34.0993 1564	34.099 31564	0.0009 03959	0.00090 3959	0.4645538 93	0.464553893
C24-12	T.Small Autosomal	36.0535 2402	36.053 52402	0.0004 19938	0.00041 9938	0.4645538 93	0.464553893
C24-12	T.Y	35.8398 8953	35.839 88953	0.0004 37602	0.00043 7602		
C24-13	T.IPC	27.2713 8329	27.271 38329				
C24-13	T.Large Autosomal	35.3319 7784	35.331 97784	0.0002 96686	0.00029 6686	1.6887729 17	1.688772917
C24-13	T.Small Autosomal	35.8835 3348	35.883 53348	0.0005 01034	0.00050 1034	1.6887729 17	1.688772917
C24-13	T.Y	36.4495 8115	36.449 58115	0.0002 43136	0.00024 3136		
C24-14	T.IPC	27.3803 73	27.380 373				
C24-14	T.Large Autosomal	37.9009 9335	37.900 99335	2.9099 8E-05	2.90998 E-05	2.6213357 45	2.621335745
C24-14	T.Small Autosomal	37.6956 6727	37.695 66727	7.6280 4E-05	7.62804 E-05	2.6213357 45	2.621335745
C24-14	T.Y	Undeter mined					
C24-15	T.IPC	27.3445 1866	27.344 51866				
C24-15	T.Large Autosomal	38.3441 4291	38.344 14291	1.9495 9E-05	1.94959 E-05		
C24-15	T.Small Autosomal	Undeter mined					
C24-15	T.Y	Undeter mined					
C24-16	T.IPC	27.3336 0291	27.333 60291				
C24-16	T.Large Autosomal	Undeter mined					
C24-16	T.Small Autosomal	38.8572 998	38.857 2998	2.2824 4E-05	2.28244 E-05		

C24-16	T.Y	Undeter mined					
C24-17	T.IPC	28.8733 1963	28.873 31963				
C24-17	T.Large Autosomal	37.4441 6428	37.444 16428	4.3975 2E-05	4.39752 E-05		
C24-17	T.Small Autosomal	Undeter mined					
C24-17	T.Y	Undeter mined					
C24-18	T.IPC	27.3604 1832	27.360 41832				
C24-18	T.Large Autosomal	32.5619 9265	32.561 99265	0.0036 27341	0.00362 7341	6.9590783 12	6.959078312
C24-18	T.Small Autosomal	32.1099 3576	32.109 93576	0.0252 42951	0.02524 2951	6.9590783 12	6.959078312
C24-18	T.Y	31.5561 5044	31.556 15044	0.0271 85619	0.02718 5619		
C24-19	T.IPC	27.6413 8412	27.641 38412				
C24-19	T.Large Autosomal	37.9600 4868	37.960 04868	2.7587 3E-05	2.75873 E-05	3.2460744 38	3.246074438
C24-19	T.Small Autosomal	37.5412 5595	37.541 25595	8.9550 5E-05	8.95505 E-05	3.2460744 38	3.246074438
C24-19	T.Y	Undeter mined					
C24-20	T.IPC	27.7349 1478	27.734 91478				
C24-20	T.Large Autosomal	Undeter mined					
C24-20	T.Small Autosomal	38.8803 9398	38.880 39398	2.2283 4E-05	2.22834 E-05		
C24-20	T.Y	Undeter mined					
C-0	T.IPC	27.6325 3975	27.632 53975				
C-0	T.Large Autosomal	Undeter mined					
C-0	T.Small Autosomal	38.6427 1927	38.642 71927	2.8523 1E-05	2.85231 E-05		
C-0	T.Y	Undeter mined					
C-0R	T.IPC	27.3327 5032	27.332 75032				

C-0R	T.Large Autosomal	Undeter mined					
C-0R	T.Small Autosomal	38.6416 2445	38.641 62445	2.8555 6E-05	2.85556 E-05		
C-0R	T.Y	Undeter mined					
C24- 11R	T.IPC	27.4735 2791	27.473 52791				
C24- 11R	T.Large Autosomal	Undeter mined					
C24- 11R	T.Small Autosomal	39.4417 6102	39.441 76102	1.2437 9E-05	1.24379 E-05		
C24- 11R	T.Y	Undeter mined					
C24- 12R	T.IPC	27.1681 8237	27.168 18237				
C24- 12R	T.Large Autosomal	34.4336 6623	34.433 66623	0.0006 68201	0.00066 8201	1.5658653 97	1.565865397
C24- 12R	T.Small Autosomal	35.1746 1395	35.174 61395	0.0010 46313	0.00104 6313	1.5658653 97	1.565865397
C24- 12R	T.Y	35.8501 9684	35.850 19684	0.0004 33276	0.00043 3276		
C24- 13R	T.IPC	27.2700 2907	27.270 02907				
C24- 13R	T.Large Autosomal	35.2976 6083	35.297 66083	0.0003 06032	0.00030 6032	2.2556765 08	2.255676508
C24- 13R	T.Small Autosomal	35.5750 0839	35.575 00839	0.0006 90309	0.00069 0309	2.2556765 08	2.255676508
C24- 13R	T.Y	35.1331 4056	35.133 14056	0.0008 64846	0.00086 4846		
C24- 14R	T.IPC	27.2171 1922	27.217 11922				
C24- 14R	T.Large Autosomal	Undeter mined					
C24- 14R	T.Small Autosomal	37.4575 6912	37.457 56912	0.0000 97683	0.00009 7683		
C24- 14R	T.Y	37.5762 5961	37.576 25961	8.2072 9E-05	8.20729 E-05		
C24- 15R	T.IPC	27.2729 8164	27.272 98164				
C24- 15R	T.Large Autosomal	37.9778 9764	37.977 89764	2.7145 8E-05	2.71458 E-05	3.8676607 61	3.867660761
C24- 15R	T.Small Autosomal	37.3881 1111	37.388 11111	0.0001 04991	0.00010 4991	3.8676607 61	3.867660761

C24-15R	T.Y	Undeter mined					
C24-16R	T.IPC	26.8284 7786	26.828 47786				
C24-16R	T.Large Autosomal	37.8012 6572	37.801 26572	3.1844 6E-05	3.18446 E-05	9.6553735 73	9.655373573
C24-16R	T.Small Autosomal	36.3536 3388	36.353 63388	0.0003 07472	0.00030 7472	9.6553735 73	9.655373573
C24-16R	T.Y	Undeter mined					
C24-17R	T.IPC	28.6393 0702	28.639 30702				
C24-17R	T.Large Autosomal	38.5412 8265	38.541 28265	0.0000 16314	0.00001 6314	0.6703719 5	0.67037195
C24-17R	T.Small Autosomal	39.5656 2042	39.565 62042	1.0936 4E-05	1.09364 E-05	0.6703719 5	0.67037195
C24-17R	T.Y	Undeter mined					
C24-18R	T.IPC	27.1054 3251	27.105 43251				
C24-18R	T.Large Autosomal	32.4471 2448	32.447 12448	0.0040 24178	0.00402 4178	7.1049356 46	7.104935646
C24-18R	T.Small Autosomal	31.9900 1312	31.990 01312	0.0285 91525	0.02859 1525	7.1049356 46	7.104935646
C24-18R	T.Y	31.0238 8	31.023 88	0.0454 10722	0.04541 0722		
C24-19R	T.IPC	27.1613 4262	27.161 34262				
C24-19R	T.Large Autosomal	37.6643 7531	37.664 37531	3.6038 7E-05	3.60387 E-05	8.4097061 16	8.409706116
C24-19R	T.Small Autosomal	36.3675 0031	36.367 50031	0.0003 03075	0.00030 3075	8.4097061 16	8.409706116
C24-19R	T.Y	Undeter mined					
C24-20R	T.IPC	27.4798 9845	27.479 89845				
C24-20R	T.Large Autosomal	Undeter mined					
C24-20R	T.Small Autosomal	Undeter mined					
C24-20R	T.Y	Undeter mined					
C-24	T.IPC	27.3716 2399	27.371 62399				

C-24	T.Large Autosomal	35.7933 5403	35.793 35403	0.0001 95521	0.00019 5521	4.7707853 32	4.770785332
C-24	T.Small Autosomal	35.2851 8295	35.285 18295	0.0009 3279	0.00093 279	4.7707853 32	4.770785332
C-24	T.Y	35.0240 2115	35.024 02115	0.0009 60768	0.00096 0768		
C-24R	T.IPC	27.4816 5512	27.481 65512				
C-24R	T.Large Autosomal	34.9722 0612	34.972 20612	0.0004 10692	0.00041 0692	1.1289242 51	1.128924251
C-24R	T.Small Autosomal	35.9582 0999	35.958 20999	0.0004 6364	0.00046 364	1.1289242 51	1.128924251
C-24R	T.Y	35.3620 224	35.362 0224	0.0006 93624	0.00069 3624		
C48-11	T.IPC	26.9218 5593	26.921 85593				
C48-11	T.Large Autosomal	Undeter mined					
C48-11	T.Small Autosomal	Undeter mined					
C48-11	T.Y	37.6931 7627	37.693 17627	7.3325 7E-05	7.33257 E-05		
C48-12	T.IPC	27.3240 1657	27.324 01657				
C48-12	T.Large Autosomal	31.8472 0612	31.847 20612	0.0069 20873	0.00692 0873	2.8372681 14	2.837268114
C48-12	T.Small Autosomal	32.3517 4179	32.351 74179	0.0196 36372	0.01963 6372	2.8372681 14	2.837268114
C48-12	T.Y	Undeter mined					
C48-13	T.IPC	27.3408 8326	27.340 88326				
C48-13	T.Large Autosomal	Undeter mined					
C48-13	T.Small Autosomal	37.4534 9121	37.453 49121	9.8097 6E-05	9.80976 E-05		
C48-13	T.Y	Undeter mined					
C48-14	T.IPC	26.8394 2032	26.839 42032				
C48-14	T.Large Autosomal	Undeter mined					
C48-14	T.Small Autosomal	36.8652 7252	36.865 27252	0.0001 80719	0.00018 0719		

