Quantification of Propofol in Blood Plasma and its Application to Future Clinical Research in Anesthesiology and Perioperative Medicine

By

David J. Bemis

A PROJECT

Submitted to

Oregon State University

Honors College

in partial fulfillment of

the requirements for the

degree of

Honors Baccalaureate of Science in Microbiology (Honors Associate)

Presented February 9th, 2021

Commencement June 2021

AN ABSTRACT OF THE THESIS OF

David J. Bemis for the degree of Honors Baccalaureate of Science in Microbiology presented on February 9th, 2021. Title: Quantification of Propofol in Blood Plasma and its Application to Future Clinical Research in Anesthesiology and Perioperative Medicine.

Abstract approved:

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Vincent T. Remcho

High Performance Liquid Chromatography-Mass Spectrometry coupled with filtration, centrifugation, and other common sample preparation techniques offers a rapid and robust method for separating and quantifying propofol, a commonly used anesthetic, from human blood and its synthetic analogs. Method development is important for not only optimizing separation and quantification for a sample in a given matrix, but also for doing so on a specific instrument. The first part of thesis focuses on this method development by determining or offering the experimental framework to determine optimal detection wavelength, flowrate, instrument (i.e. nano versus conventional HPLC), and mobile phase. The second part applies this technique to assess if alcoholic liver disease affects the minimum effective dose, recovery time, and depth of sedation in intensive care unit patients receiving propofol to be intubated due to alcohol withdrawal.

Key Words: Propofol, LC-MS, Anesthesiology, Quantification, Separation

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Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College theses. My signature below authorizes release of my project to any reader upon request.

David J. Bemis, Author

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INTRODUCTION

Propofol Uses and Side Effects

Propofol is a common anesthetic generally administered via intravenous injection. It has a short window in which it effectively functions as an anesthetic and is atypical in the world of anesthetics in that it is an anti-emetic drug. Some of the most common side effects of any anesthetic are nausea and vomiting, but propofol can help with this[1, 2, 3].

Propofol is an effective agent for maintenance of anesthesia both as the principal anesthetic—also known as total intravenous anesthesia or TIVA—and as an auxiliary one. In the latter, if a patient is anesthetized using another anesthetic but is still moving on the operating room table (a common occurrence when operating on certain parts of the body such as the genital region), the anesthesiologist might administer a dose of intravenous propofol, not necessarily to reduce pain given that the patient is already anesthetized, but instead to make the procedure safer and easier for the surgical team[1]. In the former, propofol will be administered in an infusion form to achieve total intravenous anesthesia. This is a technique of general or non-specific anesthesia in which several anesthetic agents are administered purely through an IV route rather than the more common inhalation route. This is used when an anesthesiologist would elect not to use gas anesthesia, such as nitrous oxide, which is useful to avoid for middle-ear surgery and when a patient has elevated intracranial pressure, in which volatile gas anesthetics are more dangerous. In TIVA propofol is used with opioids, muscle relaxants, or oxygen-enriched air. Propofol is also extremely useful for positioning and insertion of a laryngeal mast airway—an instrument anesthesiologists use to manage a patient's airway during anesthesia—as it lessens the laryngeal reflex which is more commonly known as the gag reflex. In a similar way, it is often used for positioning endotracheal tubes, the central goal of most intubation procedures[2].

While propofol is one of the most widely used anesthetics in the United States, this does not mean it is without its adverse side effects. It is a major vasodilator and therefore causes some arterial hypertension and myocardial depression. It can also result in a slowing of the heart rate known as bradycardia. Due to this, it is generally administered very slowly and in the lowest possible dose in patients who suffer from cardiovascular diseases or decreased intravascular volume, also known as hypovolemia. Unfortunately, it is these same patients suffering from these various types of heart disease that most commonly require the non-endoscopic procedures with which propofol is quite useful. Other less desirable effects of propofol include depression of the central nervous system by decreased cerebral blood flow and cerebral metabolic rate for oxygen, in addition to decreasing intracranial pressure. Hypoxia can occur if oxygen is not given to the patient under the effect of propofol for a prolonged period of time. Finally, no allergic reactions have been reported[2].

Propofol can cause a patient to temporarily stop breathing, referred to as apnea, immediately following injection. This is dealt with using assisted ventilation and the use of opioids in conjunction with propofol, common in TIVA, can worsen this symptom. It is worth noting that while this is a negative side effect of propofol, it is a negative side effect of many other commonly used anesthetics as well—a major reason as to why for most of the duration of surgeries and procedures that involve an anesthesiologist, their main role is airway management and ventilation[1].

There can be some pain upon intravenous injection of propofol, often described as a burning sensation. However, this is often dealt with by injecting into larger veins and injecting a small amount of lidocaine before propofol administration. Involuntary convulsions or movements can also occur due to propofol administration but this is a non-dangerous and easily

manageable negative side effect[3]. These side effects are mostly mitigated by using the water-soluble prodrug derivative of propofol, fospropofol, as well.

Benefits of Propofol for Common Endoscopic Procedures

Propofol is becoming a much more commonly used drug for endoscopic procedures due to its better recovery time, patient intra-procedure cooperation, and sedation level with no significant increase in complication rate. There is also the added benefit of it adding some degree of amnesia to the patient's memory regarding the procedure it was used for[2].

Brief Historical Context of Propofol

In 1973, Dr. John B. Glen identified propofol as a possible drug candidates to replace the previously favorited short-acting barbiturates, such as thiopental, as an anesthetic agent[1, 4]. Thiopental is the predecessor of propofol, especially in the United Kingdom. It was similar to propofol in that it had a very smooth induction but was problematic because of its narrow effective window, very painful injections, and slow metabolic profile causing it to have prolonged sedative effects long after the procedure or surgery was finished. It is also worth noting that while Thiopental had prolonged sedative effects, awakening occurred rapidly giving an anesthesiologist reduced time to react if the procedure of surgery was still occurring. At first glance these may seem contradictory, but one must consider that useful clinical sedation is not the same as sedative effects. A patient can feel fatigued or nauseous from an anesthetic long after they are conscious and the anesthetic is no longer providing meaningful sedation. This is an

extremely problematic issue, both for the patient and the surgical team. Propofol almost entirely lacks these adverse effects of Thiopental[1, 4].

Propofol's Mechanism of Action

Propofol CNS depression, from which it derives its anesthetic effects, is most likely mediated via γ-aminobutyric acid (GABA) receptors as an agonist and glutamatergic N-methyl-D-aspartate (NMDA) receptors as an antagonist[5]. This means that propofol binds to the GABA receptors to enhance cellular activity and that it blocks binding of a normal substrate in the NMDA receptors to block cellular activity[5].

What is Fosporpofol and Why Does it Matter?

Fospropofol is a water-soluble prodrug (a prodrug is an inactive or less-active form of a drug that is metabolized by the body into the active form of the drug) of Propofol, differing from Propofol by the addition of a phosphate group. The emulsion in which propofol is provided is a good growth medium for bacteria which can lead to sepsis if contaminated propofol is used, thus Propofol solutions generally contain preservatives[5]. Since fospropofol is water-soluble it eliminates the need for preservatives, egg products, and hyperlipidemia associated with propofol. Fospropofol's mechanism of action is analogous to that of propofol except it is first converted from fospropofol to propofol by endothelial alkaline phosphatases. While the mechanism of action is analogous, the time between administration of a fospropofol bolus and sedative effect is delayed due to this initial step. It also happens to be the case that although the mechanism of action is analogous, there is some decreased sedative effect for the moderate sedation desired for

endoscopic procedures when compared to normal propofol. The onset of the sedative effects of fospropofol is four to eight minutes with peak plasma concentrations occurring somewhere between 8 and 12 minutes depending on the bolus dose applied[6]. Its most common uses are for diagnostic procedures such as colonoscopies and bronchoscopies, and low acuity, low risk, surgical procedures such as osteotomies, fasciotomies, and arthroscopies. Administration of fospropofol is less painful than administration of the propofol emulsion but it can result in nausea and vomiting, something that the propofol emulsion is not commonly known to cause. Generally speaking, fospropofol is considered a safe and effective alternative to midazolam and propofol for low-acuity and low risk surgeries, and endoscopic procedures[6].

Metabolism of Propofol and its Prodrug, Fospropofol

After intravenous administration of propofol the plasma concentration will quickly reach an equilibrium. There is then an equilibrium of propofol reached between the blood plasma and organs such as the liver, kidneys, lungs, and most importantly, the brain. Due to its' highly lipophilic structure and its' quick equilibration, the effects of propofol sedation occurs extremely quickly. Generally, an anesthesiologist, nurse anesthetist, or anesthesiology assistant will have the patient count backwards from ten as they are injected with propofol[7]. It is exceedingly rare that the patient will count down past 5 or 4. After this point two important things occur in the body: propofol equilibration between the blood plasma/perfuse organs and the remaining parts of the body, and fast metabolism of propofol in the liver resulting in a total duration of anesthetic effect for a standard dose being only three to five minutes[8]. It is worth noting that body fat is also something that should be taken into consideration when dosing with propofol due to its lipophilic

nature; there is some evidence to suggest that patients with more body fat require larger doses of propofol to have an anesthetic effect lasting for the same amount of time.

A characteristic of propofol that is considered atypical among specifically volatile anesthetics is that is it undergoes extensive and rapid metabolism to several different, generally water-soluble, metabolites. Although around 30% of a dose of propofol is excreted through the lungs—the liver, brain, kidneys, and small intestines perform actual metabolism. In total 40% of propofol is metabolized in the liver. This can be considered analogous to how glucose can be metabolized throughout most of the body, but fructose can only be metabolized in the liver. In this analogy, propofol is glucose, while most other anesthetics are fructose[8].

All propofol metabolites are inactive in the human body except for 4-Hydroxypropofol (2,6-Diisopropyl-1,4-quinol), which is metabolized from propofol by the following enzymes: cytochrome P450 2B6, cytochrome P450 2C9, cytochrome P450 2C8, cytochrome P450 2C18, cytochrome P450 2C19, and cytochrome P450 2A2[8]. 4-Hydroxypropofol is approximately 1/3 as potent of an anesthetic as propofol. A diagram from the paper *Metabolic Profiles of Propofol and Fospropofol* illustrates the extensive number of the different propofol metabolites[8].

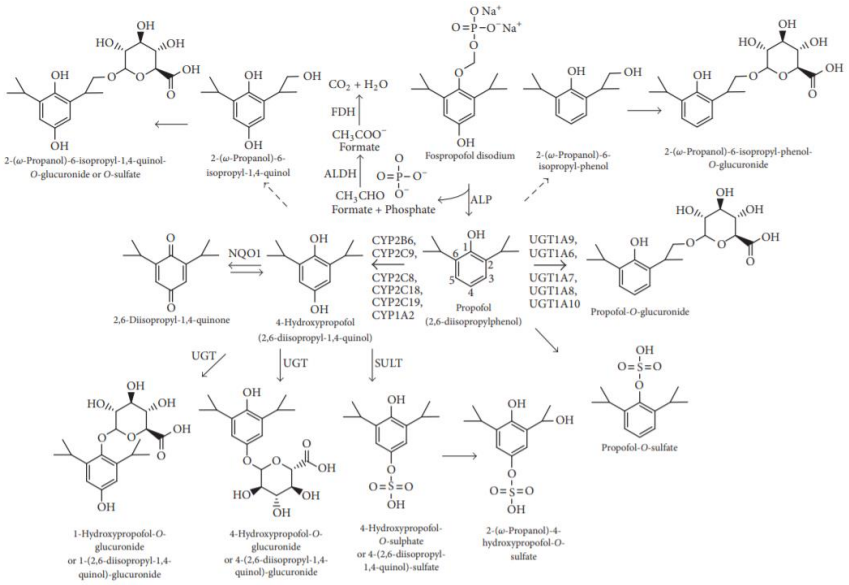

FIGURE 1: Metabolic pathway of propofol and fospropofol. Dashed arrows represent minor routes and both metabolites can undergo glucuronide and sulfate conjugation. SULT: sulfotransferase; UGT: UDP-glucuronosyltransferase; ALDH: aldehyde dehydrogenase; ALP: alkaline phosphatase; NQO1: diaphorase; CYP: cytochrome P450.

