Effects of Cleaning an Epidural Catheter Hub with Alcohol and Determination of Neurotoxicity on Rat Astrocyte Cells

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Abstract

Patient safety must always be the first concern for anesthesia providers and aligning with evidence-based research provides best practice standards. The standard for cleansing the epidural catheter hub is rudimentary and poorly established as shown by the variations in current practice. Difference in opinions exists between cleansing the epidural catheter hubs with alcohol for bolusing administrations and the risk of causing adhesive arachnoiditis and/or neurolysis/apoptosis in the epidural space. A literature review revealed research concerning skin cleansing prior to placement of neuraxial anesthesia; however, the evidence was absent regarding best practice for epidural catheter hub access. Commentary and guidelines were made based on poor outcomes of two case studies, but no research has focused on epidural catheter hub aseptic techniques and risks to date. The intention of this scholarly project was to conduct an experimental study design with five epidural catheters and pumps infusing onto commercially available rat astrocyte cells after cleansing the epidural hubs with 70% isopropyl alcohol to test the potential presence of alcohol introduced into the epidural space and the risk of adhesive arachnoiditis and neurolysis/apoptosis. Each epidural pump would run an infusion into a sample size of five commercially available rat astrocyte cells. At completion of infusion, the commercially available rat astrocyte cells would be analyzed to determine the presence of alcohol in the cells. Data would be gathered by student co-investigators and sent for analysis using a statistical analysis software package. These results are intended to provide evidence-based recommendations for cleansing epidural catheter hubs with alcohol in anesthesia practice. Due to the nature of this scholarly project and unforeseen limitations the completion of proposed methods was not possible.

Table of Contents

List of Appendices

Effects of Cleaning an Epidural Catheter Hub with Alcohol and Determination of Neurotoxicity on Rat Astrocyte Cells

Introduction

Epidural catheters are routinely placed in the clinical setting by anesthesia providers under sterile technique. These catheters pose a potential risk of neurolysis/apoptosis due to causative agents such as povidone-iodine, chlorhexidine, and alcohol entering into the epidural space (Bogod, 2014; Killen et al. 2012; Kinirons et al. 2001; Mohamed Iqbal et al., 2018). The gap in practice begins with which cleansing agent is used for the epidural catheter hub prior to injection of medication (Mohamed Iqbal et al., 2018; Paice et al., 1999; Torres de Arujo Azi et al., 2020). As alcohol has been used to cause neurolysis/apoptosis as a cancer treatment, it has been suggested that when reaching the subarachnoid or epidural spaces, 70% isopropyl alcohol is considered to be the most causative agent for neurolysis/apoptosis although specific amount has not been determined; therefore, it is not commonly used for catheter hub cleansing (Campbell et al., 2014; Health & Home Care [HHC], 2005; Home Health Visiting Nurse Association [HHVNA], 2013). The amount of 70% isopropyl alcohol necessary to cause neurolysis/apoptosis has been suggested to be at least a measurable amount of 0.1 milliliter (ml), but no minimum has been established (Campbell et al., 2014; Poddar et al., 2016). However, it is still utilized by some providers in this situation because of its superior antiseptic attributes (McKenzie & Darragh, 2011; Paton et al., 2012).

Significance and Background of Identified Problem

Anesthesia practice varies on the cleansing of epidural catheter hubs with alcohol due to the potential of adhesive arachnoiditis and neurolysis/apoptosis (Bogod, 2014; Campbell et al., 2014; HHVNA, 2013; Killen et al., 2012; Miller, 2013; Poddar et al., 2016). Concern over

alcohol entry into the subarachnoid space with spinal anesthesia after cleansing the skin with alcohol containing preparation (prep) solution has been established (American Society of Anesthesiologist [ASA], 2017; Bogod, 2014; Campbell et al., 2014; Miller, 2013). In discussion of a court case regarding arachnoiditis resulting from a splash of alcohol into the skin prep being potentially injected into the subarachnoid space, controversy exists over whether a measurable amount (greater than 0.1 ml) would be found in a spinal needle with appropriate dry time observed (ASA, 2017; Bogod, 2014; Campbell et al., 2014; Miller, 2013). This matter has been extrapolated to the potential risk of adhesive arachnoiditis or neurolysis/apoptosis occurring in the epidural space if used for cleansing the epidural catheter hub. Despite concern for neurolysis/apoptosis, cleansing with alcohol has been repeatedly proven to provide asepsis benefit for patient safety (ASA, 2017; Bogod, 2014; Campbell et al., 2014; Miller, 2013). With such a diverse stance in practice, it is necessary to investigate if cleansing the epidural hub with alcohol can provide asepsis without introducing a measurable amount into the epidural space, negating the risk of neurological injury. Certified Registered Nurse Anesthetists (CRNAs) must continue to provide safe and effective practice based on evidence-based literature. The purpose of this literature review is to assess the risk of introducing alcohol through an epidural catheter and into the epidural space after cleansing the epidural catheter hub with 70% isopropyl alcohol.