C48-14	T.Y	Undeter mined					
C48-15	T.IPC	26.9482 7843	26.948 27843				
C48-15	T.Large Autosomal	37.0143 3563	37.014 33563	6.4852 5E-05	6.48525 E-05	2.1993114 95	2.199311495
C48-15	T.Small Autosomal	37.0931 3965	37.093 13965	0.0001 42631	0.00014 2631	2.1993114 95	2.199311495
C48-15	T.Y	Undeter mined					
C48-16	T.IPC	27.1029 9683	27.102 99683				
C48-16	T.Large Autosomal	36.2150 5737	36.215 05737	0.0001 33556	0.00013 3556		
C48-16	T.Small Autosomal	Undeter mined					
C48-16	T.Y	Undeter mined					
C48-17	T.IPC	27.1106 5102	27.110 65102				
C48-17	T.Large Autosomal	37.4935 8749	37.493 58749	0.0000 42054	0.00004 2054	2.4323213 1	2.43232131
C48-17	T.Small Autosomal	37.4132 1182	37.413 21182	0.0001 02289	0.00010 2289	2.4323213 1	2.43232131
C48-17	T.Y	Undeter mined					
C48-18	T.IPC	27.2900 3716	27.290 03716				
C48-18	T.Large Autosomal	35.7516 2888	35.751 62888	0.0002 03036	0.00020 3036	2404.0864 26	2404.086426
C48-18	T.Small Autosomal	29.2582 8362	29.258 28362	0.4881 15251	0.48811 5251	2404.0864 26	2404.086426
C48-18	T.Y	27.7859 9167	27.785 99167	1.0294 46959	1.02944 6959		
C48-19	T.IPC	27.0856 6666	27.085 66666				
C48-19	T.Large Autosomal	Undeter mined					
C48-19	T.Small Autosomal	Undeter mined					
C48-19	T.Y	36.6932 7164	36.693 27164	0.0001 92237	0.00019 2237		
C48-20	T.IPC	27.2430 7632	27.243 07632				

C48-20	T.Large Autosomal	36.0955 5817	36.095 55817	0.0001 48789	0.00014 8789	282.74938 96	282.7493896
C48-20	T.Small Autosomal	31.6181 7741	31.618 17741	0.0420 70013	0.04207 0013	282.74938 96	282.7493896
C48-20	T.Y	30.8299 6559	30.829 96559	0.0547 43797	0.05474 3797		
C-48	T.IPC	27.3769 1498	27.376 91498				
C-48	T.Large Autosomal	Undeter mined					
C-48	T.Small Autosomal	35.3060 5698	35.306 05698	0.0009 12783	0.00091 2783		
C-48	T.Y	35.4867 897	35.486 7897	0.0006 15027	0.00061 5027		
C-48R	T.IPC	27.4097 4617	27.409 74617				
C-48R	T.Large Autosomal	39.4615 7837	39.461 57837	0.0000 07101	0.00000 7101	121.04355 62	121.0435562
C-48R	T.Small Autosomal	35.3639 2975	35.363 92975	0.0008 5953	0.00085 953	121.04355 62	121.0435562
C-48R	T.Y	37.1541 481	37.154 1481	0.0001 23283	0.00012 3283		
C48- 11R	T.IPC	27.2583 1032	27.258 31032				
C48- 11R	T.Large Autosomal	38.5244 9036	38.524 49036	1.6563 5E-05	1.65635 E-05	1.9869143 96	1.986914396
C48- 11R	T.Small Autosomal	38.5049 8199	38.504 98199	3.2910 2E-05	3.29102 E-05	1.9869143 96	1.986914396
C48- 11R	T.Y	Undeter mined					
C48- 12R	T.IPC	27.1800 7278	27.180 07278				
C48- 12R	T.Large Autosomal	33.5384 3307	33.538 43307	0.0015 00755	0.00150 0755	16.412641 53	16.41264153
C48- 12R	T.Small Autosomal	32.1335 4874	32.133 54874	0.0246 31355	0.02463 1355	16.412641 53	16.41264153
C48- 12R	T.Y	Undeter mined					
C48- 13R	T.IPC	27.2634 697	27.263 4697				
C48- 13R	T.Large Autosomal	Undeter mined					
C48- 13R	T.Small Autosomal	38.8661 6516	38.866 16516	2.2615 2E-05	2.26152 E-05		

C48-13R	T.Y	Undeter mined					
C48-14R	T.IPC	27.2676 0674	27.267 60674				
C48-14R	T.Large Autosomal	38.3920 0974	38.392 00974	1.8670 4E-05	1.86704 E-05	13.019276 62	13.01927662
C48-14R	T.Small Autosomal	36.5798 912	36.579 8912	0.0002 43075	0.00024 3075	13.019276 62	13.01927662
C48-14R	T.Y	Undeter mined					
C48-15R	T.IPC	27.2823 0476	27.282 30476				
C48-15R	T.Large Autosomal	Undeter mined					
C48-15R	T.Small Autosomal	39.2638 3209	39.263 83209	1.4962 8E-05	1.49628 E-05		
C48-15R	T.Y	Undeter mined					
C48-16R	T.IPC	27.1438 4079	27.143 84079				
C48-16R	T.Large Autosomal	38.3329 8874	38.332 98874	1.9693 4E-05	1.96934 E-05	0.5917752 98	0.591775298
C48-16R	T.Small Autosomal	39.5044 3268	39.504 43268	1.1654 1E-05	1.16541 E-05	0.5917752 98	0.591775298
C48-16R	T.Y	Undeter mined					
C48-17R	T.IPC	26.9532 9285	26.953 29285				
C48-17R	T.Large Autosomal	Undeter mined					
C48-17R	T.Small Autosomal	38.2074 1653	38.207 41653	4.4829 3E-05	4.48293 E-05		
C48-17R	T.Y	Undeter mined					
C48-18R	T.IPC	27.3055 3818	27.305 53818				
C48-18R	T.Large Autosomal	35.1021 7285	35.102 17285	0.0003 65175	0.00036 5175	1372.1162 11	1372.116211
C48-18R	T.Small Autosomal	29.2330 7991	29.233 07991	0.5010 62334	0.50106 2334	1372.1162 11	1372.116211
C48-18R	T.Y	27.7883 0147	27.788 30147	1.0271 57426	1.02715 7426		
C48-19R	T.IPC	27.2570 1141	27.257 01141				

C48-19R	T.Large Autosomal	Undetermined					
C48-19R	T.Small Autosomal	36.8625 7172	36.862 57172	0.0001 81227	0.00018 1227		
C48-19R	T.Y	37.2871 17	37.287 117	0.0001 08453	0.00010 8453		
C48-20R	T.IPC	27.6740 9134	27.674 09134				
C48-20R	T.Large Autosomal	34.5088 1577	34.508 81577	0.0006 24323	0.00062 4323	63.346076 97	63.34607697
C48-20R	T.Small Autosomal	31.6776 8478	31.677 68478	0.0395 48393	0.03954 8393	63.346076 97	63.34607697
C48-20R	T.Y	30.8726 4252	30.872 64252	0.0525 37508	0.05253 7508		
CC-0	T.IPC	27.6546 0777	27.654 60777				
CC-0	T.Large Autosomal	37.0646 8582	37.064 68582	0.0035 00003	0.00350 0003	1.4920227 53	1.492022753
CC-0	T.Small Autosomal	38.3316 6122	38.331 66122	0.0052 22084	0.00522 2084	1.4920227 53	1.492022753
CC-0	T.Y	37.3849 5636	37.384 95636	0.0124 06309	0.01240 6309		
CC-24	T.IPC	27.6747 5128	27.674 75128				
CC-24	T.Large Autosomal	37.4601 5549	37.460 15549	0.0025 9894	0.00259 894	2.4920094 01	2.492009401
CC-24	T.Small Autosomal	38.0712 3947	38.071 23947	0.0064 76582	0.00647 6582	2.4920094 01	2.492009401
CC-24	T.Y	Undetermined					
CC-48	T.IPC	27.7067 4706	27.706 74706				
CC-48	T.Large Autosomal	Undetermined					
CC-48	T.Small Autosomal	Undetermined					
CC-48	T.Y	Undetermined					
CC-0R	T.IPC	27.6204 4144	27.620 44144			9.3339796 07	9.333979607
CC-0R	T.Large Autosomal	37.6118 66	37.611 866	0.0023 18487	0.00231 8487		
CC-0R	T.Small Autosomal	38.4943 9621	38.494 39621	0.0045 64733	0.00456 4733		

CC-0R	T.Y	Undeter mined				8.6661920 55	8.666192055
CC-24R	T.IPC	27.7266 7122	27.726 67122			8.6661920 55	8.666192055
CC-24R	T.Large Autosomal	37.7654 6097	37.765 46097	0.0020 65366	0.00206 5366		
CC-24R	T.Small Autosomal	37.8169 2505	37.816 92505	0.0079 91993	0.00799 1993		
CC-24R	T.Y	Undeter mined				1.9688415 53	1.968841553
CC-48R	T.IPC	27.7083 5686	27.708 35686			1.9688415 53	1.968841553
CC-48R	T.Large Autosomal	Undeter mined					
CC-48R	T.Small Autosomal	39.9160 4996	39.916 04996	0.0014 09237	0.00140 9237		
CC-48R	T.Y	37.1899 0326	37.189 90326	0.0143 70607	0.01437 0607	3.8695282 94	3.869528294