Figure 1: From: Dinis-Oliveira, R. Metabolic Profiles Of Propofol And Fospropofol: Clinical And Forensic Interpretative Aspects. BioMed Research International 2018, 2018, 1-16[8].

In reference to the topic of metabolism—it is important to distinguish between the concepts of metabolism and elimination. Metabolism of propofol is the breakdown or conversion of it to less active and more tolerable metabolites while elimination is getting those new metabolites out of the body in some way. The vast majority of propofol is metabolized and then excreted via glomerular filtration in the kidneys—typical of most water-soluble metabolite the body wants to get rid of [9]. This type of filtration is the first step to producing urine. There is some small percentage, <1%, of propofol that is excreted as urine out of the body in its starting form but this is resisted by the body because generally it only wants water-soluble solutes in the urine. Fatty waste is generally excreted through feces but only about 2% of a total bolus of propofol is excreted in this fashion because of its rapid and extensive metabolism to more water-soluble compounds. It is the general consensus that the elimination half-life of propofol's metabolites is between 4 and 23 hours [9].

Storage Requirements of Propofol

A problem common to anesthetics is that they work best when lipid-soluble, as to effectively cross the fatty tissue of the brain and spinal cord, among other nervous system structures. However, if they are not water-soluble enough then they cannot be safely intravenously injected. Propofol itself is almost entirely insoluble in water and so it must be stored and injected in an emulsion in order to ensure that both ends of this paradox of IV anesthetics are met[8]. The components of the emulsion include soybean oil, glycerol, lecithin, and sodium chloride to ensure the pH is between 7 and 8.5. It is almost always in a 10 mg/mL or 1.0% form. This emulsion is a good growth environment for some types of bacteria, mostly *E. coli*, so different manufacturers add in different products to prevent bacterial growth. Cases of sepsis have been recorded in patients that were injected with improperly manufactured or stored propofol[8].

Propofol Abuse and Addiction

Propofol's quick dissipating effects combined with a common need for airway management when a patient uses it makes its' risk for abuse fairly low[9]. It is also a low incentive drug as its amnesic side effects make it such that even if it did provide a pleasurable high, the user would not remember it happening[1]. Most cases of abuse involved individuals using it to sleep, however, still other controlled substances are better at inducing this effect. It is also hard to get outside of a medical setting because the only physicians qualified to administer it as is dictated by the American Medical Association's curriculum for graduate medical training are anesthesiologists and emergency medicine physicians[10]. However, there still have been some

high-profile cases of celebrity propofol abuse, most notably Michael Jackson. Due to its lack of availability, it is mostly medical professionals, i.e. physicians and nurses who have been known to abuse it. This likely has nothing more to do with it than the fact that this group has the most access to it than any other, not that they have any particular propensity to be abusers of propofol[10].

Propofol's Variable Methods of Administration and Their Effects

Propofol has effects outside of the hypnotic and amnesic ones that make it so desirable as an anesthetic. However, there are challenges in accessing these benefits, principally its quick induction and dissipation of effects that make it so desirable as an anesthetic. It is ironic that the exact things that make it great for anesthesia present challenges for alternative uses[11].

The main way to address this challenge is alternative routes of administration outside of intravenous and alternative formulations outside of the propofol emulsion and aqueous fospropofol solution. This is also where there is a large demand for additional research, specifically regarding bioavailability. Bioavailability in various bodily fluids such as blood plasma, cerebrospinal fluid, and bile can all give valuable information on what effects should be occurring with a given dose of propofol[11].

Brief Paragraph on LC-MS

Liquid chromatography-mass spectrometry (LC-MS) is routinely used in pharmaceutical and medical research. LC-MS is well suited for applications in which samples are complex and therefore yield complicated, noisy chromatographic data[12]. A characteristic of target bodily fluids such as blood plasma or cerebrospinal fluid is that these are indeed complex matrices, making LC-MS an excellent analytical candidate. In LC-MS systems, LC is utilized to separate components of the mixture from one another, while MS is used to identify and quantify the components of the sample, rather than all of this being done with a conventional LC detector. However, this has only been the norm since the 1990s due to the development and cost-reduction of electrospray ionization in conjunction with optimized stable isotope internal standards[12].

METHODOLGY

Overview of Non-Optimized LC-MS Method

Synthetic blood plasma or centrifuged canine blood plasma will be spiked with a concentration of propofol that would typically be found during common medical procedures. This will then undergo ultrafiltration, via centrifugation in an ultrafiltration tube, and the ultrafiltrate will be spiked with a thymol as an internal standard. This will be shaken/mixed with dihydrogen sodium phosphate and cyclohexane to draw the propofol into the organic cyclohexane layer. This cyclohexane propofol solution will then be separated from the rest of the solution using pipetting and evaporated to dryness. The residue will be reconstituted with the respective mobile phase, put into an appropriate sample vial, and analyzed by HPLC-MS using the following initial parameters for LC: 0.1 uL injection volume (autosampler), 270 nm detection wavelength, and a reference wavelength between 270 and 280 nm. After the sample has exited the detector it will then enter the mass spectrometer where it will be quantified using propofol's peak area ratio with the internal standard thymol. This will be done using a selective reaction monitoring mode with an m/z at 293.1.

Synthetic Blood Plasma Formula

While separating and quantifying propofol and its metabolites from human blood plasma is the end goal, it is best to start with a less complex sample matrix such as a synthetic blood plasma and then build up to a spiked canine plasma. Table 1 shows the materials list used to make 1 L of simplified synthetic blood plasma. This mostly provides a proteinous solution with some sugars, nitrogen waste products, and salts to initially separate the propofol from. Table 2 provides what a less simplified synthetic blood plasma recipe should contain.

Albumin	40,000 mg
Fibrinogen	4,000 mg
Ammonium hydroxide	0.8515 mg
Hydrogen Chloride	3.83 mg
Creatinine	13.0 mg
Urea	180.18 mg
Glucose	1100 mg
NaOH	3333 mg

Table 1: 1 L Synthetic Blood Plasma Recipe

Ammonia	0.8515 mg
Chloride	3.83 mg
Creatinine	13 mg
Urea	180.18 mg
Glucose	1100 mg
Magnesium	1100 mg
Phosphate	142.43 mg
Pyruvate	0.9 mg
Sodium	3333 mg
Calcium	104 mg
Zinc	6.538 mg
Bilirubin	58.47 mg
Fibrinogen	4,000 mg

Albumin	40,000 mg

Table 2: 1 L True Synthetic Blood Plasma Recipe

Procedure by Experiment

Experiment 1 — Baseline Data with nano-LC using literature mobile phase

1. Spike 5 mL of synthetic blood plasma with thymol stock solution so that the final concentration of it is approximately 5,000 ng/L
2. Take 1 mL of this and put it in a labeled vial off to the side.
3. Centrifuge the remaining 4 mL of spiked synthetic blood plasma in an ultrafiltration tube at a constant rotor angle until 2 mL of ultrafiltrate is obtained.
    a. Parameters: 2500 rpm for approximately 30 minutes.
    b. Note: Do NOT forget to balance the centrifuge.
4. Take 1 mL of spiked synthetic blood plasma ultrafiltrate and put it off to the side in a labeled vial.
5. With the remaining 1 mL of spiked synthetic blood plasma ultrafiltrate add 1 mL of 0.1 M dihydrogen sodium phosphate and 5 mL of cyclohexane.
6. Put this vial of spiked synthetic blood plasma ultrafiltrate, dihydrogen sodium phosphate, and cyclohexane onto a shaker for 10 min at 200 rpm.
7. Transfer a 4 mL aliquot of the clearly defined cyclohexane layer to a clean tube with 20 uL of Tetramethylammonium hydroxide (25% in Methanol) diluted with 2-proponol in a 3:37 ratio.
    a. Abbreviation for the Tetramethylammonium hydroxide (25% in Methanol) diluted with 2-proponol in a 3:37 ratio is TMAH.

8. Evaporate this cyclohexane, TMAH, 2-proponol solution to dryness using the compressed air or nitrogen valve on a standard fume hood.
9. Redissolve residue in the mobile phase being used for chromatography—likely 75:25 Methanol:MilliQ H_2O—and place in a labeled vial off to the side.
10. Put 200 uL of each vial that has been labeled and placed off to the side into LC tubes then cap them and place them into the LC autosampler.
11. Run each sample on the LC 1-3 times with the following parameters:
    a. Detection wavelength: 270 nm
    b. Injection Volume: 0.1 uL
    c. Flow rate: 60 uL per minute
12. Analyze Data

<u>Experiment 2 — Comparison of nano-LC to conventional LC</u>

1. Spike 25 mL of synthetic blood plasma with thymol stock solution so that the final concentration of it is approximately 5,000 ng/L
2. Take 5 mL of this and put it in a labeled vial off to the side.
3. Centrifuge the remaining 20 mL of spiked synthetic blood plasma in an ultrafiltration tube at a constant rotor angle until 10 mL of ultrafiltrate is obtained.
    a. Parameters: 2500 rpm for approximately 30 minutes.
    b. Note: Do NOT forget to balance the centrifuge.
4. Take 5 mL of spiked synthetic blood plasma ultrafiltrate and put it off to the side in a labeled vial.
5. With the remaining 5 mL of spiked synthetic blood plasma ultrafiltrate add 5 mL of 0.1 M dihydrogen sodium phosphate and 25 mL of cyclohexane.
6. Put this vial of spiked synthetic blood plasma ultrafiltrate, dihydrogen sodium phosphate, and cyclohexane onto a shaker for 10 min at 200 rpm.
7. Transfer a 20 mL aliquot of the clearly defined cyclohexane layer to a clean tube with 100 uL of TMAH solution.
8. Evaporate this cyclohexane, TMAH, 2-proponol solution to dryness using the compressed air or nitrogen valve on a standard fume hood.
9. Redissolve residue in the mobile phase being used for chromatography—likely 75:25 Methanol:MilliQ H2O—and place in a labeled vial off to the side.
10. Put 5 mL of each vial that has been labeled and placed off to the side into LC tubes then cap them and place them into the LC autosampler.
11. Run each sample on the LC 1-3 times with the following parameters:
    a. Detection wavelength: 270 nm

b. Injection Volume: 0.5 mL

c. Flow rate: 60 uL per minute

12. Analyze Data

Experiment 3 —Mobile phase standard calibration on nano-LC

1. Make solutions consisting of increasing proportions of MeOH and decreasing proportions of MilliQ Water in increments of 10% per experimental group.
    a. This is to be done from 30:70 MeOH:MQH_2O to 70:30 MeOH:MQH_2O
    b. All experimental groups (MeOH:MQH_2O) → 30:70, 40:60, 50:50, 60:40, 70:30
2. Take 1000 uL of each of the 5 solutions and place them into separate labeled 5 mL vials.
3. Spike each 1000 uL solution with 50 uL of the 100,000 ng/mL Thymol stock solution.
    a. Final concentration is 5,000 ng/mL which is considerably higher than the propofol or thymol concentrations we will later be trying to quantify.
4. Run each sample on the nano-LC with the following parameters:
    a. Detection Wavelength: 270 nm
    b. Injection Volume: 0.1 uL
    c. Flow Rate: 60 uL/min
    d. Mobile Phase: Match mobile phase with sample so if the 60:40 MeOH:MQH_2O sample is being run, the mobile phase should be 60% MeOH and 40% MQH_2O.
5. Analyze Data