PICOT Evidence Review Questions

This systematic literature review was driven by two PICOT questions. The first addresses the clinical problem: When bolusing in-dwelling epidural catheters (P), does cleansing the Luer tip catheter injection port with 70% isopropyl alcohol (I) place patients at risk for neurological injury (O)?

The second addresses the clinical innovation: In epidural catheters (P), what is the effect of cleansing the injection port with 70% isopropyl alcohol pad and bolusing with 2% lidocaine with epinephrine 1:200,000 (I) on gas chromatography (GC) analysis results, and is there neurolysis/apoptosis of commercially available rat astrocyte cells with a subsequent infusion of 0.2% ropivacaine plain (O)?

Search Strategy

The search strategy included five online databases: CINHAL, Cochrane Collection Plus, Google Scholar, Centers for Disease and Control and Prevention (CDC) and PubMed. Initially, a total of 3,653 articles populated. After examining the titles, abstracts, and articles for applicability, 14 articles consisting of systematic reviews, policies, editorials, and clinical trials met inclusion criteria; articles not focused on or beyond the scope of the problem were excluded. Six additional sources were added based on commonly referenced criteria throughout the literature for a total of 20 articles. The key search terms and MESH combinations comprised: *epidural catheters* AND *isopropyl alcohol,* AND, *epidural* AND *alcohol* AND *neurolysis*, AND, *neuraxial blockade* AND *toxicity*, AND, *antiseptic* AND *neuraxis*, AND, *alcohol* AND *adhesive arachnoiditis*, AND, *cleaning* AND *catheter* AND *epidural*, AND, *epidural* AND *micropore filter*. The MESH terms comprised: *neurolysis, anesthesia, epidural, anti-infective agents, propanol, adhesive arachnoiditis*. The search limits included: academic journals, free full text, English language, peer review, systematic review, abstract available, and linked full text.

GRADE Level of Evidence

The quality of the literature concerning disinfecting epidural catheter hubs with isopropyl alcohol related to the risk of neurolysis/apoptosis was examined using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria. The articles

found were a mix of systematic reviews, open label research, guidelines and practice advisories, case studies, and editorials with only one random controlled trial (RCT). For this reason, initially, the research GRADE level of evidence was low at 2. Additionally, concerns of risk of bias, inconsistency, indirectness, imprecision, and publication bias decreased the GRADE rating down to very low at 1. Risk of bias was seen with small sample size, few numbers of reported cases, unvalidated measures used, expert opinions and inference from other aseptic practices, and inadequate blinding when attempted. Inconsistencies were found in aseptic technique and product used, varying opinions on amount of alcohol risking neurolysis/apoptosis, and provider preference-based practices versus evidence-based research. Conclusions regarding neurolysis/apoptosis after swabbing an epidural catheter hub with isopropyl alcohol not officially studied resulted in indirectness, along with inferences from unrelated uses of isopropyl alcohol. Not directly studying disinfecting epidural catheter hubs versus skin prep and small sample size, case incidences, and studies led to imprecision regarding safety and efficacy of isopropyl alcohol use as an antiseptic for epidural catheters. The possibility of publication bias was noted as some publications were for policies and practice guidelines of individual hospitals, several did not address the potential of bias, and others received direct commercial funding, although potential bias was denied. Based on studies not directly researching isopropyl alcohol as an antiseptic for epidural catheter hubs and associated risks, strong relationship of intervention and positive dose-response gradient cannot be observed, thus the quality cannot be graded up. This leaves the GRADE of evidence at a very low +1. Ultimately, the literature quality is very low, and as the problem is not specifically addressed, clinical practice recommendations could not be made. Upon completion of a scholarly project, practice recommendations will be made based on findings.