Table 16. Raw quantification data for ibuprofen-spiked blood samples

Sample Name	Target Name	C _T	C _T Mean	Quantity	Quantity Mean	Degradation Index	Degradation Index Mean
I0-1	T.IPC	27.6890 7356	27.689 07356				
I0-1	T.Large Autosomal	27.1043 3197	27.104 33197	0.5033 42628	0.50334 2628	1.1068617 11	1.106861711
I0-1	T.Small Autosomal	29.1309 6237	29.130 96237	0.5571 30694	0.55713 0694	1.1068617 11	1.106861711
I0-1	T.Y	28.3784 3513	28.378 43513	0.5815 60791	0.58156 0791		
I0-2	T.IPC	27.5029 2969	27.502 92969				
I0-2	T.Large Autosomal	36.6199 6078	36.619 96078	9.2625 2E-05	9.26252 E-05	6.6474242 21	6.647424221
I0-2	T.Small Autosomal	35.6850 9674	35.685 09674	0.0006 15719	0.00061 5719	6.6474242 21	6.647424221
I0-2	T.Y	35.8359 1461	35.835 91461	0.0004 39282	0.00043 9282		
I0-3	T.IPC	27.4101 9821	27.410 19821				
I0-3	T.Large Autosomal	36.8362 4268	36.836 24268	7.6178 6E-05	7.61786 E-05	1.6538002 49	1.653800249
I0-3	T.Small Autosomal	37.2126 1978	37.212 61978	0.0001 25984	0.00012 5984	1.6538002 49	1.653800249

I0-3	T.Y	35.3347 3969	35.334 73969	0.0007 12107	0.00071 2107		
I0-4	T.IPC	27.3581 4095	27.358 14095				
I0-4	T.Large Autosomal	35.3782 1579	35.378 21579	0.0002 84542	0.00028 4542	0.6661803 13	0.666180313
I0-4	T.Small Autosomal	36.8193 0923	36.819 30923	0.0001 89556	0.00018 9556	0.6661803 13	0.666180313
I0-4	T.Y	37.7901 0391	37.790 10391	6.6785 2E-05	6.67852 E-05		
I0-5	T.IPC	27.5198 04	27.519 804				
I0-5	T.Large Autosomal	36.9041 7862	36.904 17862	7.1641 7E-05	7.16417 E-05	1.5380388 5	1.53803885
I0-5	T.Small Autosomal	37.3415 9851	37.341 59851	0.0001 10188	0.00011 0188	1.5380388 5	1.53803885
I0-5	T.Y	37.0927 3529	37.092 73529	0.0001 30802	0.00013 0802		
I0-6	T.IPC	27.2764 3204	27.276 43204				
I0-6	T.Large Autosomal	31.9128 0746	31.912 80746	0.0065 22448	0.00652 2448	1.1707726 72	1.170772672
I0-6	T.Small Autosomal	33.2610 2448	33.261 02448	0.0076 36304	0.00763 6304	1.1707726 72	1.170772672
I0-6	T.Y	32.7763 5956	32.776 35956	0.0083 85617	0.00838 5617		
I0-7	T.IPC	27.4497 5471	27.449 75471				
I0-7	T.Large Autosomal	31.8504 5815	31.850 45815	0.0069 00561	0.00690 0561	0.3318004 91	0.331800491
I0-7	T.Small Autosomal	34.4206 81	34.420 681	0.0022 89609	0.00228 9609	0.3318004 91	0.331800491
I0-7	T.Y	38.5269 2795	38.526 92795	0.0000 32827	0.00003 2827		
I0-8	T.IPC	27.7450 695	27.745 0695				
I0-8	T.Large Autosomal	37.9783 4396	37.978 34396	2.7134 9E-05	2.71349 E-05	10.596921 92	10.59692192
I0-8	T.Small Autosomal	36.4181 366	36.418 1366	0.0002 87546	0.00028 7546	10.596921 92	10.59692192
I0-8	T.Y	35.4447 6318	35.444 76318	0.0006 40453	0.00064 0453		
I0-9	T.IPC	27.5397 9492	27.539 79492				

I0-9	T.Large Autosomal	35.9514 8468	35.951 48468	0.0001 69482	0.00016 9482	0.4944147 47	0.494414747
I0-9	T.Small Autosomal	37.6052 1698	37.605 21698	8.3794 4E-05	8.37944 E-05	0.4944147 47	0.494414747
I0-9	T.Y	Undeter mined					
I0-10	T.IPC	28.1527 0233	28.152 70233				
I0-10	T.Large Autosomal	Undeter mined					
I0-10	T.Small Autosomal	Undeter mined					
I0-10	T.Y	Undeter mined					
Trio Standard 2	T.IPC	28.5706 8634	28.390 10239				
Trio Standard 2	T.Large Autosomal	22.9732 7805	23.014 08386	5			
Trio Standard 2	T.Small Autosomal	25.7371 8643	25.673 48671	5			
Trio Standard 2	T.Y	24.5311 9278	24.601 90964	5			
Trio Standard 2	T.IPC	28.2095 1653	28.390 10239				
Trio Standard 2	T.Large Autosomal	23.0548 9159	23.014 08386	5			
Trio Standard 2	T.Small Autosomal	25.6097 8699	25.673 48671	5			
Trio Standard 2	T.Y	24.6726 284	24.601 90964	5			
I0-1R	T.IPC	27.6005 3253	27.600 53253				
I0-1R	T.Large Autosomal	27.0355 0148	27.035 50148	0.5356 50492	0.53565 0492	1.1024558 54	1.102455854
I0-1R	T.Small Autosomal	29.0749 0921	29.074 90921	0.5905 30992	0.59053 0992	1.1024558 54	1.102455854

I0-1R	T.Y	28.1523 2468	28.152 32468	0.7231 85241	0.72318 5241		
I0-2R	T.IPC	26.7622 3564	26.762 23564				
I0-2R	T.Large Autosomal	33.9891 6245	33.989 16245	0.0009 98589	0.00099 8589	6.2323865 89	6.232386589
I0-2R	T.Small Autosomal	33.4579 6967	33.457 96967	0.0062 23592	0.00622 3592	6.2323865 89	6.232386589
I0-2R	T.Y	34.5128 5553	34.512 85553	0.0015 72544	0.00157 2544		
I0-3R	T.IPC	27.2312 9654	27.231 29654				
I0-3R	T.Large Autosomal	35.5788 1165	35.578 81165	0.0002 3736	0.00023 736	0.9053797 72	0.905379772
I0-3R	T.Small Autosomal	36.6984 9396	36.698 49396	0.0002 14901	0.00021 4901	0.9053797 72	0.905379772
I0-3R	T.Y	38.9529 1519	38.952 91519	2.1772 3E-05	2.17723 E-05		
I0-4R	T.IPC	27.3655 8151	27.365 58151				
I0-4R	T.Large Autosomal	36.6329 1931	36.632 91931	9.1546 7E-05	9.15467 E-05	0.8324580 79	0.832458079
I0-4R	T.Small Autosomal	37.6965 7135	37.696 57135	7.6208 8E-05	7.62088 E-05	0.8324580 79	0.832458079
I0-4R	T.Y	Undeter mined					
I0-5R	T.IPC	27.3307 1136	27.330 71136				
I0-5R	T.Large Autosomal	36.4259 9869	36.425 99869	0.0001 10373	0.00011 0373	2.8744220 73	2.874422073
I0-5R	T.Small Autosomal	36.3234 6344	36.323 46344	0.0003 1726	0.00031 726	2.8744220 73	2.874422073
I0-5R	T.Y	36.4372 406	36.437 2406	0.0002 46046	0.00024 6046		
I0-6R	T.IPC	27.3277 4734	27.327 74734				
I0-6R	T.Large Autosomal	30.6417 2554	30.641 72554	0.0205 75184	0.02057 5184	1.0416165 59	1.041616559
I0-6R	T.Small Autosomal	32.2675 2472	32.267 52472	0.0214 31454	0.02143 1454	1.0416165 59	1.041616559
I0-6R	T.Y	31.9846 8399	31.984 68399	0.0179 86489	0.01798 6489		
I0-7R	T.IPC	27.6433 0292	27.643 30292				

I0-7R	T.Large Autosomal	35.2187 0422	35.218 70422	0.0003 28669	0.00032 8669	0.2285631	0.2285631
I0-7R	T.Small Autosomal	37.7104 0344	37.710 40344	7.5121 7E-05	7.51217 E-05	0.2285631	0.2285631
I0-7R	T.Y	36.3919 7922	36.391 97922	0.0002 57018	0.00025 7018		
I0-8R	T.IPC	27.6366 4818	27.636 64818				
I0-8R	T.Large Autosomal	36.8832 8171	36.883 28171	7.3007 7E-05	7.30077 E-05	4.6009941 1	4.60099411
I0-8R	T.Small Autosomal	36.2684 7458	36.268 47458	0.0003 35908	0.00033 5908	4.6009941 1	4.60099411
I0-8R	T.Y	38.0163 6505	38.016 36505	5.3698 6E-05	5.36986 E-05		
I0-9R	T.IPC	27.4792 3279	27.479 23279				
I0-9R	T.Large Autosomal	37.5997 1619	37.599 71619	3.8207 6E-05	3.82076 E-05	0.4692019 52	0.469201952
I0-9R	T.Small Autosomal	39.0898 2086	39.089 82086	1.7927 1E-05	1.79271 E-05	0.4692019 52	0.469201952
I0-9R	T.Y	Undeter mined					
I0-10R	T.IPC	27.7876 2436	27.787 62436				
I0-10R	T.Large Autosomal	37.1509 6283	37.150 96283	5.7318 8E-05	5.73188 E-05	0.2147201 15	0.214720115
I0-10R	T.Small Autosomal	39.4519 1193	39.451 91193	1.2307 5E-05	1.23075 E-05	0.2147201 15	0.214720115
I0-10R	T.Y	Undeter mined					