Experiment 4 — Increase detection wavelength parameter (270 nm → 280 nm)

1. Spike 5 mL of synthetic blood plasma with thymol stock solution so that the final concentration of it is approximately 5,000 ng/L
2. Take 1 mL of this and put it in a labeled vial off to the side.
3. Centrifuge the remaining 4 mL of spiked synthetic blood plasma in an ultrafiltration tube at a constant rotor angle until 2 mL of ultrafiltrate is obtained.
    a. Parameters: 2500 rpm for approximately 30 minutes.
    b. Note: Do NOT forget to balance the centrifuge.
4. Take 1 mL of spiked synthetic blood plasma ultrafiltrate and put it off to the side in a labeled vial.
5. With the remaining 1 mL of spiked synthetic blood plasma ultrafiltrate add 1 mL of 0.1 M dihydrogen sodium phosphate and 5 mL of cyclohexane.
6. Put this vial of spiked synthetic blood plasma ultrafiltrate, dihydrogen sodium phosphate, and cyclohexane onto a shaker for 10 min at 200 rpm.
7. Transfer a 4 mL aliquot of the clearly defined cyclohexane layer to a clean tube with 20 uL of TMAH solution.
8. Evaporate this cyclohexane, TMAH, 2-proponol solution to dryness using the compressed air or nitrogen valve on a standard fume hood.
9. Redissolve residue in the mobile phase being used for chromatography—likely 75:25 Methanol:MilliQ H2O, but subject to change depending on the results of experiment 3—and place in a labeled vial off to the side.
10. Put 200 uL of each vial that has been labeled and placed off to the side into LC tubes then cap them and place them into the LC autosampler.

11. Finally, make up a solution that of the same concentration as the mobile phase (TBD) and spike it so that its concentration is 5,000 ng/mL of thymol.
    a. This sample will function as a blank. If the thymol appears to be better detected at 270 nm in this, but at 280 nm in the final treated synthetic blood plasma sample then it will be clear that there is some inconsistency. This same reasoning should hold true for most of the groups.
12. Run each sample on the LC 1-3 times with the following parameters:
    a. Parameter set 1
        i. Detection wavelength: 270 nm
        ii. Injection Volume: 0.1 uL
        iii. Flow rate: 60 uL per minute
        iv. Mobile Phase: TBD in exp. 3
    b. Parameter set 2:
        i. Detection wavelength: 280 nm
        ii. Injection Volume: 0.1 uL
        iii. Flow rate: 60 uL per minute
        iv. Mobile Phase: TBD in exp. 3
13. Analyze Data

<u>Experiment 5 — Flowrate experiment</u>

Sample Prep Portion of Experiment

1. Spike 5 mL of synthetic blood plasma with thymol stock solution so that the final concentration of it is approximately 5,000 ng/L
2. Centrifuge the spiked synthetic blood plasma in an ultrafiltration tube at a constant rotor angle until 2 mL of ultrafiltrate is obtained.
    a. Parameters: 2500 rpm for approximately 30 minutes.
    b. Note: Do NOT forget to balance the centrifuge.
3. With the spiked synthetic blood plasma ultrafiltrate add 1 mL of 0.1 M dihydrogen sodium phosphate and 5 mL of cyclohexane.
4. Put this vial of spiked synthetic blood plasma ultrafiltrate, dihydrogen sodium phosphate, and cyclohexane onto a shaker for 10 min at 200 rpm.
5. Transfer a 4 mL aliquot of the clearly defined cyclohexane layer to a clean tube with 20 uL of TMAH solution.
6. Evaporate this cyclohexane, TMAH, 2-proponol solution to dryness using the compressed air or nitrogen valve on a standard fume hood.
7. Redissolve residue in the mobile phase being used for chromatography—likely 75:25 Methanol:MilliQ H2O, but subject to change depending on the outcome of experiment 3.
8. Put 250 uL of this redissolved residue solution into a labeled chromatography vial.
9. Make a solution of 5,000 ng/mL of thymol in mobile phase.
    a. This sample will function as a blank and will help to find any inconsistencies in the data or errors in the sample preparation.

Data Collection Portion of Experiment

1. Run each sample for 3 trials for each of the following flow rate groups: 40 uL/min, 50 uL/min, 60 uL/min, 70 uL/min, 80 uL/min
2. Ensure that the following parameters stay constant for every trial:
    a. Detection wavelength: TBD exp. 4
    b. Injection Volume: 0.1 uL
    c. Mobile Phase: TBD in exp. 3

Experiment 6 – Samples Analyzed Using LC-MS

System Setup Consideration When Interfacing a LC and MS: A conventional LC setup produces at least 1 mL/min of liquid which about 1 L/min when converted into the gasous phase required for Atmospheric Pressure Ionization (API). Electrospray ionization (ESI) is a common application of API and is what we wish to use. A conventional MS can only accept approximately 1 mL/min of gas, therefore we have elected to interface the nano-LC with the MS rather than the conventional HPLC as this helps to mitigate this issue.

(Basic course of LC-MS & source → https://www.agilent.com/cs/library/support/documents/a05296.pdf)

1. Perform the same sample preparation procedure done in experiments 1, 4, and 5 but with the appropriate modifications to detection wavelength, flowrate, and mobile phase (from experiment 1, 3, 4, & 5).
2. Set the MS to selective reaction monitoring mode with an m/z at 293.1.
3. Ensure that the LC detector outlet goes into the MS inlet rather than a waste beaker.
4. Run 3 samples from each reconsititued LC vial.
    a. Starting concentration range (before sample preparation) is from 50 ng/mL to 550 ng/mL in increments of 50 ng/mL with a thymol internal standard at concentration 100 ng/mL. This means there should be 11 concentration groups.
5. Analyze data and construct a calibration plot.

Data Analysis and Troubleshooting

When analyzing the chromatograms obtained from the experiments above many simple assessments can be made about different components of each one. This is how the optimal parameter is determined.

If the pressure on a chromatogram is much higher than it should be at a given flow rate then the operator must check for column contamination or plugged instrument components like the packing or a frit. The former and middle can be dealt with by backflushing and washing the column. If this does not affect the pressure then the operator is forced to clear plugged frits or change the frit altogether. If it is too low then the operator must check for a leak in the system.

If split peaks are present on the chromatogram then the operator should lock for possible void volume that may be affecting flow path, the possibility that the sample may be taking multiple paths through the column, poorly packed bed, or a high pH sample or solvent system that is dissolving the column silica.

In the event that one is obtaining broad peaks or tailed peaks then the operators should look for column contamination, aging, or loading effects. In addition, they should look at secondary column interactions which can be caused by residual silanol interactions. This can be solved by using a low pH solvent system.

If there is inconsistent retention time between trials of the same or different concentrations then one should look for column contamination, insufficient time for the system to equilibrate, the column-mobile phase combination used, the flow rate, and the gradient delay volume if applicable.

If detection issues are occurring, especially when there are missing peaks, one must ensure that the detection parameters, specifically the excitation and detection wavelength are optimal for what one is trying to detect. This can always be done by finding literature excitation and detection values. It is possible that the detection wavelength for the analyte one is trying to quantify can be improper for other components of the solution and can thus result in their being fewer peaks than expected, but this should not affect the final outcome since these components are not being quantified.

Results and Discussion

Experiment 1 — Baseline Data with nano-LC using literature mobile phase

The baseline results for this sample preparation and separation technique were confusing to say the least. For some chromatograms, there was a thymol peak at a retention time of 3.75 minutes in the thymol spiked synthetic blood plasma organic reconstitute. While there were peaks in similar positions for some of the non-spiked synthetic blood plasma organic reconstitute, they were much smaller and could therefore be chalked up to impurities leftover from the sample preparation technique. The peaks in both cases were broader than ideal so it very well could be that multiple substrates are contained in each peak, but the spiked peak being much higher is a good sign that the thymol was being detected. However, there were also some chromatograms for the thymol spiked and non-spiked synthetic blood plasma reconstitute that did not possess these thymol peaks.

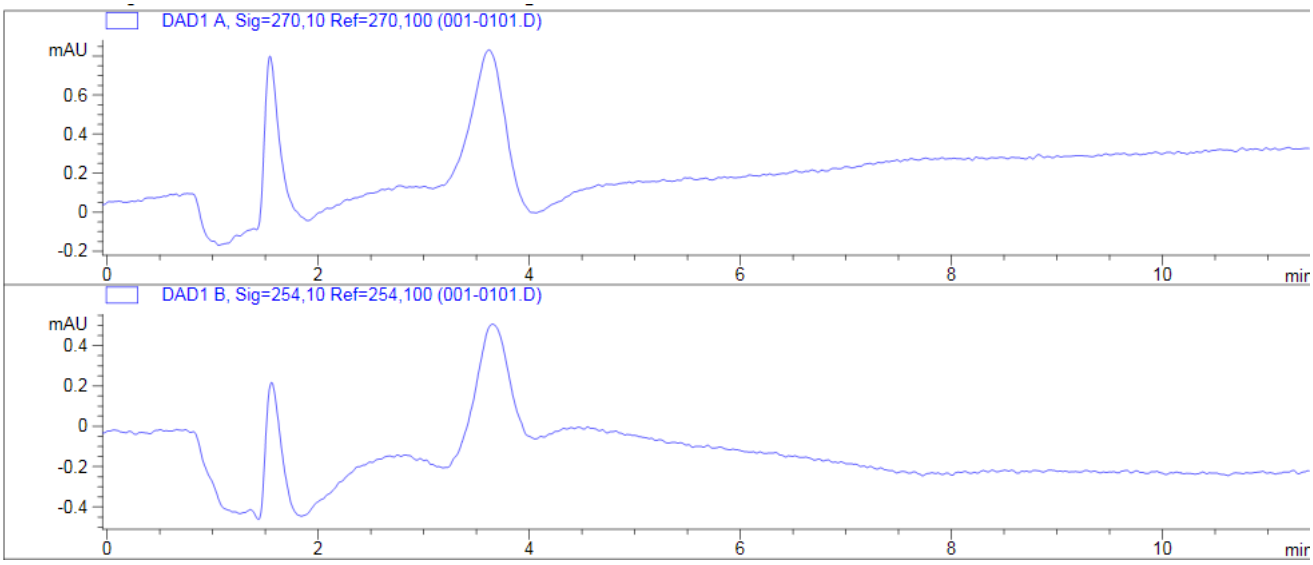

Figure 2: Thymol-spiked synthetic blood plasma organic reconstitute chromatogram.