Literature Review & Synthesis of Evidence

Overview

Based on literature review, the use of alcohol to cleanse epidural catheter hubs is controversial, as concern exists with potentially introducing alcohol into the epidural space and causing neurolysis/apoptosis or adhesive arachnoiditis (Bogod, 2014; Campbell et al., 2014; Health & Home Care [HHC], 2005; Home Health Visiting Nurse Association [HHVNA], 2013 McKenzie & Darragh, 2011; Paton et al., 2012). This association has only been documented with direct contact of alcohol into the epidural space, as seen with cancer treatments that purposely inject varying amounts and percentages of alcohol into the epidural space to cause neurolysis/apoptosis for therapeutic effects (Straube et al., 2014; Poddar et al., 2016)**.** Conflicting practice exists because evidence has established alcohol as a known antiseptic medium preventing the introduction of bacterial agents. The purpose of this literature review is to assess the evidence related to cleansing the epidural catheter hub with 70% isopropyl alcohol and risk for neurolysis.

Operational Definitions

For the purposes of this scholarly project the following terms will be defined as:

- Gas Chromatography (GC) is a technique that analyzes volatile compounds by separating the sample by boiling points through the process of vaporization to individual components Shimadzu Excellence in Science, 2020).
- Gas Chromatography Mass Spectrometry (GC/MS) utilizes the same concept as the GC but in addition ionizes the compound and separates the ions into a mass to charge ratio measuring the intensity of each ion (Shimadzu Excellence in Science, 2020).

- Neurological injury will encompass neurolysis/apoptosis or adhesive arachnoiditis. Neurolysis is the degeneration of nerve fibers by erosive chemical or physical substances (Tariq et al., 2002). Apoptosis is cell death predetermined through genetics which can be manipulated by extrinsic initiators (Renehan et al., 2001). Adhesive Arachnoiditis is defined as an inflammatory injury that leads to intrathecal scarring which eventually can cause disruption in blood supply resulting in atrophy (Killen et al., 2012).

Infection Control

In correspondence with the CDC considering the cleaning of epidural catheter ports, they recommend aseptic technique when the possibility of introducing infection into blood, urine, or cerebrospinal fluid could occur (CDC, 2011; The Division of Healthcare Quality Promotion Centers for Disease Control and Prevention, personal communication, February 26, 2021). The Anesthesia Patient Safety Foundation (APSF) responded similarly citing the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) “Scrub the Hub” initiative which recommended the use of 70% isopropyl alcohol or chlorhexidine gluconate alcohol solution every time a hub is accessed, as all are potential portals for infection. Hubs listed included injection ports of bottles, intravenous (IV) bags, administration sets, needleless connectors, and the hub of a catheter (JCAHO, 2013; M. Warner, personal communication, February 24, 2021). Alternatively, micropore filters used at the end of epidural catheter hubs are another technique to prevent bacterial growth; however, they have proven ineffective (ASA, 2017; De Cicco et al., 1995; Martin et al., 2017).

According to the Standards of Nurse Anesthesia Practice of the American Association of Nurse Anesthesiology (AANA), specifically Standard 10: Infection Control and Prevention, it is the responsibility for the anesthesia provider to follow infection prevention policies and

minimize the risk of infection (AANA, 2019). Alcohol, along with chlorhexidine and povidone iodine, has been established in the literature as use for skin cleansing prior to neuraxial placement, successfully reducing the rate of infection risk (ASA, 2017; Bogod, 2014; Campbell et al., 2014; McKenzie & Darragh, 2011; Paton et al., 2012). The evidence concerning aseptic superiority of chlorhexidine versus povidone iodine is contradictory and poorly established. However, when chlorhexidine and povidone iodine were individually added to the alcohol cleansing solution, bacterial growth was eliminated versus being mixed in an aqueous solution without alcohol (Campbell et al., 2014; Tôrres de Araújo Azi et al., 2020). Infection rate has been studied by examination of epidural catheters, but as a result of skin antisepsis not epidural catheter hub antisepsis (ASA, 2017; Bogod, 2014; Campbell et al., 2014; McKenzie & Darragh, 2011; Paton et al., 2012). Thus, a gap in the literature was discovered concerning the specific use of alcohol wipes to cleanse epidural catheter hubs. This should be studied to establish evidence based practice recommendations. Despite antiseptic benefits, there is concern over introducing alcohol into the epidural space related to adhesive arachnoiditis and/or neurolysis/apoptosis (Bogod, 2014; HHC, 2005; HHVNA, 2013; Killen et al., 2012; Poddar et al. 2016).