Appendix B : HPLC Raw Data

Table 17. Raw HPLC data for Standard 1

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.48	9392	1988	0.016	0.046
2	0.6	40237	7712	0.07	0.179
3	0.706	230401	72708	0.4	1.692
4	0.857	54546	9391	0.095	0.219
5	0.96	155397	9342	0.27	0.217
6	1.24	57535	9228	0.1	0.215
7	1.451	117476	9639	0.204	0.224
8	1.56	55764	9741	0.097	0.227
9	1.655	483634	20876	0.84	0.486
10	3.244	1515821	65638	2.633	1.527
11	3.497	1134038	96839	1.97	2.253
12	3.572	2548178	842686	4.426	19.607
13	4.165	5592934	699974	9.715	16.286
14	4.464	2661845	192669	4.624	4.483
15	4.782	4042013	229898	7.021	5.349
16	5.034	4045603	287618	7.027	6.692
17	5.437	6440432	287371	11.187	6.686
18	5.596	2912591	290169	5.059	6.751
19	5.712	10517064	290737	18.268	6.764
20	6.323	2067347	287704	3.591	6.694
21	6.638	7178459	288171	12.469	6.705
22	6.821	5709639	287892	9.918	6.698
Total		57570346	4297991	100	100

Table 18. Raw HPLC data for Standard 2

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.48	9392	1988	0.016	0.046
2	0.6	40237	7712	0.07	0.179
3	0.706	230401	72708	0.4	1.692
4	0.857	54546	9391	0.095	0.219
5	0.96	155397	9342	0.27	0.217
6	1.24	57535	9228	0.1	0.215
7	1.451	117476	9639	0.204	0.224

8	1.56	55764	9741	0.097	0.227
9	1.655	483634	20876	0.84	0.486
10	3.244	1515821	65638	2.633	1.527
11	3.497	1134038	96839	1.97	2.253
12	3.572	2548178	842686	4.426	19.607
13	4.165	5592934	699974	9.715	16.286
14	4.464	2661845	192669	4.624	4.483
15	4.782	4042013	229898	7.021	5.349
16	5.034	4045603	287618	7.027	6.692
17	5.437	6440432	287371	11.187	6.686
18	5.596	2912591	290169	5.059	6.751
19	5.712	10517064	290737	18.268	6.764
20	6.323	2067347	287704	3.591	6.694
21	6.638	7178459	288171	12.469	6.705
22	6.821	5709639	287892	9.918	6.698
Total		57570346	4297991	100	100

Table 19. Raw HPLC data for Standard 3

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.594	41648	6607	0.371	0.342
2	0.713	115146	28438	1.026	1.471
3	0.857	127558	6778	1.137	0.35
4	1.408	90406	5031	0.806	0.26
5	1.573	289900	53608	2.584	2.772
6	3.161	127993	50990	1.141	2.637
7	3.302	106758	49686	0.952	2.569
8	3.511	2003882	757860	17.864	39.191
9	4.131	1817074	553494	16.199	28.622
10	4.759	1580272	58948	14.088	3.048
11	5.013	1352210	217590	12.055	11.252
12	5.577	2046588	59153	18.245	3.059
13	5.693	1226500	51471	10.934	2.662
14	6.293	47727	6291	0.425	0.325
15	6.818	243768	27836	2.173	1.439
Total		11217430	1933781	100	99.999

Table 20. Raw HPLC data for Standard 4

Peak #	Retention Time	Area	Height	Area %	Height %
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1	0.577	22579	4653	0.153	0.154
2	0.708	160869	47884	1.088	1.58
3	0.847	116562	8930	0.788	0.295
4	1.056	40660	7802	0.275	0.257
5	1.165	123346	7308	0.834	0.241
6	1.494	450495	100893	3.047	3.33
7	3.095	256596	100931	1.736	3.331
8	3.246	204538	95598	1.383	3.155
9	3.313	40570	15352	0.274	0.507
10	3.464	2661304	978265	18	32.283
11	4.108	2381943	861219	16.111	28.421
12	4.426	601232	45589	4.067	1.504
13	4.744	961427	56839	6.503	1.876
14	5.002	1582101	356970	10.701	11.78
15	5.572	1895952	55464	12.824	1.83
16	5.687	752402	48596	5.089	1.604
17	5.936	181908	29576	1.23	0.976
18	6.048	187991	21449	1.272	0.708
19	6.282	86710	6291	0.586	0.208
20	6.536	78473	18202	0.531	0.601
21	6.684	624581	60002	4.225	1.98
22	6.821	1217831	100224	8.237	3.307
23	9.256	154595	2223	1.046	0.073
Total		14784665	3030260	100	100.001

Table 21. Raw HPLC data for Standard 5

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.472	7665	1996	0.048	0.055
2	0.59	42265	7760	0.264	0.213
3	0.705	277255	96294	1.733	2.642
4	0.855	95481	8199	0.597	0.225
5	1.016	49560	7173	0.31	0.197
6	1.148	31081	6640	0.194	0.182
7	1.28	66118	6070	0.413	0.167
8	1.459	872559	238291	5.455	6.538
9	3.06	680318	257954	4.253	7.077
10	3.217	494067	225832	3.089	6.196
11	3.292	32609	12687	0.204	0.348

12	3.44	2561204	951699	16.013	26.111
13	4.098	2009463	775894	12.563	21.287
14	4.742	1104299	41395	6.904	1.136
15	5	1969947	671861	12.316	18.433
16	5.569	1238912	33451	7.746	0.918
17	5.683	307747	25576	1.924	0.702
18	5.93	61592	5228	0.385	0.143
19	6.274	196113	24987	1.226	0.686
20	6.68	1990553	108296	12.445	2.971
21	6.812	1905906	137576	11.916	3.775
Total		15994714	3644859	99.998	100.002

Table 22. Raw HPLC data for aspirin-spiked blood sample 0-1

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.326	4607	492	0.008	0.013
2	0.576	12238	1538	0.022	0.041
3	0.646	81219	20156	0.148	0.533
4	0.742	459065	58201	0.836	1.54
5	0.906	1363	535	0.002	0.014
6	1.232	1185	205	0.002	0.005
7	1.52	11998	880	0.022	0.023
8	1.762	24898	1425	0.045	0.038
9	2.056	4981	803	0.009	0.021
10	2.141	7529	927	0.014	0.025
11	3.372	2277350	83452	4.146	2.208
12	4.368	8486798	216175	15.452	5.721
13	4.453	1046213	227262	1.905	6.014
14	4.895	7688948	366373	13.999	9.696
15	5.052	1102203	259353	2.007	6.863
16	5.175	3163287	288961	5.759	7.647
17	5.373	2383829	279192	4.34	7.388
18	5.48	1461012	278452	2.66	7.369
19	5.633	5152250	288816	9.381	7.643
20	5.911	4491713	277280	8.178	7.338
21	6.118	1444464	273766	2.63	7.245
22	6.339	4196480	292105	7.64	7.73
23	6.467	3348295	283625	6.096	7.506
24	6.813	8072831	278817	14.698	7.378

Total		54924756	3778791	99.999	99.999
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Table 23. Raw HPLC data for aspirin-spiked blood sample 0-6

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.685	135611	36379	0.264	1.116
2	0.742	137590	41826	0.268	1.284
3	0.908	1382	574	0.003	0.018
4	1.232	3596	559	0.007	0.017
5	1.491	27254	1826	0.053	0.056
6	1.773	62733	3077	0.122	0.094
7	2.008	14652	2784	0.029	0.085
8	2.08	46982	2880	0.092	0.088
9	3.176	1214824	53575	2.367	1.644
10	3.374	740810	75315	1.443	2.311
11	4.717	10951653	212122	21.336	6.509
12	4.898	3340570	365486	6.508	11.216
13	5.056	1842822	247553	3.59	7.597
14	5.354	4543504	268106	8.852	8.227
15	5.659	5068311	275111	9.874	8.442
16	5.862	6092044	278894	11.869	9
17	6.114	2226471	273857	4.338	8.404
18	6.361	3567748	277359	6.951	8.511
19	6.462	2394847	281101	4.666	8.626
20	6.627	2774930	276248	5.406	8.477
21	6.8	6140136	284127	11.962	8.719
Total		51328470	3258759	100	99.999

Table 24. Raw HPLC data for aspirin-spiked blood sample 0-10

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.569	18371	2116	0.035	0.049
2	0.744	170699	38826	0.329	0.892
3	0.909	33459	2886	0.064	0.066
4	1.378	68541	3803	0.132	0.087
5	1.52	29840	3951	0.058	0.091
6	1.81	168228	5776	0.324	0.133
7	2.128	77526	4919	0.149	0.113

8	3.207	1327306	55955	2.559	1.285
9	3.372	736330	77189	1.419	1.773
10	4.712	10977257	212868	21.16	4.889
11	4.894	3470020	371677	6.689	8.536
12	5.052	1849601	247535	3.565	5.685
13	5.35	4564984	270099	8.8	6.203
14	5.408	904412	270157	1.743	6.205
15	5.564	2460225	271045	4.742	6.225
16	5.648	1692670	272101	3.263	6.249
17	5.728	1299843	270923	2.506	6.222
18	5.812	1171135	271346	2.258	6.232
19	5.872	1950818	271240	3.76	6.23
20	6.01	1431829	272072	2.76	6.249
21	6.138	2344144	271912	4.519	6.245
22	6.352	4020782	315959	7.751	7.257
23	6.46	3295321	280631	6.352	6.445
24	6.798	7813323	289041	15.061	6.638
Total		51876664	4354027	99.998	99.999