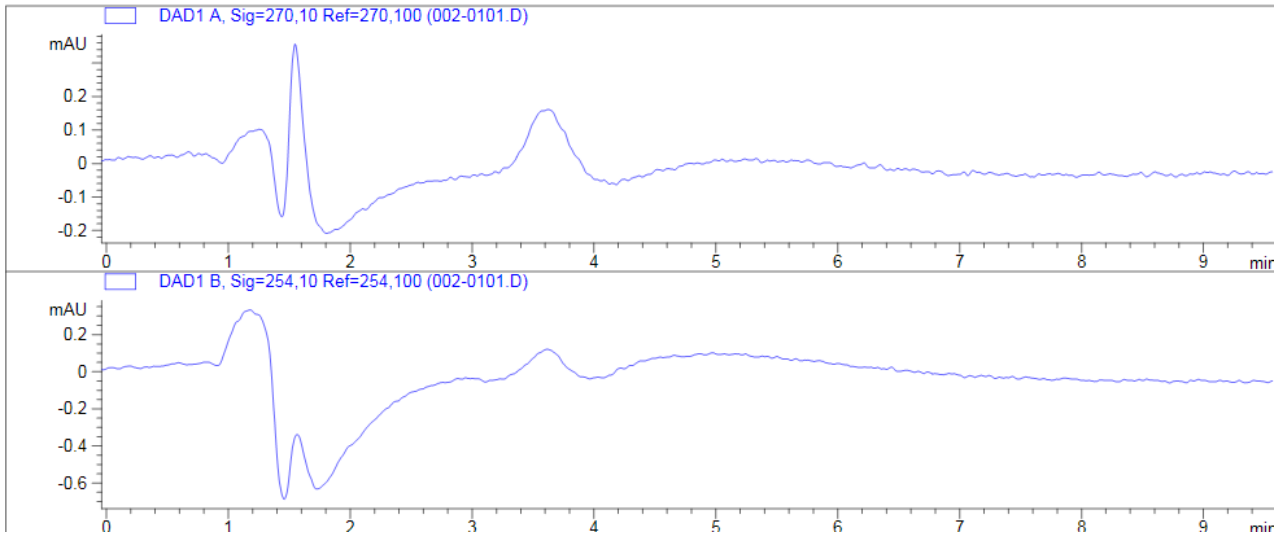

Figure 3: Non-spiked synthetic blood plasma organic reconstitute chromatogram.

It is also the case that in both the trials for the thymol spiked and non-spiked raw synthetic blood plasma there were large, broad negative peaks. While this did make it difficult to compare to the groups that had gone through sample preparation, this was not necessarily bad data. If one could clearly identify a thymol peak in the groups not having gone through sample preparation then there would be no need to perform sample preparation. Therefore, this experiment confirmed the necessity of the underlying goal for this thesis—to develop a method to separate and quantify propofol from blood plasma.

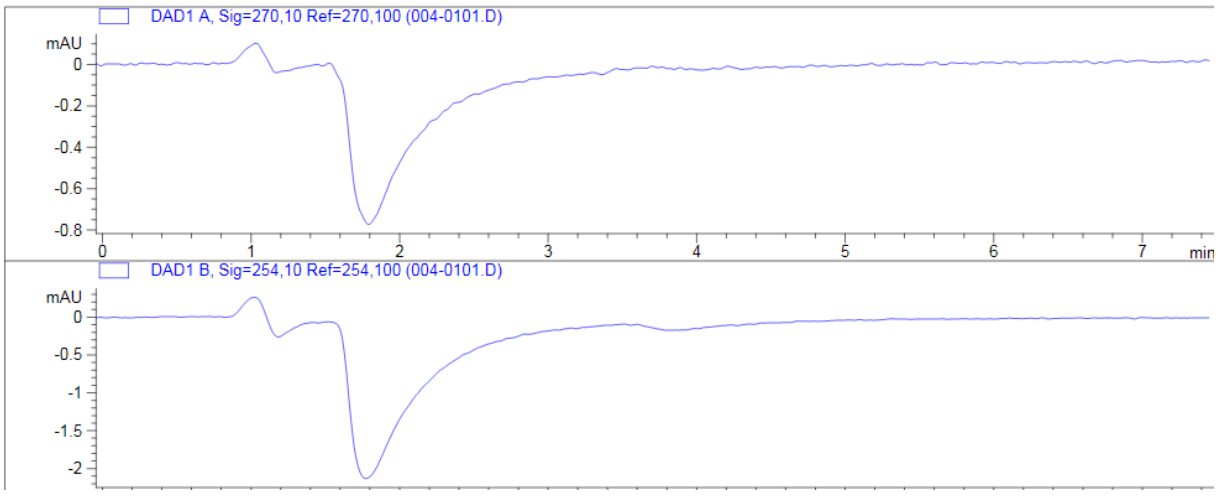

Figure 4: Thymol-spiked raw synthetic blood plasma chromatogram.

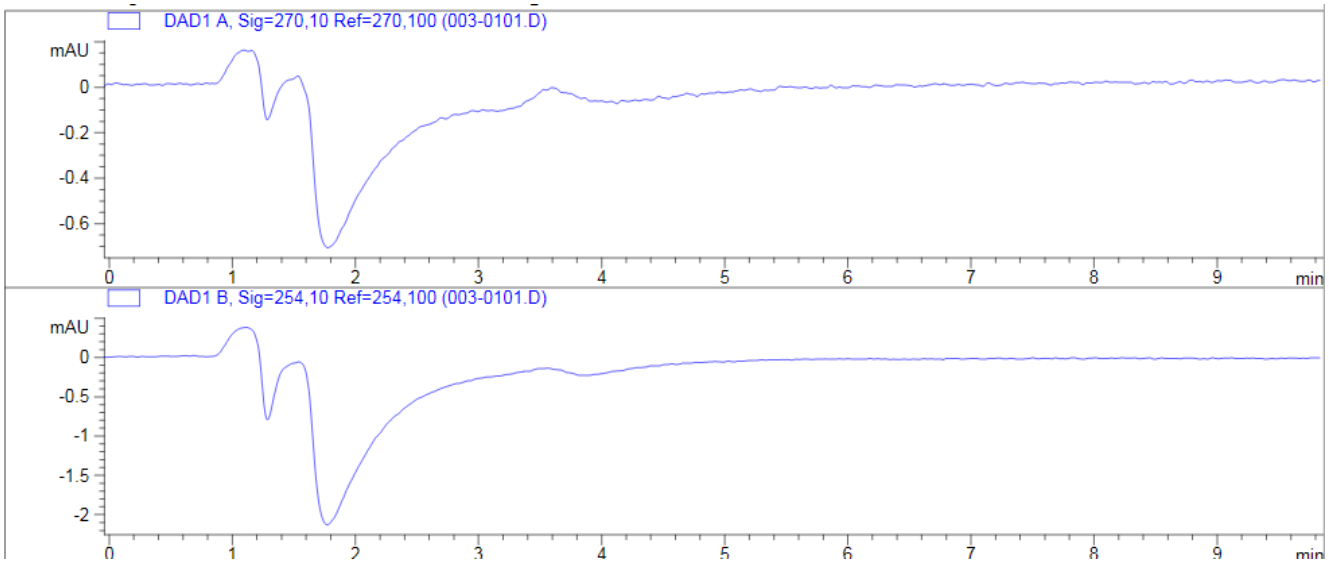

Figure 5: Non-spiked raw synthetic blood plasma chromatogram.

One should also note the instrumentation challenges in this and the following experiments. Throughout any experiment performed on this instrument, some inconsistencies should be expected because the instrument itself is inconsistent. There are some things that can be done to counter this, for example: washing the autosampler needle between every sample run, however, this can only do so much. If the electromagnetic pump valve starts causing the instrument to have significant pressure fluctuations, as it will in the next experiment, and the operator must fix it and finish the experiment the next day, this will likely result in slight variation in conditions when performing different trials. These variations can ultimately impact the chromatogram consistency, especially when dealing with a nano-scale liquid chromatographer.

This experiment clearly illustrates a need for further experimentation to be done regarding optimal excitation and emission wavelengths for the detector. This is meant to improve the absorbance values, especially the difference between the thymol spiked and non-spiked

values. This also illustrated the need for a mobile phase calibration curve as negative peaks generally reflect either an improper proportion of organic to polar phase, especially when using methanol, or an improper organic phase altogether. While there is literature suggesting that there are other viable organic phases that can be used to separate propofol and thus thymol, such as a 2:3 ratio of acetonitrile to MilliQ H_2O, there are also many which use methanol as the organic phase[12]. This would indicate there is something wrong with the method we are using that does not inherently have to do with methanol being the mobile phase, but the negative peaks do warrant its further exploration, thus the reasoning for performing experiment 3.

Experiment 2 — Comparison of nano-LC to conventional LC

This experiment essentially functioned as an instrument audit. Literature asserts what the differences should be between a nano-LC and a conventional LC often are and why this is the case. Some of these differences, and to what extent they exist, can mean that there is some instrument troubleshooting that ought to occur before advancing to the next experimental stage.

One of the common established differences between nano-LC and conventional HPLC is that the former often has broader peaks in its' chromatographic data. This is mostly due to Pre- and Post-column dead volumes which are generally caused by inappropriate or poor tubing and connections. Specifically, the frits used in standard-bore columns are often used in nano-LC setups and they will almost always impart an inappropriately high dead volume for the system. It is worth noting that these poor connections can also result in reduced separation resolution. If the conventional LC has much narrower peaks than the nano-LC, then the chromatographer ought to check the tubing and fittings for system compatibility.

Another established difference is that nano-LC have significantly smaller injection volumes which can result in a loss of detectability. This can be hard to fix because larger injection volumes will often cause peak broadening. While for this application, broad peaks are not necessarily the end of the world, it is important to be able to ensure separation is occurring. If peaks are too broad, and combining into one or masking other smaller peaks while passing through the LC detector, then this is a major issue. Comparing the nano-LC to conventional will help ensure that there is appropriate detectability in the former approach. If there are four peaks in the conventional chromatogram and two in the nano-LC then, assuming other items such as tubing and fittings are appropriate, then the injection volume ought to be increased. This is illustrated in figure 5 and figure 6 where it can be observed that increasing the injection volume

from 0.4 uL (fig. 5) to 0.5 uL (fig. 6) results in another peak appearing at approximately 3.75 minutes. This is likely due to a loss of detectability of that analyte at the smaller injection volume. In short, this experiment helps to ensure an injection volume in which separation can be observed on the chromatogram is being used.