Neurolysis/Apoptosis or Adhesive Arachnoiditis

Adhesive arachnoiditis has been established as a major neurological concern related to the possibility of introducing chlorhexidine or alcohol into the epidural space if used as cleansing agents for the skin (Bogod, 2014; Campbell et al., 2014; Killen et al., 2012; Miller, 2013; Poddar et al., 2016). When considering this risk, concern was only noted if a measurable amount (greater than 0.1 ml) was found (ASA, 2017; Bogod, 2014; Campbell et al., 2014; Miller, 2013). As mentioned previously, it was highly suspect that even this small amount would be found on introduction of a spinal needle into the subarachnoid space with appropriate dry time observed

(Bogod, 2014; Campbell et al., 2014; Miller, 2013). Accidental injections of chlorhexidine in alcohol consisting of 8 ml directly into the epidural space have been determined as the causative agent for adhesive arachnoiditis in case studies (Bogod, 2014; Miller, 2013).

Conversely, alcohol has been purposely injected into the epidural space as a neurolytic epidural block (Poddar et al., 2016; Straube et al., 2014). The minimum amount to cause neurolysis/apoptosis has not been established, but varying amounts of 3-15 ml at 7%-100% alcohol has been used for pain relief since the 1930s (Poddar et al., 2016; Straube et al., 2013). Caution was given to narrow benefit versus risk of alcohol injection, and no undesired neurotoxicity effects were reported, although some studies failed to report serious adverse effects. Neurolysis/apoptosis from 3-15 ml at 7%-100% alcohol injection appears to be transient with reports lasting two weeks to one year (Poddar et al., 2016; Straube et al., 2013). Despite concern for neurolysis/apoptosis in neuraxial injections, it was repeatedly found that the asepsis benefit of cleansing with alcohol outweighed the neurotoxicity risk for neuraxial blocks (ASA, 2017; Bogod, 2014; Campbell et al., 2014; Miller et al., 2013).

Literature Gap

There is a gap in the literature regarding the actual practice of anesthesia providers cleansing epidural catheter hubs prior to bolusing. However, there is correlation of cleansing epidural catheters when disconnections occur. According to a practice survey of Scottish anaesthetists, providers reported using 70% isopropyl alcohol swabs to cleanse the epidural catheter 37% of the time when disconnections occurred (Paton et al. 2012). In another survey from the United Kingdom, anaesthetists reported cleansing a disconnected epidural catheter with an antiseptic and allowing it to dry prior to reconnecting 78% of the time; the specific type of antiseptic used is not specified (McKenzie & Darragh, 2011). However, some epidural policies

contraindicate the use of alcohol for epidural catheter hub cleansing due to the potential risk of introducing alcohol into the epidural space and causing neurolysis/apoptosis, despite citing conflicting evidence-based practice (HHA 2010; HHVNA, 2013). Since the literature is nonexistent in epidural catheter hub cleansing practices, further research is needed to develop evidence-based practice.

Project Aims

The purpose of this scholarly project is to determine if 70% isopropyl alcohol pad is an appropriate disinfecting agent to cleanse the hub of the in-dwelling epidural catheter without risk of neurolysis/apoptosis by the anesthesia provider. A secondary aim is to establish evidence-based recommendation on the results from this scholarly project. The specific objectives for this scholarly project are:

1. Determine and acquire resources needed to implement the research design at AdventHealth University (AHU) Chemistry and Microbiology Laboratory (lab) by Fall 2021.
2. Using hemocytometer, determine if there is an effect between cleansing the epidural catheter hub with 70% isopropyl alcohol pad and neurolysis in the AdventHealth University Chemistry and Microbiology Lab by Spring 2022.
3. Gas chromatography determine the amount of 70% isopropyl alcohol at the end of the epidural catheter and in commercially available rat astrocyte cells after cleansing the epidural catheter hub and allowing for differing amounts of dry times at the AdventHealth University Chemistry and Microbiology Lab by Spring 2022.
4. After cleansing the epidural catheter hub with 70% isopropyl alcohol, determine the relationship in amount of 70% isopropyl alcohol at the end of the epidural catheter and in

commercially available rat astrocyte cells after injecting 2% lidocaine with epinephrine 1:200,000 top up dose and reconnecting the epidural catheter to the epidural pump for completion of 0.2% ropivacaine plain infusion at 12 ml/hr for 8-10 hours at the AdventHealth University Chemistry and Microbiology Lab by Spring 2022.