Table 25. Raw HPLC data for aspirin-spiked blood sample A0-6

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.262	2348	374	0.004	0.006
2	0.407	7531	980	0.012	0.015
3	0.576	12608	1734	0.02	0.027
4	0.655	99907	28121	0.161	0.439
5	0.736	557860	140833	0.897	2.199
6	0.904	1204	522	0.002	0.008
7	1.271	342644	22792	0.551	0.356
8	1.656	371543	10671	0.597	0.167
9	2.349	104978	8877	0.169	0.139
10	2.821	909649	83890	1.462	1.31
11	3.062	648746	64308	1.043	1.004
12	3.226	670197	75528	1.077	1.179
13	3.375	875838	103856	1.408	1.622
14	3.523	953515	133671	1.532	2.087
15	3.576	417586	126673	0.671	1.978
16	3.886	2828480	195245	4.546	3.049
17	4.344	6464968	269914	10.39	4.214

18	4.452	1961663	280437	3.153	4.379
19	4.55	2219108	277956	3.567	4.34
20	4.722	2404565	286475	3.865	4.473
21	4.902	4162307	451595	6.69	7.051
22	5.055	1513783	296536	2.433	4.63
23	5.177	3783177	340931	6.08	5.323
24	5.377	4191049	313470	6.736	4.894
25	5.551	1269817	298588	2.041	4.662
26	5.633	1977084	300244	3.178	4.688
27	5.736	1113112	292510	1.789	4.567
28	5.838	1953448	291728	3.14	4.555
29	5.911	3281021	292773	5.273	4.571
30	6.103	1340203	280540	2.154	4.38
31	6.199	1618734	284820	2.602	4.447
32	6.34	2968776	285473	4.771	4.457
33	6.532	3207753	280997	5.155	4.387
34	6.808	7985463	281535	12.834	4.396
Total		62220665	6404597	100.003	99.999

Table 26. Raw HPLC data for aspirin-spiked blood sample A0-8

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.343	5329	636	0.01	0.013
2	0.576	15230	1922	0.028	0.038
3	0.655	32784	15315	0.059	0.302
4	0.736	304087	69394	0.55	1.367
5	0.905	1227	528	0.002	0.01
6	1.26	149583	8356	0.271	0.165
7	1.504	43857	5313	0.079	0.105
8	1.649	152289	6304	0.275	0.124
9	2.016	101511	5786	0.184	0.114
10	2.805	578558	46869	1.047	0.923
11	2.954	318744	46136	0.577	0.909
12	3.191	693707	56245	1.255	1.108
13	3.344	1988618	328595	3.597	6.474
14	3.558	620026	91819	1.122	1.809
15	3.874	2044728	132076	3.699	2.602
16	4.047	2272343	381316	4.11	7.512
17	4.541	4977507	203519	9.004	4.01

18	4.717	2365397	230093	4.279	4.533
19	4.892	3148137	381683	5.695	7.52
20	5.051	1902658	254030	3.442	5.005
21	5.308	4540340	283995	8.213	5.595
22	5.394	2418806	283859	4.375	5.592
23	5.541	1203547	279789	2.177	5.512
24	5.66	2820933	282491	5.103	5.565
25	5.846	4949797	281521	8.954	5.546
26	6.086	1586541	276871	2.87	5.455
27	6.279	4294891	286228	7.769	5.639
28	6.43	3185655	277673	5.763	5.47
29	6.744	3179248	277704	5.751	5.471
30	6.815	5386078	279764	9.743	5.512
Total		55282156	5075830	100.003	100

Table 27. Raw HPLC data for aspirin-spiked blood sample 24-1

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.43	11191	1121	0.022	0.028
2	0.576	10631	1956	0.02	0.048
3	0.744	504028	39947	0.969	0.983
4	0.91	1353	569	0.003	0.014
5	1.181	4082	343	0.008	0.008
6	1.544	5985	632	0.012	0.016
7	1.781	27042	1491	0.052	0.037
8	2.096	9302	635	0.018	0.016
9	3.025	786982	45451	1.513	1.119
10	3.186	637803	57034	1.226	1.404
11	3.384	754518	80131	1.451	1.972
12	4.713	10911764	213212	20.98	5.248
13	4.897	3385623	347935	6.509	8.565
14	4.984	682757	237593	1.313	5.849
15	5.054	1160723	247020	2.232	6.081
16	5.349	4559300	269472	8.766	6.633
17	5.416	897843	267822	1.726	6.593
18	5.653	5574050	271791	10.717	6.69
19	5.808	1167759	270534	2.245	6.659
20	5.872	1555757	270249	2.991	6.652
21	6.013	1816086	270950	3.492	6.67

22	6.142	2211929	271794	4.253	6.69
23	6.353	4212914	327719	8.1	8.067
24	6.464	2760883	278973	5.308	6.867
25	6.806	8188938	286204	15.745	7.045
26	9.296	171269	1841	0.329	0.045
Total		52010512	4062419	100	99.999

Table 28. Raw HPLC data for aspirin-spiked blood sample 24-6

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.392	2676	531	0.005	0.012
2	0.581	13270	1780	0.025	0.039
3	0.655	38108	10993	0.073	0.242
4	0.744	403083	40632	0.774	0.895
5	0.91	1764	657	0.003	0.014
6	1.28	3927	506	0.008	0.011
7	1.402	5819	795	0.011	0.018
8	1.528	5461	808	0.01	0.018
9	1.798	44949	1747	0.086	0.038
10	3.392	2534819	187222	4.87	4.124
11	4.068	4275720	207053	8.215	4.561
12	4.714	6748919	216238	12.967	4.763
13	4.902	3171734	309670	6.094	6.821
14	5.058	1966945	247290	3.779	5.447
15	5.355	4436621	268663	8.525	5.918
16	5.417	1027926	268489	1.975	5.914
17	5.495	1162030	269766	2.233	5.942
18	5.56	1296720	270452	2.492	5.958
19	5.654	1703237	276874	3.273	6.099
20	5.735	1430505	271858	2.749	5.989
21	5.823	1039040	270895	1.996	5.967
22	5.917	2209221	271281	4.245	5.976
23	6.019	1297108	270647	2.492	5.962
24	6.12	2339637	271405	4.495	5.979
25	6.371	6411643	320734	12.319	7.065
26	6.814	8474171	282652	16.282	6.226
Total		52045053	4539638	99.996	99.998

Table 29. Raw HPLC data for aspirin-spiked blood sample 24-8

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.176	1112	303	0.002	0.006
2	0.261	5559	589	0.011	0.012
3	0.578	18767	1901	0.036	0.04
4	0.743	194494	42446	0.375	0.893
5	0.91	30748	2614	0.059	0.055
6	1.259	32368	3104	0.062	0.065
7	1.395	34521	3653	0.067	0.077
8	1.536	27976	3684	0.054	0.077
9	1.777	220417	5247	0.425	0.11
10	3.196	1275302	54544	2.457	1.147
11	3.388	990450	107103	1.908	2.253
12	4.068	4142981	160171	7.981	3.369
13	4.714	6815017	214518	13.129	4.512
14	4.901	3324588	363521	6.405	7.646
15	4.989	799553	238845	1.54	5.024
16	5.057	1048852	247555	2.021	5.207
17	5.24	3029196	261022	5.836	5.49
18	5.356	1659359	269739	3.197	5.674
19	5.416	898594	268139	1.731	5.64
20	5.568	2321569	270065	4.472	5.68
21	5.654	1824414	273981	3.515	5.763
22	5.735	1299753	271196	2.504	5.704
23	5.824	1040116	271095	2.004	5.702
24	5.879	2209373	271312	4.256	5.707
25	6.02	1427404	271377	2.75	5.708
26	6.163	2465410	270977	4.75	5.7
27	6.377	6264392	316981	12.068	6.667
28	6.813	8505706	288640	16.386	6.071
Total		51907991	4754322	100.001	99.999

Table 30. Raw HPLC data for aspirin-spiked blood sample A24-1

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.583	18851	2113	0.035	0.046
2	0.657	54321	16845	0.099	0.364
3	0.736	403478	88078	0.739	1.906
4	0.908	1776	640	0.003	0.014
5	1.267	230016	14760	0.421	0.319

6	1.64	243496	8950	0.446	0.194
7	1.992	74492	6519	0.136	0.141
8	2.298	80106	7234	0.147	0.156
9	2.801	686027	57746	1.257	1.249
10	3.344	2079905	144676	3.81	3.13
11	3.448	324772	85863	0.595	1.858
12	3.56	649328	93065	1.189	2.013
13	3.872	2107823	144667	3.861	3.13
14	4.047	1682295	198323	3.081	4.291
15	4.386	3130728	182052	5.734	3.939
16	4.539	1935684	202899	3.545	4.39
17	4.711	2220670	221674	4.067	4.796
18	4.891	3191314	370374	5.845	8.013
19	5.05	1883319	251191	3.45	5.435
20	5.393	6526969	278286	11.955	6.021
21	5.541	1458520	277776	2.671	6.01
22	5.645	2132235	280292	3.905	6.064
23	5.856	5737430	281072	10.509	6.081
24	6.084	1190418	276489	2.18	5.982
25	6.253	5695126	293474	10.431	6.349
26	6.501	2261860	278742	4.143	6.031
27	6.738	3054536	278082	5.595	6.016
28	6.814	5396129	279482	9.884	6.047
29	9.328	144931	704	0.265	0.015
Total		54596555	4622068	99.998	100

Table 31. Raw HPLC data for aspirin-spiked blood sample A24-3

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.581	8805	1748	0.016	0.034
2	0.734	530523	105456	0.959	2.056
3	0.907	1160	506	0.002	0.01
4	1.262	328105	18771	0.593	0.366
5	1.642	338182	9830	0.611	0.192
6	2.304	74266	7004	0.134	0.137
7	2.809	576471	37722	1.042	0.735
8	3.05	583489	55116	1.055	1.075
9	3.358	1353905	88102	2.447	1.718
10	3.503	720771	105032	1.303	2.048