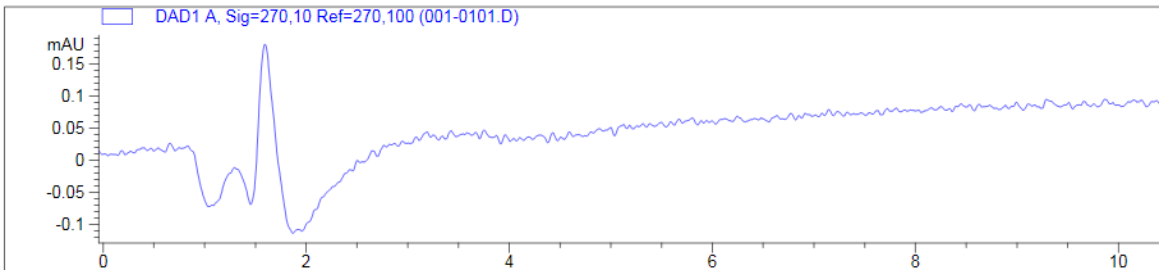

Figure 6: Thymol sbp organic reconstitute with an injection volume of 0.4 uL

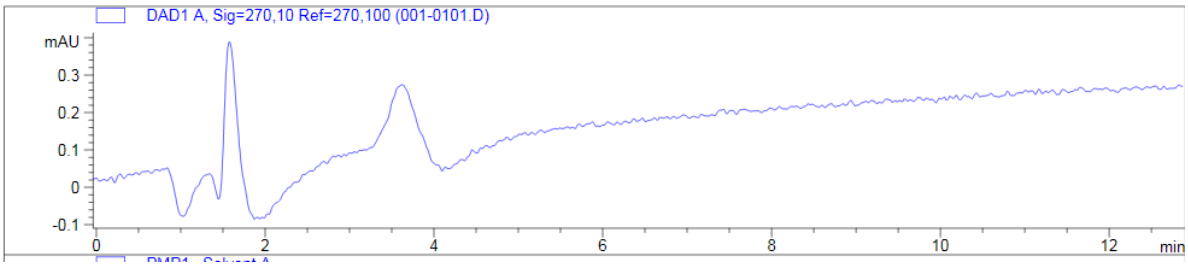

Figure 7: Thymol sbp organic reconstitute with an injection volume of 0.5 uL

Experiment 3 —Mobile phase standard calibration on nano-LC

After running samples for the 70:30, 60:40, 50:50, 40:60, and 30:70 MeOH to MilliQ H_2O groups, the results seem to indicate that that the 75:25 ratio that had been used in the previous procedures was inappropriate. The high mAU values were found to be in the 60:40 ratio. While peak height is not necessarily the only thing to consider when choosing a mobile phase, on a nano-scale instrument it is a useful item to consider. Most peaks will not be 100's of mAU high—instead they will generally be less than 10 mAU.

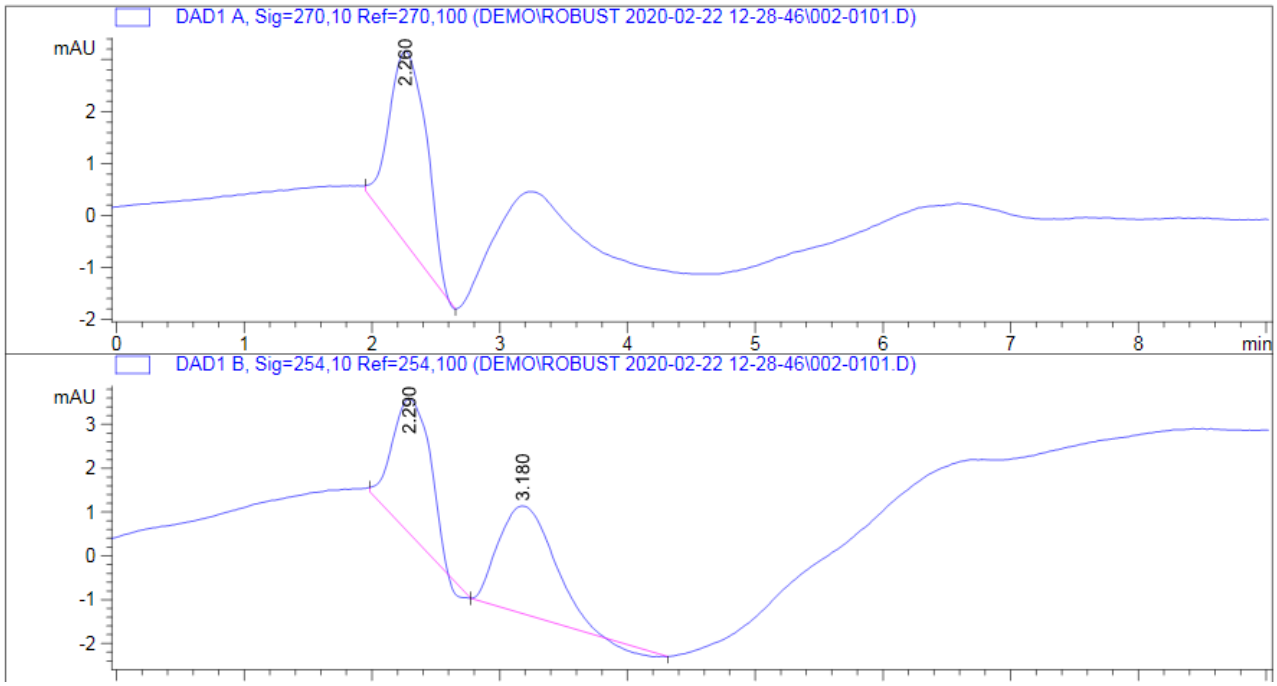

Figure 8: 60:40 methanol to milliQ Water chromatogram.

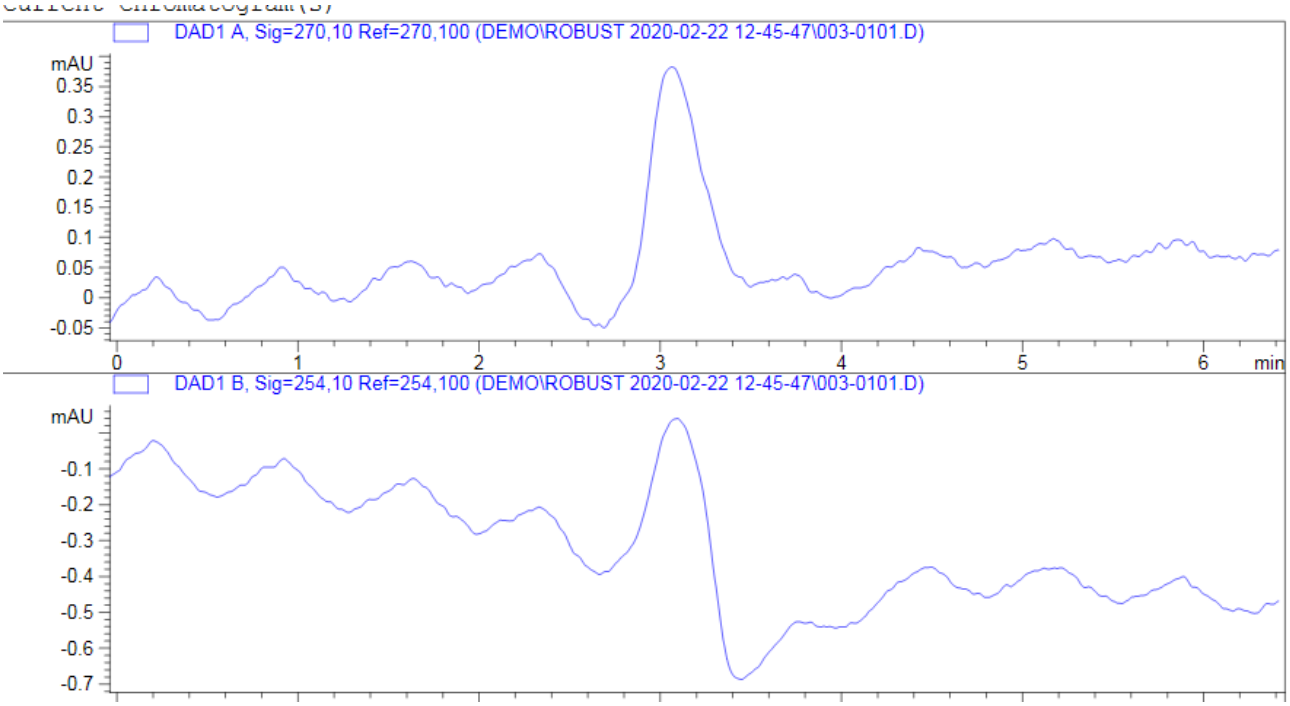

Figure 9: 50:50 methanol to milliQ Water chromatogram. Extremely poor separation is occurring.

An example of why this may be important was illustrated during this same experiment. The electro-magnetic proportioning valve (EMPV) began to fluctuate outside its normal range during this experiment. Typically, it will oscillate by approximately one or two Barr but during this experiment it began to oscillate by approximately 10 Barr. This increased fluctuation can create a noisy baseline on the chromatogram, as clearly illustrated in the trial below. This is a very significant issue if the analyte peak is the same size as the fluctuating noising baseline itself, which is not impossible on a nano-scale setup such as the one used in this experiment. However, since the peaks found in the 60:40 trial were approximately 2.5 mAU high, versus the 0.05 mAU fluctuation, this became a non-issue.

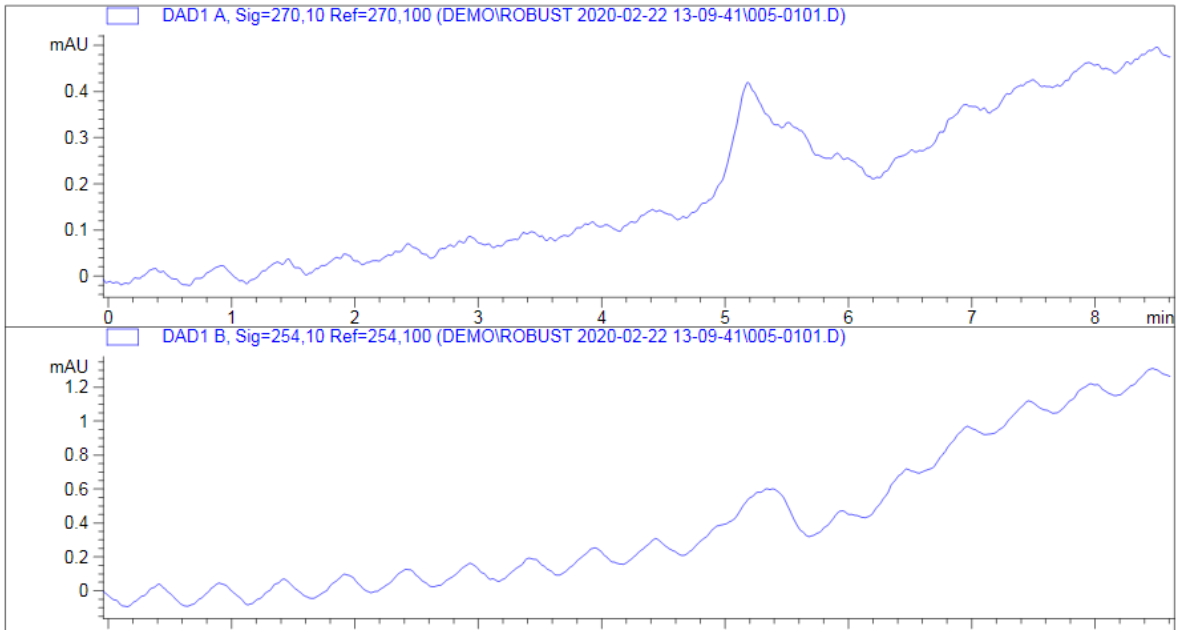

Figure 10: 30:70 methanol to milliQ Water chromatogram.

Analytical chemists strive for optimal conditions. While one can argue that using the 60:40 MEOH:MilliQ H_2O mobile phase mitigates the oscillating pressure from the EMPV under this scenario, one cannot argue it will do so under all scenarios. Instead of testing all scenarios, one can instead simply fix the EMPV.