5. Make evidence-based recommendations to anesthesia providers for the appropriate use of 70% isopropyl alcohol as a cleansing agent for epidural catheter hubs when bolusing in-dwelling epidural catheters by Fall 2022.

Theoretical Framework

This scholarly project was guided by the Plan, Do, Study, Act theoretical framework, which focuses on assessing an implemented change (Agency for Healthcare Research and Quality [AHRQ], 2020). It deconstructs the change or implemented experimentation as with this scholarly project allowing for reassessment and improvement of methods based on outcomes (Agency for Healthcare Research and Quality, 2020).

Plan

The purpose of the planning stage of this scholarly project aimed to determine if there is an effect between cleansing the epidural catheter hub with 70% isopropyl alcohol pad and neurolysis/apoptosis. Planning was completed and concluded that GC and GC/MS were the instrumentation which the Solid Phase Microextraction (SPME) fiber would be used to determine alcohol concentration at the end of an epidural catheter hub after a bolus through it. The sample would then be exposed to rat astrocyte cells and observed for cell death at AHU's Microbiology and Chemistry Lab in the Summer of 2021.

Do

In Fall of 2021, this scholarly project proposal was submitted for feedback and approval to IRB, SRC, and EHS. Once IRB determined the quantitative scholarly project status as non-human research implementation began. All methods, results, and findings were recorded in this phase beginning in Fall 2021.

Study

After implementation of the scholarly project was completed, the results were analyzed in the Fall of 2022. This revealed limitations, deviating from planned project aims. Results were inconsistent affecting the validity of any recommendation for practice.

Act

Finally, this scholarly project's limitations and outcome were evaluated. New methods and recommendations were identified for future research to produce consistent results and evidence-based recommendations. Findings, lessons learned, and recommendations were disseminated to stakeholders in Spring of 2022.

Methods

This scholarly project did not require the use of human subjects; instead, the use of commercially available rat astrocyte cells were to be utilized; therefore, there were no recruitment methods, potential risks, and benefits, nor ethical considerations, including informed consent needed for this scholarly project. This experimental study design took place in Orlando, Florida, at AHU's Chemistry and Microbiology Labs.

Proposed Methods

The methodology will purposively test 70% isopropyl alcohol content in commercially available rat astrocyte cells correlated to risk of neurolysis/apoptosis after cleansing injection

port of epidural catheters and bolusing 2% lidocaine with epinephrine 1:200,000 and infusing 0.2% ropivacaine plain through five separate epidural pumps at differing dry times (0, 5,10, 15, and 20 seconds). The sample size will include five epidural catheter pumps with 10 pieces of data related to different times at which the injection port is cleansed, with five pieces of data dedicated to a control group without alcohol exposure, resulting in a total of 50 pieces of data. Commercially available rat astrocyte cells will be obtained through co-investigators and used in compliance with the lab safety protocols.

The preliminary study GC will be used to establish a method calibration curve containing samples consisting of 0.5 parts per million (ppm), 5 ppm, 25 ppm, 50 ppm, and 75 ppm isopropyl alcohol. Co-investigators will prepare these different concentrations of isopropyl alcohol with 18 Ohms deionized (DI) water and 2-propanol deuterated medium as the internal standard. Vials with just 18 Ohms water will be prepared as blanks to prevent cross-contamination. The isopropyl alcohol will then be extracted from the vials using the SPME. The SPME fiber will be placed into the GC to determine the extracted concentration of isopropyl alcohol to establish validity of analysis to assess alcohol mass by concentration compared to 2-propanol deuterated medium internal standard. This process will be repeated by alternating isopropyl alcohol sample vials and blank vials.

An epidural catheter hub will then be cleansed with 70% isopropyl alcohol for 30 seconds then differing amounts of drying time (0, 5, 10, 15, and 20 seconds), and then 18 Ohms water will be injected through the epidural catheter to ensure isopropyl alcohol calibration curve is not confounded by 2% lidocaine with epinephrine 1:200,000 or 0.2% ropivacaine plain. The sample will then be collected using SPME and analyzed for isopropyl alcohol content using GC as standard described above. The cleansed hub samples will be alternated with blank 18 Ohms vials

to prevent cross contamination. These samples will then be compared to the established calibration curve.