11	3.561	376597	101938	0.681	1.987
12	3.872	2262790	170454	4.09	3.323
13	3.952	589592	161004	1.066	3.139
14	4.114	1626722	165106	2.941	3.219
15	4.406	3771231	215057	6.817	4.193
16	4.541	1169051	213228	2.113	4.157
17	4.721	2502086	245035	4.523	4.777
18	4.892	2910683	375649	5.262	7.324
19	5.051	1893027	252602	3.422	4.925
20	5.252	3426982	274761	6.195	5.357
21	5.542	4628769	280953	8.367	5.477
22	5.64	2022869	284155	3.657	5.54
23	5.763	2795621	277896	5.054	5.418
24	5.898	2647789	277941	4.786	5.419
25	6.082	1717919	276762	3.105	5.396
26	6.251	5808055	290450	10.499	5.663
27	6.508	1595151	278290	2.884	5.426
28	6.737	3589339	279063	6.488	5.441
29	6.813	5271176	278705	9.529	5.434
30	9.424	197836	908	0.358	0.018
Total		55318962	5129244	99.999	100.004

Table 32. Raw HPLC data for aspirin-spiked blood sample 48-5

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.317	8167	636	0.015	0.011
2	0.656	152470	35717	0.275	0.606
3	0.738	397257	91944	0.718	1.559
4	0.912	1476	573	0.003	0.01
5	1.295	209303	14229	0.378	0.241
6	1.677	185910	9249	0.336	0.157
7	1.88	91222	6423	0.165	0.109
8	2.128	28691	6055	0.052	0.103
9	2.256	41433	6204	0.075	0.105
10	2.348	36475	6426	0.066	0.109
11	3.021	1002959	63923	1.812	1.084
12	3.122	343516	61427	0.621	1.042
13	3.386	2156778	330807	3.896	5.611
14	3.52	473505	94058	0.855	1.595

15	3.577	311688	94324	0.563	1.6
16	3.768	1283375	124530	2.318	2.112
17	3.882	876471	140462	1.583	2.382
18	3.972	750258	145939	1.355	2.475
19	4.064	1492161	338111	2.696	5.734
20	4.414	3357842	199350	6.066	3.381
21	4.548	1609033	207936	2.907	3.527
22	4.721	2374091	232297	4.289	3.94
23	4.9	2872642	339688	5.19	5.761
24	5.056	2115847	251446	3.822	4.265
25	5.325	4581464	280931	8.277	4.765
26	5.409	911612	272270	1.647	4.618
27	5.491	1302315	272173	2.353	4.616
28	5.552	915093	272862	1.653	4.628
29	5.652	2109673	278898	3.811	4.73
30	5.736	1440601	273749	2.603	4.643
31	5.879	3270289	273099	5.908	4.632
32	6.018	1043694	272167	1.885	4.616
33	6.154	2478896	272213	4.478	4.617
34	6.366	7073218	340470	12.778	5.774
35	6.808	8054379	285544	14.551	4.843
Total		55353804	5896130	100	100.001

Table 33. Raw HPLC data for aspirin-spiked blood sample A48-1

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.45	2676	472	0.005	0.011
2	0.576	9186	1847	0.017	0.042
3	0.734	398681	79214	0.733	1.798
4	0.908	1273	527	0.002	0.012
5	1.274	231159	12600	0.425	0.286
6	1.482	67064	7379	0.123	0.168
7	1.643	165138	8536	0.303	0.194
8	2	83735	6408	0.154	0.145
9	2.303	75111	6841	0.138	0.155
10	2.81	597200	41130	1.097	0.934
11	3.208	1031300	59026	1.895	1.34
12	3.349	815817	99514	1.499	2.259
13	3.556	1048202	101503	1.926	2.304

14	3.868	2170561	158801	3.988	3.605
15	3.951	563707	152625	1.036	3.465
16	4.049	1051469	166971	1.932	3.79
17	4.405	3642395	204480	6.693	4.642
18	4.537	1598828	205364	2.938	4.662
19	4.718	2450540	236463	4.503	5.368
20	4.889	2937274	359793	5.397	8.168
21	5.049	1754096	249181	3.223	5.657
22	5.241	4097907	281901	7.529	6.399
23	5.393	1424825	271745	2.618	6.169
24	5.64	7577463	285775	13.923	6.487
25	5.897	2233384	275905	4.104	6.263
26	6.086	1973401	275972	3.626	6.265
27	6.259	4367531	293904	8.025	6.672
28	6.416	3474005	283037	6.383	6.425
29	6.811	8387821	277860	15.412	6.308
30	9.456	193171	331	0.355	0.008
Total		54424920	4405105	100.002	100.001

Table 34. Raw HPLC data for aspirin-spiked blood sample AC-48

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.437	8403	1047	0.015	0.02
2	0.576	9611	1670	0.017	0.033
3	0.652	117201	31721	0.206	0.621
4	0.737	311793	91184	0.549	1.784
5	0.908	34086	6191	0.06	0.121
6	1.264	210300	12638	0.37	0.247
7	1.524	57136	6228	0.101	0.122
8	1.661	158563	7472	0.279	0.146
9	2.031	78640	6311	0.138	0.123
10	2.307	83462	6929	0.147	0.136
11	3.128	1366645	67293	2.405	1.317
12	3.205	353889	68251	0.623	1.335
13	3.362	1022345	118235	1.799	2.313
14	4.053	5022731	187646	8.84	3.671
15	4.362	3838905	222300	6.756	4.349
16	4.446	1179177	233506	2.075	4.569
17	4.713	3728834	247216	6.562	4.837

18	4.891	3827239	387739	6.736	7.586
19	5.046	1371325	264673	2.413	5.179
20	5.172	2303482	299976	4.054	5.869
21	5.258	2092939	296544	3.683	5.802
22	5.37	2031879	284982	3.576	5.576
23	5.473	942469	281612	1.659	5.51
24	5.547	1218175	283909	2.144	5.555
25	5.653	5168359	292338	9.096	5.72
26	5.912	2783591	280311	4.899	5.485
27	6.108	1848802	276792	3.254	5.416
28	6.299	4308160	286499	7.582	5.606
29	6.479	4666128	281191	8.212	5.502
30	6.809	6676467	278559	11.75	5.45
Total		56820736	5110963	100	100

Table 35. Raw HPLC data for caffeine-spiked blood sample CC-0

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.385	24849	4236	0.025	0.053
2	0.649	8623237	1075334	8.791	13.479
3	0.871	14869324	906941	15.159	11.368
4	1.785	1404625	105788	1.432	1.326
5	3.937	13576657	783638	13.841	9.823
6	4.492	7309731	281546	7.452	3.529
7	4.708	3450043	305239	3.517	3.826
8	4.838	4167746	522791	4.249	6.553
9	5.029	3562131	360846	3.632	4.523
10	5.308	9144889	394348	9.323	4.943
11	5.535	2058480	366360	2.099	4.592
12	5.642	1687571	362625	1.72	4.545
13	5.818	7474561	358060	7.62	4.488
14	6.097	2337688	329450	2.383	4.13
15	6.331	4945076	400605	5.041	5.021
16	6.436	1661351	357547	1.694	4.482
17	6.528	2422927	352930	2.47	4.424
18	6.627	1531318	320175	1.561	4.013
19	6.759	7751926	379974	7.903	4.763
20	7.838	84454	9393	0.086	0.118
Total		98088584	7977826	99.998	99.999

Table 36. Raw HPLC data for caffeine-spiked blood sample C0-9

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.256	2715	599	0.003	0.008
2	0.393	24181	2001	0.028	0.025
3	0.571	17721	3524	0.021	0.045
4	0.686	3612028	543485	4.186	6.883
5	1.327	706748	25322	0.819	0.321
6	1.751	558497	22528	0.647	0.285
7	3.355	17617687	931687	20.416	11.799
8	3.554	754995	116912	0.875	1.481
9	4.041	12050989	1487481	13.965	18.838
10	4.303	2426880	200563	2.812	2.54
11	4.532	3053608	241036	3.539	3.053
12	4.711	2678836	273508	3.104	3.464
13	4.88	2849592	330079	3.302	4.18
14	5.045	2438098	310751	2.825	3.935
15	5.189	3579426	321637	4.148	4.073
16	5.388	4362063	335424	5.055	4.248
17	5.536	1431779	305913	1.659	3.874
18	5.643	1728108	312600	2.003	3.959
19	5.841	5128720	312655	5.943	3.96
20	5.996	1278523	300316	1.482	3.803
21	6.068	1404521	294610	1.628	3.731
22	6.256	6018855	525900	6.975	6.66
23	6.43	3695309	324602	4.282	4.111
24	6.752	8703911	372286	10.086	4.715
25	9.36	171807	691	0.199	0.009
Total		86295597	7896110	100.002	100

Table 37. Raw HPLC data for caffeine-spiked blood sample C0-19

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.694	4723871	635063	5.327	7.239
2	1.326	1067980	40980	1.204	0.467
3	1.728	667828	35082	0.753	0.4
4	3.348	15761882	799528	17.774	9.114
5	3.502	502877	136742	0.567	1.559
6	3.56	445348	135919	0.502	1.549

7	4.04	11902442	1274327	13.422	14.527
8	4.254	2904781	244703	3.276	2.789
9	4.414	1057649	222540	1.193	2.537
10	4.531	2178287	267261	2.456	3.047
11	4.711	2669427	287000	3.01	3.272
12	4.876	3513836	447668	3.962	5.103
13	5.044	2355311	312507	2.656	3.562
14	5.197	3132358	364325	3.532	4.153
15	5.305	2752553	380746	3.104	4.34
16	5.392	2822019	352919	3.182	4.023
17	5.544	1520914	322745	1.715	3.679
18	5.652	2964688	343488	3.343	3.916
19	5.808	1667798	321211	1.881	3.662
20	5.856	3709799	320380	4.183	3.652
21	6.082	1989782	301590	2.244	3.438
22	6.274	5551727	538638	6.26	6.14
23	6.427	3786289	330260	4.27	3.765
24	6.745	9032117	356715	10.185	4.066
Total		88681563	8772337	100.001	99.999