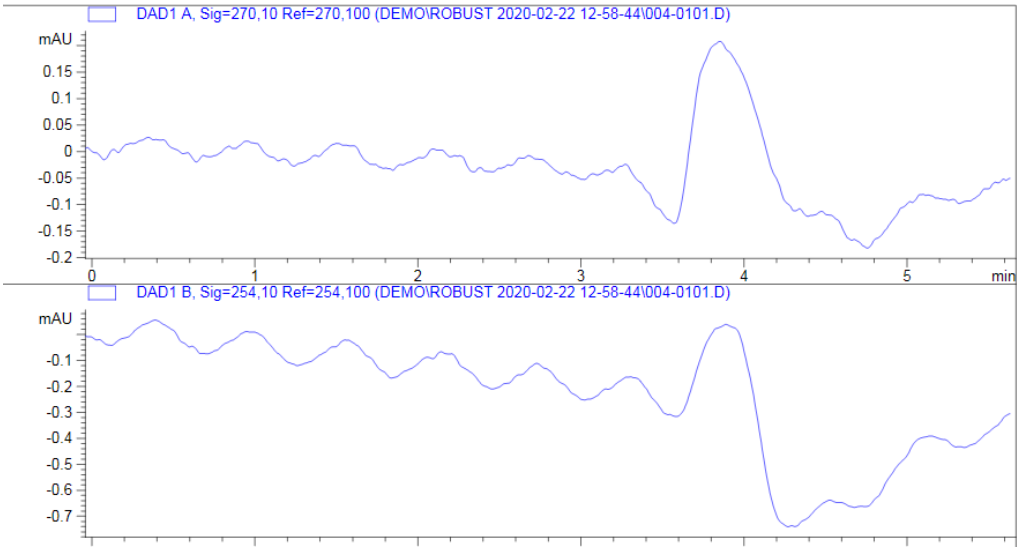

Figure 11: 40:60 methanol to milliQ Water chromatogram. This is a good example of what pressure instability can do to a nano-LC's chromatogram.

The first step to fixing the EMPV was figuring out exactly what may be wrong with this. ChemStation is a user-friendly program that already has a build-in automated EMPV test that can determine most issues one can be having with it. It was performed 3 times and all three times it failed to be completed. All steps up to the test, some of which were blocking the EMPV outlet with a blank nut and flushing the aqueous and organic phase channels running into it, were completed successfully but the test continuously timed out.

While one cannot be sure, the occurrence of this issue soon after running messy, proteinous, synthetic blood samples on the system would lead one to suspect that there is likely some particulate contamination within the EMPV. The system will be flushed with methanol for eight hours, MilliQ H_2O for eight hours, and a 50:50 ratio of MeOH: MilliQ H_2O for another eight hours. While it is characteristic of scientists performing cleaning procedures on their chromatographers to use harsher solvents like concentrated nitric acid, tetrahydrofuran, etc. to clean the instrument, it is known for an absolute fact that everything run on this instrument in the past year has been water-soluble because of its use by only one scientist, excluding a few experiments performed by a fellow lab member, and the documentation done by both of those people. It can be asserted that if this cleaning procedure does not fix the EMPV pressure oscillation then it is not particulate build-up that is the principal cause.

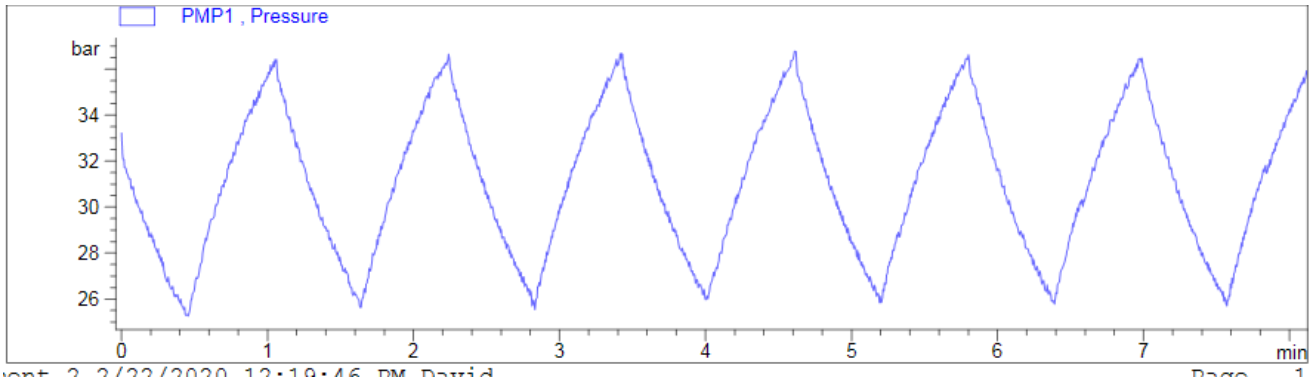

Figure 12: Example of inappropriate pressure fluctuation from the EMPV.

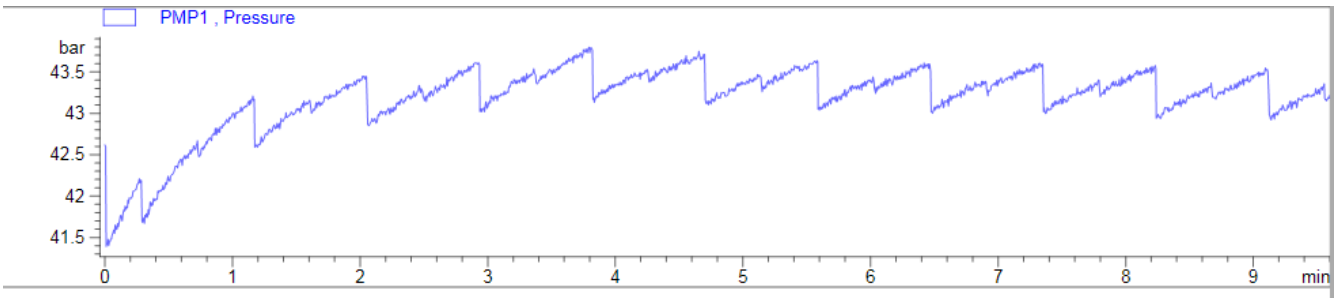

Figure 13: Example of appropriate pressure fluctuation from EMPV. Note the y-axis scale on both this figure and figure 9.

Experiment 4 — Increase detection wavelength parameter (270 nm → 280 nm) (**Theoretical Results**)

The literature regarding propofol absorbance at various wavelengths, and at various pHs, suggests that increasing the detection wavelength parameter will not likely have a significant effect. While figure 13 below from "**Multi-wavelength spectrophotometric determination of propofol acidity constant in different acetonitrile-water mixtures**," asserts there would be almost no difference, the lowest pH this paper obtained an absorbance spectrum was at 8.08[13]. The figure also shows that small differences in pH, such as the difference between 8.08 and 7.35-7.45—the pH of blood, can significantly affect the absorbance values at certain wavelengths so this experiment is still important to do.

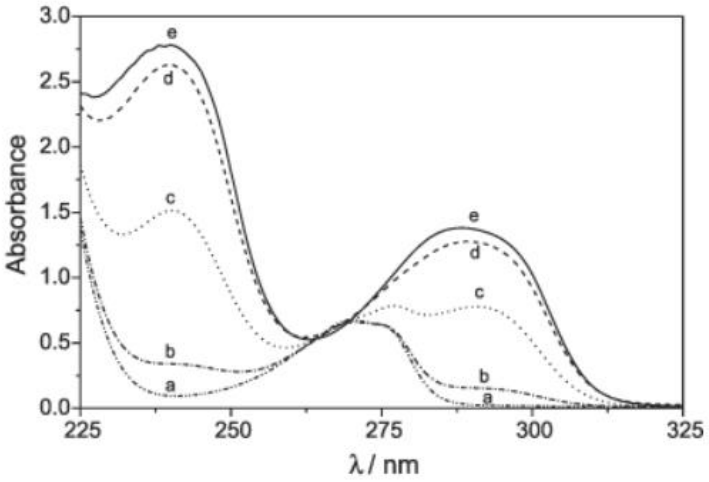

Figure 1. Spectra of propofol in water at different pH. pH: (**a**) 8.08; (**b**) 10.45; (**c**) 11.69; (**d**) 12.69; (**e**) 13.65. Propofol concentration: 5.0×10^{-4} mol L^{-1}.

Figure 14: Spectrum of propofol absorption in water at various wavelengths and pHs from ***"Mulit-wavelength spectrophotometric determination of propofol acidity constant in different acetonitrile-water mixtures."***

It is also important to consider the absorbance of thymol, the internal standard, and other blood components as well. If there is a high absorbance value at 280 nm but no detectable

absorbance for thymol then this would be an inappropriate wavelength for this method. In addition, if there is such a high absorbance for the various blood plasma components that it makes the thymol and propofol peaks appear as potential background noise then this would also be an inappropriate wavelength for this method. It is due to all of the above considerations that while this experiment may not provide incredibly important data for the method, it is still an important experiment to perform.

Experiment 5 — Flowrate experiment

In practical chromatography, flowrate is a balancing act in which the operator must ensure that proper separation is occurring but that there are also narrow peaks. Proper separation is promoted by the sample spending more time in the column and thus at a lower flowrate. Narrow peaks are promoted by the sample passing in front of the detector more rapidly and thus at a higher flowrate. There are two additional considerations as well. The first is that with increased flowrate also comes increased pressure. A system operating at an inappropriately high pressure will result in leaks. This pressure consideration is especially important on a nano-LC setup since there is a lower tolerance for inappropriate pressure and thus a propensity for leaks. The second is more practical in nature. An operator only has so much time to perform experiments and if the flow-rate is so low that each sample takes 30 plus minutes to run then this experiment can quickly balloon into multiple days. As a rule of thumb, doing a whole experiment in one sitting is preferred as it leaves fewer variables that could affect the results outside of the independent variables assigned during experimental design.

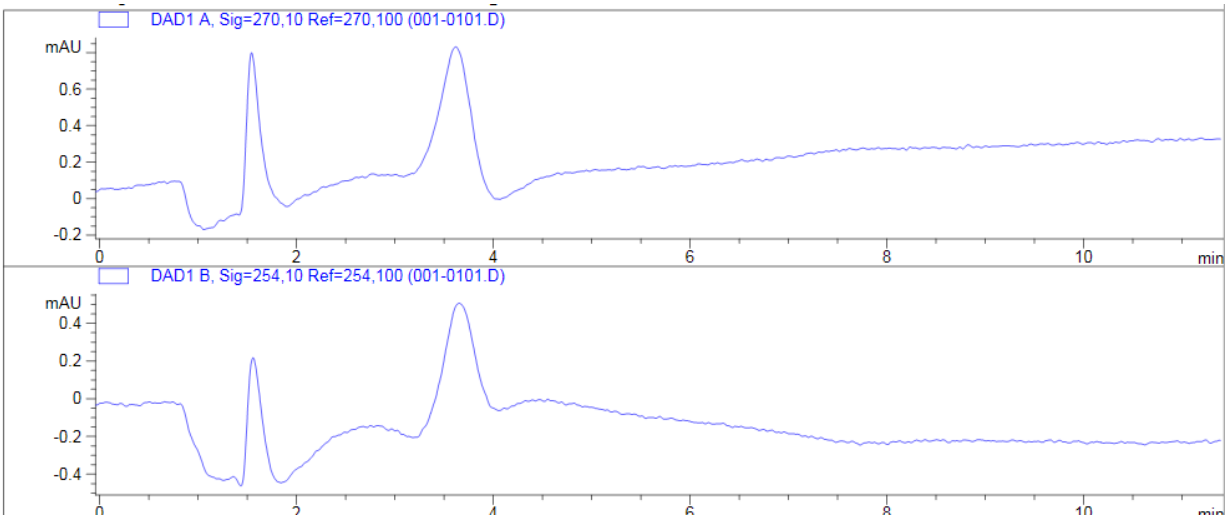

Figure 15: Thymol-spiked synthetic blood plasma organic reconstitute ran at 60 ul/min.