For confluence and passage of commercially available rat astrocyte cells, all the necessary equipment will be cleansed with 70% isopropyl alcohol and placed in the laboratory fume hood. The commercially available rat astrocyte cells will then be removed from liquid nitrogen washed and thawed in a 37°C water bath. The outside of the cell containers will be sprayed with 70% isopropyl alcohol prior to being placed in the laboratory fume hood. Once this process is completed the cells will be seeded into sterile tissue culture dishes in Dulbecco's Modified Eagle's Medium (DMEM) and 15% Fetal Bovine Serum (FBS) medium for incubation at 37°C, 5% CO_2, and 90% humidity. Cells will then be grown to 100% confluence and then passaged to be divided amongst additional sterile tissue culture dishes for further confluence. These dishes will then either be frozen in liquid nitrogen for later use or repassaged for additional dishes.

For the primary study, five separate epidural pumps will be labeled and programmed to infuse 0.2% ropivacaine plain 100 ml bag at 12 ml/hr.; epidural catheters will then be primed and connected to 0.2% ropivacaine plain infusion. The distal tip of the epidural catheter will be embedded into a sterile tissue culture dish of commercially available rat astrocyte cells. At differing hour marks corresponding epidural catheters will be disconnected, and the hub will be cleansed for 30 seconds. Epidural catheter hubs will then be allowed to dry at differing 0, 5, 10, 15, and 20 seconds.

Next, a 5 ml bolus of 2% lidocaine with epinephrine 1:200,000 will be administered through each epidural catheter into the commercially available rat astrocyte cells and reconnected to continuous infusion. To establish a control, separate epidural catheters will be

placed on commercially available rat astrocyte cells and receive the 0.2% ropivacaine plain infusions, but the epidural catheter hubs will not be cleansed with 70% isopropyl alcohol prior to administration of the 5 ml bolus of 2% lidocaine with epinephrine 1:200,000.

Finally, ropivacaine plain 100 ml infusions are complete (8-10 hours), epidural catheters will be removed from commercially available rat astrocyte cells. Other passaged commercially available rat astrocytes will be exposed to differing percentages of isopropyl alcohol to determine at what concentration cell death occurs. Once both methods expose the commercially available rat astrocytes to 70% isopropyl alcohol trypan blue, and using a hemocytometer, 200 microliters of the cells will then be used to analyze the dishes' live versus dead count. Lastly, Fluorescent Microscopy will be used to validate cells as commercially available rat astrocytes using antibody specific stains. The process will be repeated five times with each epidural pump for a total of 50 data sets. The compiled data from the lab will then be analyzed using a statistical analysis software package.

To ensure rigor of the project, consistency will be maintained with limited extraneous handling; cells will also be placed in an incubator in the lab under lock and key. This scholarly project will require epidural catheters and pumps, lab fume hood, pipette, glass vials and beakers, sterile tissue culture dishes, Nitric Acid, liquid Nitrogen, DMEM, FBS, trypan blue solution, 2-propanol deuterated, SPME fiber, 70% isopropyl alcohol swabs, isopropyl alcohol, 18 Ohms, incubators, microscope and slides, and a GC.

Equipment must be tested and validated for reliability of results. Thus, epidural pumps will be compared to one another for proper functioning, and the incubators will be assessed for proper temperature. Results will be validated through concurrent methods of GC and neurolysis/apoptosis analysis of commercially available rat astrocyte cells. Data will then be run

through a statistical analysis software package with repeated measures analysis of variants (ANOVA) for quantitative analysis. Data information will be stored on a personal AHU's Microsoft Teams account; password protected documents will be accessed only by co-investigators and the primary investigator. It will be available for 7 years then destroyed as per AHU's institutional review board (IRB).

Finalized Methods

SPME Fiber Absorption Optimization Time

In order to determine the optimum time for absorption of isopropyl alcohol by the SPME fiber. Approximately 0.1 ml of pure isopropyl alcohol placed in a 10 ml headspace vial which was capped with a teflon coated septa and sealed with a crimp top seal. The SPME fiber was first conditioned for 2 minutes (min) at 220 ^{o}C in the injection port of the GC; after which the SPME fiber was cooled for 2 min and then inserted into the headspace of the vial containing isopropyl alcohol. The fiber was allowed to remain at various sampling times (15, 20, 25 min). A plot of the IPA peak response versus sampling time. See Appendix B. This study was repeated and consistent with the results shown in this graph. As the graph shows, the optimum absorption time was achieved at 20 min absorption time; beyond the 20 min time the response decreased.