Table 38. Raw HPLC data for caffeine-spiked blood sample C24-16

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.683	3431074	434406	4.128	5.511
2	1.328	487869	21445	0.587	0.272
3	3.349	15021316	814182	18.072	10.329
4	3.5	913835	134163	1.099	1.702
5	4.038	10822347	1242702	13.02	15.765
6	4.255	1861869	228062	2.24	2.893
7	4.328	1288162	222772	1.55	2.826
8	4.533	2596363	255004	3.124	3.235
9	4.684	2499972	258256	3.008	3.276
10	4.875	3370151	423243	4.055	5.369
11	5.043	2185380	302620	2.629	3.839
12	5.261	5962342	388063	7.173	4.923
13	5.386	2361282	326355	2.841	4.14
14	5.542	1467538	311881	1.766	3.956
15	5.643	4834763	349223	5.817	4.43
16	5.898	2310490	309556	2.78	3.927

17	5.989	1115140	293559	1.342	3.724
18	6.07	1525574	291067	1.835	3.692
19	6.251	5701180	543549	6.859	6.895
20	6.419	4074377	367747	4.902	4.665
21	6.739	9137899	364692	10.994	4.626
22	9.36	150708	251	0.181	0.003
Total		83119631	7882798	100.002	99.998

Table 39. Raw HPLC data for caffeine-spiked blood sample C24-18

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.682	3813439	511498	4.514	6.366
2	1.327	689130	26343	0.816	0.328
3	1.735	445967	20778	0.528	0.259
4	3.348	14849235	819300	17.576	10.197
5	4.038	11926737	1310782	14.117	16.313
6	4.253	2625909	232219	3.108	2.89
7	4.529	3118230	245373	3.691	3.054
8	4.712	2695352	288311	3.19	3.588
9	4.874	3214723	398153	3.805	4.955
10	5.043	2131205	299562	2.523	3.728
11	5.252	4952481	354052	5.862	4.406
12	5.384	2909061	337671	3.443	4.202
13	5.534	1894046	309407	2.242	3.851
14	5.64	1911194	315706	2.262	3.929
15	5.844	4686752	322081	5.547	4.008
16	5.968	1744345	308007	2.065	3.833
17	6.06	1278996	297206	1.514	3.699
18	6.238	6249308	560901	7.397	6.981
19	6.419	2083415	355752	2.466	4.427
20	6.503	2234729	332958	2.645	4.144
21	6.734	9030059	389044	10.688	4.842
Total		84484313	8035104	99.999	100

Table 40. Raw HPLC data for caffeine-spiked blood sample CC-24

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.464	7390	1090	0.013	0.023
2	0.581	11731	2024	0.021	0.042
3	0.736	378348	78038	0.69	1.626

4	0.908	43256	5998	0.079	0.125
5	1.064	38242	5773	0.07	0.12
6	1.273	135553	9514	0.247	0.198
7	1.528	52145	6326	0.095	0.132
8	1.659	175707	7559	0.32	0.157
9	2.016	65320	6516	0.119	0.136
10	2.29	92841	7221	0.169	0.15
11	2.825	675076	56369	1.231	1.174
12	3.116	950315	80722	1.732	1.682
13	3.206	232136	61670	0.423	1.285
14	3.355	1313183	217183	2.394	4.525
15	3.505	825062	102083	1.504	2.127
16	3.886	2150687	132244	3.921	2.755
17	4.049	1849401	242318	3.372	5.049
18	4.385	3089148	184068	5.632	3.835
19	4.538	1960380	207339	3.574	4.32
20	4.713	2347922	225299	4.28	4.694
21	4.891	3102683	372692	5.656	7.765
22	5.049	1771706	252633	3.23	5.264
23	5.319	4909727	290277	8.951	6.048
24	5.397	2393881	280770	4.364	5.85
25	5.543	927351	276857	1.691	5.768
26	5.713	3197969	279396	5.83	5.821
27	5.813	4523751	279420	8.247	5.822
28	6.101	2237262	275585	4.079	5.742
29	6.341	4024667	287424	7.337	5.988
30	6.531	4686905	285716	8.544	5.953
31	6.807	6683437	279535	12.184	5.824
Total		54853182	4799659	99.999	100

Table 41. Raw HPLC data for caffeine-spiked blood sample C48-4

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.376	2060	425	0.002	0.005
2	0.464	2722	632	0.003	0.007
3	0.693	5413226	829348	6.054	9.02
4	1.337	1138522	41389	1.273	0.45
5	1.746	627093	23801	0.701	0.259
6	3.353	16516061	919416	18.472	10

7	3.552	913278	142505	1.021	1.55
8	3.864	3375227	266450	3.775	2.898
9	4.04	10611005	1483623	11.868	16.137
10	4.314	1813881	216037	2.029	2.35
11	4.412	902046	210377	1.009	2.288
12	4.53	1922003	243961	2.15	2.653
13	4.714	2702556	288075	3.023	3.133
14	4.881	3050397	369805	3.412	4.022
15	5.042	3265126	326966	3.652	3.556
16	5.185	2943158	355341	3.292	3.865
17	5.303	1221033	326278	1.366	3.549
18	5.379	3003698	333813	3.359	3.631
19	5.535	1609607	312143	1.8	3.395
20	5.642	3488231	324616	3.901	3.531
21	5.903	3071585	299233	3.435	3.255
22	5.993	1415358	301697	1.583	3.281
23	6.071	1956562	295123	2.188	3.21
24	6.268	5771243	552690	6.455	6.011
25	6.428	3042561	333828	3.403	3.631
26	6.748	9631579	396595	10.772	4.314
Total		89409818	9194167	99.998	100.001

Table 42. Raw HPLC data for caffeine-spiked blood sample C48-6

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.566	21606	2277	0.024	0.025
2	0.69	4265480	680920	4.832	7.491
3	1.324	827239	32669	0.937	0.359
4	1.733	568980	21111	0.645	0.232
5	3.354	16049319	904765	18.181	9.954
6	3.503	846787	123880	0.959	1.363
7	4.04	12094784	1441446	13.701	15.858
8	4.309	2551072	220381	2.89	2.425
9	4.401	1288598	229863	1.46	2.529
10	4.531	2044994	261143	2.317	2.873
11	4.715	2894606	323393	3.279	3.558
12	4.882	2934071	323100	3.324	3.555
13	5.041	2427897	316303	2.75	3.48
14	5.188	2986402	363117	3.383	3.995

15	5.262	5796233	363238	6.566	3.996
16	5.54	1475976	311742	1.672	3.43
17	5.644	2116341	331249	2.397	3.644
18	5.798	2363073	310815	2.677	3.419
19	5.899	2204788	312949	2.498	3.443
20	5.989	1287683	300476	1.459	3.306
21	6.084	1881471	304733	2.131	3.353
22	6.257	6345105	580127	7.188	6.382
23	6.428	1729995	344345	1.96	3.788
24	6.515	2357219	320994	2.67	3.531
25	6.748	8769179	364042	9.934	4.005
26	9.336	146230	492	0.166	0.005
Total		88275128	9089570	100	99.999

Table 43. Raw HPLC data for caffeine-spiked blood sample C48-12

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.675	3253052	458213	3.147	3.689
2	1.418	292617	11983	0.283	0.096
3	1.866	261721	11979	0.253	0.096
4	3.346	18742213	1067269	18.129	8.591
5	3.658	1673829	156295	1.619	1.258
6	3.785	1242834	144283	1.202	1.161
7	4.035	8158589	1354875	7.892	10.906
8	4.256	3035028	285858	2.936	2.301
9	4.694	5238391	261913	5.067	2.108
10	4.873	3829823	389088	3.704	3.132
11	5.044	9800202	2616004	9.479	21.058
12	5.273	5499528	761043	5.32	6.126
13	5.363	2176879	681335	2.106	5.485
14	5.45	7581138	1354732	7.333	10.905
15	5.611	5218496	421727	5.048	3.395
16	5.896	2470634	341750	2.39	2.751
17	5.991	2621463	356728	2.536	2.872
18	6.209	9181768	1024360	8.881	8.246
19	6.412	3905183	353491	3.777	2.846
20	6.73	9200287	369797	8.899	2.977
Total		103383675	12422723	100.001	99.999

Table 44. Raw HPLC data for caffeine-spiked blood sample C48-18

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.248	1028	122	0.001	0.001
2	0.397	5388	569	0.006	0.007
3	0.69	3631211	520538	4.107	6.27
4	1.32	737291	30178	0.834	0.363
5	1.736	476353	23012	0.539	0.277
6	3.348	14904477	817590	16.856	9.848
7	3.5	834471	128026	0.944	1.542
8	3.746	1562564	151883	1.767	1.829
9	4.037	9094739	1263911	10.286	15.223
10	4.329	4117526	406149	4.657	4.892
11	4.535	3116618	343742	3.525	4.14
12	4.678	2655352	362991	3.003	4.372
13	4.873	3172155	400850	3.588	4.828
14	5.042	2348599	312781	2.656	3.767
15	5.25	5443793	409674	6.157	4.934
16	5.382	3387520	359273	3.831	4.327
17	5.637	4280371	414054	4.841	4.987
18	5.72	1255124	329583	1.419	3.97
19	5.846	4062665	335455	4.595	4.04
20	6.233	8509666	566805	9.624	6.827
21	6.415	2709309	376688	3.064	4.537
22	6.51	2239815	345848	2.533	4.166
23	6.733	9874989	402719	11.168	4.851
Total		88421024	8302441	100.001	99.998