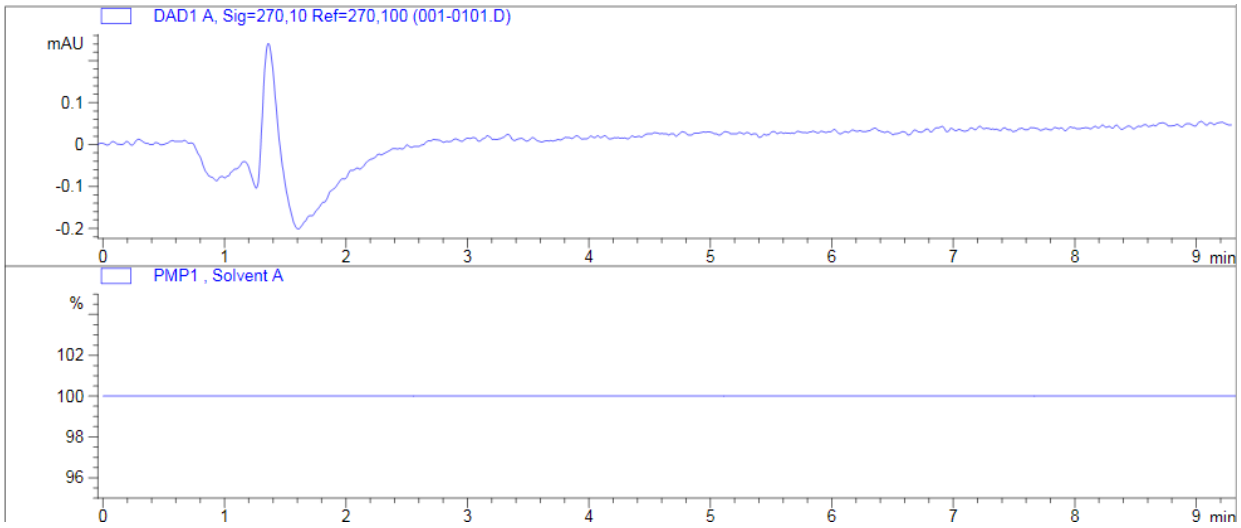

Figure 16: Thymol-spiked synthetic blood plasma organic reconstitute ran at 70 ul/min. There is a noticable lack of seperation at the faster flowrate.

It is due to all the above considerations that a flowrate experiment is worth doing, especially on a set up as finicky and sensible to changes in pressure as a nano-LC. The flow-rate used up to this point has been 60 uL/min. This is the recommended flowrate used for the inner diameter (I.D.) of the column purchased for this nano-LC setup, in an optimal system, i.e. one with a tubing I.D. that matches the column I.D.. However, something that a chromatographer should keep in mind when optimizing a protocol for a system: Optimal in general may not be optimal for the system being used. Therefore, testing flowrates near 60 uL/min (between 40 uL/min and 80 uL/min) may offer some better balance of separation, peak width, and run time.

Experiment 6 – Samples Analyzed Using LC-MS (**Theoretical Results**)

So long as no operator errors or system-related setbacks occurred then there would likely be rapid, accurate, and precise separation and quantification of propofol using the nano-LC-MS setup. The literature clearly shows that LC-MS is well suited to this medical application, in which there is a complex sample leading to noisy chromatographic data, but that is once a method is developed. All experiments leading up to this have been in order to develop this method so that the LC-MS can occur smoothly, as would have hopefully occurred in this experiment. The data from this would also illustrate that the LC and MS were properly interfaced if the experiment went well. If not then the previous experiments would help to make the interfacing of the LC and MS the main the first variable to look further into, outside of the general finickiest of the nano-LC instrument, as everything else should be roughly optimal.

Figures 16 and 17 are a chromatogram and spectrum respectively from an important paper that uses a similar separation system as was optimized in this work. While there are some small differences between the ultimate method used in this thesis and this paper, such as the mobile phase being 75% methanol and 25% milliQ water with an addition of 0.025% NH_4OH, likely due to column aging, they are somewhat representative of data that might be obtained if not for the COVID-19 pandemic. Chromatogram A corresponds to spectrum A & A'.

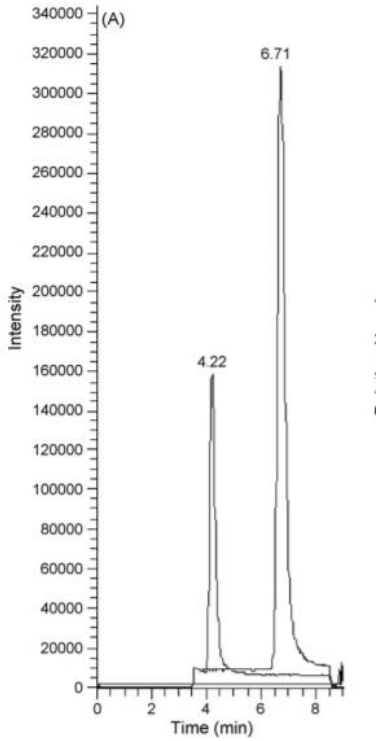

Figure 17: Chromatogram of thymol (4.22 min) and propofol (6.71 min) separated from human blood plasma. Concentration of propofol is 1.35 ug/mL[14].

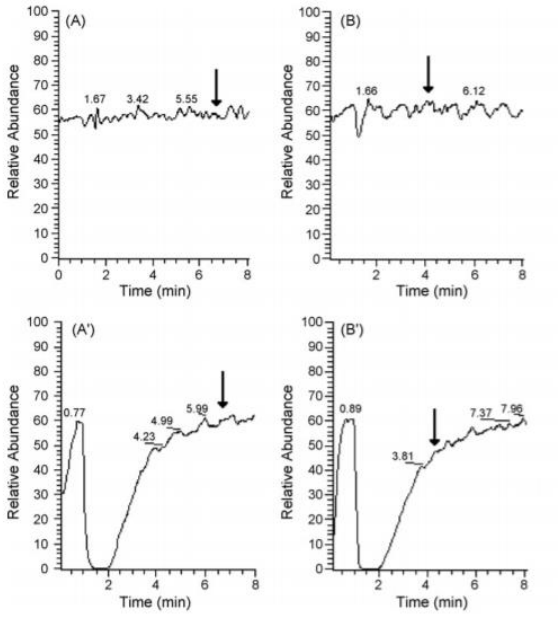

Figure 18: Ion suppression study spectrums from mass spectrometer. Propofol is A & A'; Thymol B and B'. [14]

All in all, data obtained from this experiment would be a culmination of many other experiments and would if the method had been developed to the point of readiness for additional research purposes such as clinical studies or quantification of analytes in a sample taken from a patient. The data from this experiment, if performed, would hopefully look like that which was derived from Cohen et al.

Conclusion

Despite the many scientific and health-related obstacles faced throughout this project, significant progress was made towards developing an optimum method to separate and quantify propofol in blood plasma using LC-MS instrumentation. Parameters such as mobile phase ratio, detection wavelength, and flowrate were optimized while relevant baseline data and comparisons between nano and conventional LC were obtained. For many of the experiments thymol was used as the analyte due to its low cost in comparison to propofol although it would be the internal standard in real world applications. However, because of the molecular similarity between the two this was an appropriate choice.

There are many future experiments and instrument modifications that could give us valuable insights into the developed method. Comparing the nano-LC instrument in the Remcho lab to another nano-LC instrument could help us determine if the former is so temperamental by the nature of it being a nano-LC, or because of its age and previously heavy use. Replacing the EMPV could also lead to more consistent results by stabilizing the pressure samples are run at to a greater extent than previously. Experimenting not only with smaller mobile phase ratios (ratios like 65:34 and 62:38 rather than increments of ten), but also solvent systems entirely different from the current methanol-water one could also lead to more optimal separation and quantification. Applying this method to a real-world clinical experiment would also be valuable because this method was not developed for the sake of development, but instead to be used in a clinical setting. A section highlighting an experiment for just this can be found below.

Although additional experiments can be performed, this project not only produced a more optimal separation and quantification technique for propofol on the instruments in the Remcho laboratory but it also created a more capable and proficient scientist.

Clinical Experiment Using a Fully Developed Methodology

Introduction

One of the most common conditions that any intensivist must treat is alcohol withdrawal. Each year in the US there are approximately 500,000 instances of moderate or severe alcohol withdraw that warrant some level of pharmacological or medical treatment[15]. Since most of these patients are chronic alcoholics, a patient population known for its numerous comorbidities, it is medically necessary to examine how these other physiological issues they may be suffering from during their hospital stay may impact treatment for the chief complaint.

Alcoholics and even typical heavy drinkers often exhibit various liver diseases due to the disastrous effect heavy, chronic alcohol consumption has on it. Alcoholic liver disease is the principal disease which they experience—an estimated 10 to 15 percent of patients within this population will progress to the final stage of this diseases, liver cirrhosis[16, 17].

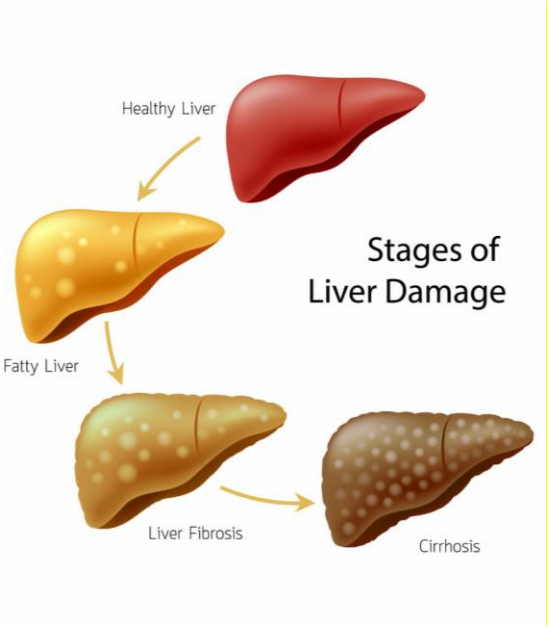

Figure 19: Stages of Liver Damage[23]

Another consideration when treating alcohol withdrawal is the frequency of intubation, which generally involves the use of propofol during the insertion of a laryngeal tube. Propofol is also used as a secondary treatment for patients suffering from refractory delirium tremens induced by the alcohol withdrawal, but only when the patient does not improve from the more conventional treatment of a high dose of benzodiazepines. It is worth noting that the dose of propofol used to treat this also can result in the need for intubation due to concerns regarding patient airway and inhalation control. In addition to this, when a patient is suffering from status epilepticius, the seizures that are commonplace to alcohol withdrawal, propofol can be used to treat it. Similar to its use in Refractory delirium tremens, too much propofol will result in a need for intubation.