Calibration Curve

To determine identification of pure isopropyl alcohol and the internal standard 2-methyl-1-propanol, a solution was created by combining 2.5 microliters of each compound into a 10 milliliter vial and closed with a teflon coated septa and crimp top seal caps. The co-investigators used the SPME fiber direct headspace technique and GC/MS analysis to determine retention times and ratio. See Appendix C.

A stock solution was prepared by diluting 0.500 grams of isopropyl alcohol in a 500 milliliter volumetric flask containing 18 Ohms deionized water creating a stock solution. This stock solution was used to establish 0.01 ppm, 0.03 ppm, 0.05 ppm, 0.07 ppm, & 0.1 ppm standard solutions used for the calibration curve. The direct headspace of each standard solution was analyzed using the SPME fiber technique in the GC/MS.

Ten milliliters of each isopropyl alcohol concentration were placed into a 20 milliliter glass vial with the addition of five microliters of 2-methyl-1propanol. The vials were then closed with a teflon coated septa and crimp top seal caps. Next the vials were placed into an isowater bath at 35 ^{o}C until warm. Separately, the vials were removed and punctured for SPME fiber placement and absorption of 20 min. Once this was completed, the SPME fiber was removed and placed into the GC/MS for 3 min for headspace analysis. Between each isopropyl alcohol sample run the SPME fiber and GC/MS were conditioned as blanks to prevent crossover contamination.

The peak height and retention time of each isopropyl alcohol sample were recorded and compared with each other along with single ions determined by the GC/MS library method identifying the results as isopropyl alcohol and 2-methyl-1-propanol. These results were then charted in a spreadsheet to establish the calibration curve.

Epidural Cleansing and Bolusing

The SPME fiber and GC/MS were conditioned as blanks to ensure no anomalies were present prior to the sample extraction and analysis. Five milliliters of 18 Ohms deionized water was placed in a 10 milliliter vial, capped with teflon coated septa and crimptop seal. The SPME fiber was placed in the sample for 20 min then removed and analyzed in the GC/MS to establish a blank; this was repeated between each sample. To prevent contaminants and cross over, 18 Ohms deionized water was bolused through the epidural catheter before and in between each dry

time. Each epidural catheter hub was scrubbed with the 70% isopropyl alcohol pad for 30 seconds and allowed to dry for the previously determined times (0, 5, 10, 15, and 20 seconds). Five microliters of 18 Ohms deionized water was then bolused through the epidural catheter into a 10 milliliter vial after the dry time was completed and sealed with a teflon coated septa and crimptop seal once 2.5 microliters of the internal standard was placed in the vial. The vial was treated as all other preparations for SPME fiber headspace sampling. The SPME fiber was then placed in the GC/MS for analysis where the peak height and retention times were recorded and compared.

Planning

For effective planning, implementation, and data analysis, co-investigators will collaborate with key stakeholders: Dr. Martin Rivera, DNP, CRNA Assistant Professor of Nurse Anesthesia; Dr. Nadia Edwin, PhD Assistant Professor of Chemistry at AHU; Dr. Sebastian Farrell, PhD Vice Chair of Sciences at AHU; Dr. Erik Williams, DNAP, CRNA; Dr. Anael Santos Jr, PhD Professor of Biochemistry at AHU. Key stakeholders were sought out for their expertise in their respective fields relevant to the progression and implementation of the scholarly project and IRB submission. Resources necessary include AHU's Chemistry and Microbiology Labs and equipment, epidural pumps obtained from AdventHealth Biomedical Department, 20 gram Braun Perifix epidural catheters were donated from Smiths Medical, sterile 70% isopropyl alcohol pads were obtained from Consumer Value Stores Pharmacy, inc., and 0.2% ropivacaine plain for infusions and 2% lidocaine with epinephrine 1:200,000 for boluses were obtained from AdventHealth Pharmacy for scholarly project purposes. Storage lock box for medication and epidural pumps obtained by AHU Doctorate of Nurse Anesthesia Practice (DNAP) and approved by AHU's Environmental Health and Safety. Liquid nitrogen, sterile

tissue culture dishes, Nitric Acid, liquid Nitrogen, DMEM, FBS, trypan blue solution were to be obtained but ultimately not needed as this scholarly project did not advance to later stages.