Table 45. Raw HPLC data for caffeine-spiked blood sample C48-20

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.344	4979	308	0.006	0.004
2	0.687	5445542	826339	6.094	9.781
3	1.366	1271552	45871	1.423	0.543
4	1.6	761998	38398	0.853	0.454
5	3.351	16011772	868690	17.919	10.282
6	3.505	1044105	157144	1.168	1.86
7	4.041	12305535	1309043	13.771	15.494
8	4.256	2939204	252874	3.289	2.993
9	4.403	966174	225364	1.081	2.667

10	4.532	2178839	260018	2.438	3.078
11	4.716	3036091	308544	3.398	3.652
12	4.876	3117305	378502	3.489	4.48
13	5.044	2452856	306562	2.745	3.628
14	5.179	2331898	323375	2.61	3.827
15	5.257	1398525	330275	1.565	3.909
16	5.31	1574480	329489	1.762	3.9
17	5.397	2770369	338749	3.1	4.009
18	5.642	3411892	332966	3.818	3.941
19	5.725	4453939	307458	4.985	3.639
20	6.083	3012890	305744	3.372	3.619
21	6.235	6104822	538506	6.832	6.374
22	6.421	3836102	334849	4.293	3.963
23	6.738	8793102	329379	9.841	3.899
24	9.296	131248	382	0.147	0.005
Total		89355219	8448829	99.999	100.001

Table 46. Raw HPLC data for ibuprofen-spiked blood sample I0-1

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.336	12578	860	0.016	0.011
2	0.568	8994	1548	0.011	0.019
3	0.687	3262067	513988	4.031	6.392
4	1.315	591920	25491	0.731	0.317
5	1.737	410867	19747	0.508	0.246
6	3.351	14423231	824593	17.824	10.255
7	3.504	455068	110774	0.562	1.378
8	3.557	351501	106881	0.434	1.329
9	4.04	10218147	1272611	12.627	15.827
10	4.255	2030156	217203	2.509	2.701
11	4.531	3379864	228641	4.177	2.844
12	4.708	2416534	246026	2.986	3.06
13	4.876	2995417	377868	3.702	4.699
14	4.966	1379853	321446	1.705	3.998
15	5.044	1242990	306516	1.536	3.812
16	5.263	5624577	375933	6.951	4.675
17	5.388	2483950	319815	3.07	3.977
18	5.541	1310296	306845	1.619	3.816
19	5.641	3006838	340622	3.716	4.236

20	5.847	4069526	309139	5.029	3.845
21	5.992	1142719	300455	1.412	3.737
22	6.053	1422616	299324	1.758	3.723
23	6.245	5922692	513815	7.319	6.39
24	6.42	3317376	330871	4.1	4.115
25	6.736	9322139	369310	11.52	4.593
26	9.256	118067	394	0.146	0.005
Total		80919983	8040716	99.999	100

Table 47. Raw HPLC data for ibuprofen-spiked blood sample I0-3

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.24	1837	340	0.002	0.004
2	0.351	3915	427	0.005	0.005
3	0.686	3930433	596348	4.624	6.638
4	1.341	681572	27049	0.802	0.301
5	1.711	569871	26418	0.67	0.294
6	3.35	15052904	828861	17.711	9.226
7	3.499	481429	130649	0.566	1.454
8	3.56	490444	131004	0.577	1.458
9	4.039	12154021	1297755	14.3	14.445
10	4.254	2273629	246797	2.675	2.747
11	4.398	2214929	260953	2.606	2.905
12	4.531	1662079	250262	1.956	2.786
13	4.714	2785535	297824	3.277	3.315
14	4.873	2783210	341255	3.275	3.798
15	4.965	1345037	296384	1.583	3.299
16	5.042	1350314	292285	1.589	3.253
17	5.196	2668417	307774	3.14	3.426
18	5.309	2301272	305538	2.708	3.401
19	5.39	1149011	303213	1.352	3.375
20	5.471	1286093	299246	1.513	3.331
21	5.537	1431319	301060	1.684	3.351
22	5.64	2064125	325291	2.429	3.621
23	5.797	4833814	299483	5.687	3.333
24	6.063	2538681	297508	2.987	3.311
25	6.247	6258242	547404	7.363	6.093
26	6.421	1648612	323288	1.94	3.598
27	6.505	2155713	310306	2.536	3.454

28	6.738	8877547	339469	10.445	3.779
Total		84994005	8984191	100.002	100.001

Table 48. Raw HPLC data for ibuprofen-spiked blood sample I0-7

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.225	3130	454	0.004	0.007
2	0.418	11089	925	0.015	0.013
3	0.654	1916369	270093	2.509	3.922
4	1.821	395453	13018	0.518	0.189
5	3.349	13138630	810115	17.199	11.763
6	4.036	10011635	1252446	13.106	18.186
7	4.249	1827502	200910	2.392	2.917
8	4.696	5052113	221865	6.613	3.222
9	4.87	2975540	334631	3.895	4.859
10	4.958	2443680	689512	3.199	10.012
11	5.038	1086919	296697	1.423	4.308
12	5.249	5465144	367702	7.154	5.339
13	5.389	1111758	292380	1.455	4.245
14	5.632	6203616	348833	8.121	5.065
15	5.846	3073700	295108	4.024	4.285
16	6.075	3220472	301348	4.216	4.376
17	6.241	4970044	506000	6.506	7.347
18	6.405	4124161	363413	5.399	5.277
19	6.737	9360796	321486	12.254	4.668
Total		76391751	6886936	100.002	100

Table 49. Raw HPLC data for ibuprofen-spiked blood sample I0-8

Peak #	Retention Time	Area	Height	Area %	Height %
1	0.302	1309	224	0.002	0.003
2	0.657	1472481	194937	1.995	2.732
3	1.248	32925	2747	0.045	0.039
4	1.904	314890	14412	0.427	0.202
5	3.351	13582684	823645	18.406	11.543
6	4.038	10213710	1276215	13.841	17.886
7	4.253	1845182	189250	2.5	2.652
8	4.698	4888961	220641	6.625	3.092
9	4.873	2992092	316616	4.055	4.437
10	4.961	1521803	342810	2.062	4.804

11	5.041	1149047	278636	1.557	3.905
12	5.175	2327828	279834	3.155	3.922
13	5.339	2411215	283162	3.268	3.968
14	5.398	1070371	280579	1.45	3.932
15	5.539	2331721	291231	3.16	4.082
16	5.634	2194350	321890	2.974	4.511
17	5.715	4633962	304761	6.28	4.271
18	5.992	1233420	289076	1.671	4.051
19	6.058	1381175	289872	1.872	4.063
20	6.252	5520747	443575	7.481	6.217
21	6.423	3855067	328645	5.224	4.606
22	6.741	8818632	362541	11.95	5.081
Total		73793572	7135299	100	99.999

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ACADEMIC VITA

Dipesh Patel

Education

The Pennsylvania State University – 2019-2023

Eberly College of Science & Schreyer Honor College Bachelor of Science Biology - Vertebrate Physiology

- Minor in Psychological Sciences and Classics and Ancient Mediterranean Studies
- Honors in Biology
- The Pennsylvania State University Dean's List Spring 2020 - Present

Relevant Experiences

Undergraduate Research

Undergraduate Researcher in Roy Lab – October 2021-Present

Reference: Dr. Reena Roy, Lab Coordinator and Instructor

- Working towards acquiring data for forensic science and biological cases regarding over-the-counter drugs such as aspirin, caffeine, and ibuprofen, in male human blood
- Researching impacts of these over-the-counter drugs on data and chemical analysis of DNA composition and identification of the donor blood

Undergraduate Researcher in Neiderhiser Lab – December 2020-October 2021

Reference: Mari Greecher, Undergraduate Research Coordinator

- Ordered, de-identified, and coded relevant medical information into RedCap for over 50 prenatal medical records and delivery records of mothers
- Acquired a greater understanding of medical labs, vaccines, medical / surgical procedures, and prenatal environmental impacts on the development of the fetus

Hazlet Fire and Rescue Squad Involvement

Emergency Medical Technician – June 2021-Present

Reference: Chief Stephen Schmidt

- Attended EMT classes with RWJ Barnabas Health over the summer of 2021 and passed at the top of the class with an average of 94% and earned my license in August of 2021
- Working with Hazlet Rescue and Fire Squad in aiding about 15 patients per shift and doing around 4, 12 hour shifts a week during my time off school
- Improving my patient care skills by learning from senior EMTs as well as gaining more field

Riverside Pediatrics Medical Group Involvement

Intern / Volunteer

Reference: Dr. Naimat Bokhari, MD.

- Aided over 100 patients everyday in making appointments and filling out relevant paperwork
- Assisted Certified Medical Assistants in filling out private and VFC vaccines into the online NJIIS system as well as updating any online vaccination forms in the patient e-files

Undergraduate Pediatric Shadowing May 2020- Present

Reference: Dr. Naimat Bokhari, MD.

- Shadowing Dr. Patel, Dr. Satwani, and Dr. Azhar, all of whom are certified pediatricians at Riverside Pediatrics Medical Group
- Interacting with and examining about 15 to 20 patients everyday alongside the doctors during my shadowing period
- Learning about the methods of examination, vaccines, as well as various medical conditions and their associated diagnoses and treatments

Research Grants Received

Wieland Research Funds - \$2,200

Conferences Attended

International Society for Forensic Genetics – 2022

International Symposium on Human Identification -2022

International Society for Applied Biological Sciences – 2022

Other Involvements / Skills

Public Relations Executive Board position in Alpha Epsilon Delta since April 2022

Programming Executive Board position in Eberly College of Science Student Council since April 2022

Member of the Pennsylvania State University Student Red Cross Club since Fall 2019

Excellent with Adobe applications, Microsoft softwares, Google programs, RedCap, SAS, Strata, NJIIS, EPIC

Laboratory proficiencies in GC-MS, DNA Extraction, Quantification, and Amplification, and Powerplex Fusion 6c and Y23 Systems