Alcohol is also a central nervous system depressant that functions by binding to specific sites on the GABA receptor complex and promoting their activity in addition to inhibiting glutamate, a major excitatory amino acid, from binding to NMDA receptors and thus inhibiting NMDA activity[18]. This is a similar mechanism of action to propofol's, which is not counterintuitive considering how they are both depressants[18].

One must consider the frequency of propofol administration in the treatment of patients suffering from alcohol withdrawal, the frequency of late-stage alcohol liver disease, i.e. liver cirrhosis, in this same population, the fact that 40% of propofol is metabolized in the liver, and that inappropriately high doses of propofol can result in intubation which can be monetarily costly for the patient and hospital, and temporally costly for the medical team. From this emerges a scholarly interest in how the administration of a given propofol dosage in patients suffering from alcoholic liver disease result in more or less extreme clinical effects when compared to a normal healthy patient.

When examining the plasma propofol levels of patients suffering from liver failure, one must first consider that IV administration of propofol quickly reaches an equilibrium between perfuse organs and the blood plasma, then it is quickly metabolized by the liver, making most of the clinical effects of a total single dose of propofol last only between three and five minutes. Knowing this, it is reasonable to ask if a patient with liver failure will have a higher equilibrium concentration in their organs. This is a clinically relevant question because if this is the case then a given dose of propofol would elicit a greater clinical effect than it would in other patients. It is also reasonable to ask if the effects of a given dose of propofol will last longer because of the potential reduced ability for the liver to metabolize propofol; much of why propofol is so useful is because of how quickly it's effects dissipate compared to other anesthetics. If this advantage is taken away it is also possible that other anesthetics would offer more advantages and it could affect whether a physician deems it necessary or advantageous to use fospropofol, a widely used, slower acting prodrug of propofol. If experimental data is obtained that supports this line of thinking, it could ultimately save hospitals and patient money, not only because there would be experimental data to support using less of the drug on a given patient population, but also because administering too much propofol can cause physiological complications, which require the time and attention of physicians. When something requires physician attention it can be an assumption that it will be a costly expense, either because of their loss of time to perform other activities that produce profits for the hospital or by having them run tests and do procedures that cost the patient money.

In a similar vein, there are many circumstances in which continuous propofol administration is performed—intubation can be one of those things if an anesthesiologist wishes to keep the patient unconscious throughout the duration of the laryngeal tube being in their

esophagus. If a patient suffering from liver failure is kept in this state during alcohol withdrawal, continuous propofol administration at an inappropriately high rate, even if it may be considered typical for patients with a healthy liver, could cause serious complications as well if a physician does not catch it soon enough.

In short, it can be costly and dangerous for a physician to administer too much anesthetic, so research on if an appropriate propofol dosage in patients with a healthy liver is different from those suffering from advanced liver failure could be economically and clinically useful. This is only emphasized further by the frequency at which this patient population, i.e. alcohol withdrawal coupled with liver cirrhosis, is treated in by intensivists in ICU's across the US.

Study Design (Include a procedure in here)

Propofol administration will be used during rapid sequence intubation by physicians performing endotracheal intubation. Rapid sequence intubation is performed since patients in this study, and requiring intubation in general, will not have fasted. This is an archetypical situation found in an emergency department or intensive care unit and reduces the risk of patients vomiting and subsequent aspiration of vomit[19]. This style of intubation, as opposed to common procedural anesthetization, does not require additional airway management like bag-value-mask ventilation[19]. The dosage for viable patients within this study will be equal to the recommended common dosing range for a rapid sequence intubation that is not performed in an operating room of 1.0 to 3.0 mg/kg. This will not only allow one to analyze the plasma propofol but also average the requisite dose of propofol required to elicit the clinical effects sough for an intubation.

The physician administering this experiment will take a small 1 mL blood sample every minute from the point of 1 minute before the propofol dose is administered, until 20 minutes after it has been administered. After each blood sample it will immediately be put on ice and then all samples will be frozen in a -80 °C and transported to the lab with which the samples will be analyzed in. They will be analyzed using an analogous, but more perfected technique, to that described and refined in the first section of this thesis. It is worth noting that an intubation will occur for experimental group 1, those undergoing severe alcohol withdraw which have a history of the alcoholic cirrhosis stage of alcoholic liver disease who are requiring intubation. A bispectrality index monitor will also be used to record the general level of sedation each patient is under in a quantitative fashion. This instrument assigns a number between 0 and 100 that indicates how sedated a given patient is. Since it is not calibrated for a given patient it is a rough value, but may still yield important clinical insights. Note that the other quantitative

measurement taken will be the minimum amount of propofol required to put the patient past the threshold of "appropriately sedated" as determined by the board certified anesthesiologist administering the propofol.

Defined Experimental Groups

- **Group 1** – 7 Patients undergoing severe alcohol withdraw which have a documented history of the alcoholic cirrhosis stage of alcoholic liver disease who are requiring intubation. This group will have consented to being a part of and fully understand the implications of participating in this study.
    - This is the experimental group.
- **Group 2** – 7 volunteers who suffer from similar risk factors as the those undergoing alcohol withdraw induced intubation and alcoholic liver cirrhosis, but have normal, healthy functioning livers. This group will have consented to being a part of and fully understand the implications of participating in this study. This group, like group 3, will not be intubated. Risk factors included in this group would be patients suffering from anxiety, obesity, hypertension, chronic bronchitis, epilepsy, and peptic ulcer disease[20, 21, 22].
    - This is the control group.
- **Group 3** – 7 healthy volunteers who are sedated via propofol administered but will not be intubated. They will also have consented to being a part of and fully understand the implications of participating in this study.
    - This group is the control group for the control group. This helps us determine if a change in propofol plasma concentration, bispectral index monitor value, or clinical response to propofol is due to liver functioning or from one of the other

numerous risk factors that the patient undergoing severe alcohol withdraw likely has. If this group has a lower concentration of plasma propofol at a given point and time, but at that same point group 1 and 3 have a different plasma propofol concentration, this could indicate that there the difference in propofol metabolism, is likely due to other comorbidities, not liver function.

Procurement of Research Subjects

This study is to be approved by the ethics committee and/or institutional review board at the hospital or university this study is conducted at. Written consent of all subjects must be obtained before participation in the study begins. While group 2 and 3 consist entirely of volunteers, group 1 will need to be recruited in the emergency department and/or intensive care unit. A large proportion of emergency department patients at any given institution will be those which are suffering from alcohol withdrawal so procurement should not be challenging. A subgroup of those suffering from alcohol withdrawal will have a history of the alcoholic cirrhosis stage of alcoholic liver disease. There is usually some time between admission to the emergency department and intubation as it is not required in all cases of alcohol withdraw, therefore a clinical research assistant or medical student will try to secure participation of all patients who fit this group and will only initiate the experiment if intubation does end of being required.

IRB must determine that each of the following requirements are satisfied

- Physical and psychological risks to subjects are minimized.

    - Patients from group 1 which have the experiment performed on them will already be undergoing intubation, of which propofol sedation is a typical component of. Therefore, no additional physical or psychological risks are added, except for the loss of 21 mL of blood. This is a very small amount of blood, but clinicians will use their judgment to determine if the patient is in such a poor condition that this could potentially harm them. If there is any chance it could, then they will not be a participant in the study.
    - Patients from groups 2 and 3 will have a physician and nurse attending to them throughout the experiment to ensure any medical complication can be immediately dealt with. There is an extremely low likelihood of any negative outcomes occurring. There are few psychological risks to this study as propofol has an amnesic affect so they are unlikely to remember the propofol administration or sampling of blood.
- Physical and psychological risks to subjects are reasonable in relation to anticipated benefits to those subjects and to the importance of the general knowledge that may reasonably be expected to result.
    - Considering how widely used propofol is as a means to intubate patients, and how often patients with some form of alcoholic liver disease require intubation, it can be asserted that the importance of knowledge obtained from this study, and the benefit to many of the group 1 patients that could be categorized as “frequent fliers,” outweighs the physical and psychological risks the subjects may be subjected to.
- Selection of subjects is equitable.

- It is not unreasonable to worry that potential subjects for group 1 could be susceptible to coercion or undue influence from those conducting this study. After all, the first interaction which the study coordinators will have with a potential participant is when they are already in the emergency department for alcohol withdrawal. With this there is an implication that the patient will be under some degree of intoxication. It is because of this that a neutral, passive, and concise standardized script will be made for the study coordinator to ask the patient if they are interested in participating. If the IRB deems that this alone cannot assure equitable selection of subjects and that subject consent will in invalid due to their intoxicated state, then the study coordinators will seek out subjects who are habitually admitted to a given hospital for alcohol withdrawal and obtain consent to participation in the study for their next case of alcohol withdrawal, at the end of their current hospital stay for the same chief complaint.
- For groups 2 and 3, posters will be put up in the hospital and surrounding area looking for study participants. From those that volunteer to potentially be part of the study, a group which is representative of the population of interest for groups 2 and 3 will be selected by the study coordinators and independently verified by the diversity office.

- Informed consent will be obtained, including at least the following items being communicated to potential participants or their authorized surrogates:
  - A 1-2 page document with the pieces of information seen below on it will be given to the participant. A study coordinator, clinical research assistant, or medical student will explain each item and ensure that any possible questions a

participant has are answered before consenting to the experiment. Pieces of information:

- purposes of the research, its expected duration, and the nature of any interventions/experiments.
- anticipated risks and benefits of participation and the reasonable alternatives to participation in the research protocol;
- confidentiality provisions relating to the research records;
- any compensation and/or treatment available for research related injuries;
- the right to not participate and to discontinue participation at any time without penalty.

- Informed consent will be documented appropriately as is customary for IRB approved research studies.

Data Analysis

Propofol Plasma Concentration and Bispectral Index Monitor Values

After separation and quantification of propofol in blood plasma occurs for all samples by LC-MS, each point will be averaged with the other points at that time and in that group. For example, all samples taken for group 1 at the time of 5 minutes will be averaged. These will then be plotted on a line graph and compared to the other 2 groups. There should be three total lines on the graph, one for each group. This will allow for easy evaluation of how propofol was metabolized over time and how it relates to liver function and other risk factors. This will also be compared against the data gathered from the bispectral index

monitor, which will be averaged and graphed in an identical manner, to determine if there are large differences in depth of anesthesia versus blood plasma propofol levels across different groups at various points throughout the sedation.

Propofol Dosage Required to Induced Sedation by Group

This portion will consist of a relatively uncomplicated data analysis. Each individual participants required dosage for appropriate propofol sedation will be recorded during experimentation and then divided into their respective groups. Group 1, 2, and 3 will then be compared to see if there is a difference between the average dosage required (in terms of mg/kg) and conclusions will be drawn based on this. Since $n > 3$ for each group, differences will be considered statistically significant. Analysing this in the form of mg/kg is important since this allows one to standardize the results across weights.

Conclusion

Studying the impact that alcoholic liver disease resulting in liver cirrhosis has on propofol metabolizing and sedation could improve patient safety, reduce economic costs for the consumer base, and the hospital system. There is a clear biological mechanism by which there could be a relationship between the two, principally 40% of propofol being metabolized in the liver (57% of all non-excreted propofol) thus warranting exploration. The sound study design and multiple quantitative measurements taken will hopefully illustrate the relationship between liver cirrhosis and propofol induced sedation, if one exists.

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