All compounds, solvents, and volatile organic compounds (VOC) in this scholarly project used in this study had purity greater than 99% and were obtained from Sigma-Aldrich in Milwaukee, Wisconsin. SPME 7 micron polydimethysiloxane (PDMS) and 85 micron polyacrylate fibers were obtained from Supelco along with SPME fiber holder and syringes. Hamilton 10 microliter and 25 microliter syringes, gastight 5 milliliter sample lock syringes, were obtained from Hamilton Company in Reno, Nevada. Adjustable pipette 0.5-10 microliter and 10 microliter pipette tips were obtained from Biohit. While 10 and 20 milliliter glass vials, teflon coated septa, and crimp top seal caps were obtained from National Scientific. The 18 Ohms water used in this project was obtained from University of South Florida and Chemworld.

Instrumentation Theory

SPME was used in conjunction with GC and GC/MS for instrumentation of this scholarly project. The SPME technique was chosen to analyze isopropyl alcohol in a water matrix due to direct injection of water into the GC or GC/MS is detrimental to both instruments. SPME offers a sensitive technique to analyze isopropyl alcohol in water. In this technique a fiber coated with a known absorbent is inserted into a vial containing the water/isopropyl alcohol mixture. The fiber can be inserted directly into the matrix. For the analysis, two different fibers were tested, namely 7 micron polydimethylsiloxane (PDMS) (*green)*, 85 micron polyacrylate (*white*). The most successful of which was PDMS. However, for reasons previously cited, the fiber was inserted into the headspace of the vial for a prescribed time. Any analyte in the headspace will be absorbed onto the fiber. The fiber is then inserted into the heated injection port of the GC or GC/MS where the analyte is desorbed and detected by either instrument.

SPME was used for isopropyl alcohol sampling; while the GC and G/CMS were used to detect isopropyl alcohol in samples. The GC and GC/MS instruments both use a heated column containing a solid phase absorbent to separate the components in a volatile or semi-volatile mixture. Once the mixture is injected or desorbed into the injection port of the GC, the liquid sample is immediately vaporized and forced onto the separating GC column using helium (USA only uses helium) or other inert gasses. The temperature of the column is usually programmed to increase from a set value (40 ^{o}C in this case) to a final temperature that exceeds the boiling points of the analytes in the sample (isopropyl alcohol and 2-methyl-1-propanol). In this way, the sample remains in the gaseous state as it travels to the detector.

For actual separation of each analyte from the mixture, their unique molecular weight and structural arrangement allows them to interact with the sorbent of the column differently. Consequently, each analyte will be retained on the column at different retention times. The GC was equipped with a flame Ionization detector (FID). Analytes are directed from the GC column to the FID which converts the analytes to ions using a flame. The ions generate a voltage. The more concentrated the analyte, the higher the voltage and the signal corresponding to each analyte. A plot of the voltage (peak response) versus the retention of each analyte is called a chromatogram. Peak response is proportional to the concentration of the analyte. The higher the response (ie. peak height or peak area), the more concentrated the analyte.

Specifically for the GC/MS, the principle of peak separation is the same. Each analyte is bombarded by fast moving electrons which fragment the molecule into different positive and negative ions. Most GC/MS, including the one used for this scholarly project, detect just the positive ions. A plot of the intensity (y-axis) of each ion versus mass-to-charge ratio (x-axis) is called mass spectrum. The identity of each analyte can be confirmed by matching its mass

spectrum against a database of standardized mass spectra and by analysis of a pure sample of that analyte and retention time (reference the pure peaks and library picture from GC/MS).

Samples were analyzed using a Shimadzu GC-2010 GC that was controlled by Class-VP Chromatography Data Station, version 4.2. The GC was equipped with a split/splitless injector, a flame ionization detector (FID), and a Restek Corporation capillary column (Rtx-5, 30.0 m × 0.25 mm i.d. × .25 DF). The column temperature was initially set to 40 ^{o}C for 1 min. Following this, the temperature was increased at a rate of 20 ^{o}C /min to 90 ^{o}C for 1 min, then ramped to a final temperature of 200 ^{o}C for 2 min at 40 ^{o}C/min. Both the injector and the detector were held at a constant temperature of 200 ^{o}C. The GC was operated with air, N_2, and H_2 respectively set at 20 pounds per square inch (psi), 40 psi, and 50 psi.

The Shimadzu QP-2010 SE GC/MS was equipped with a split/splitless injector, mass spectrometer detector and a Restek Corporation capillary column (SH-Rxi-5Sil MS, 30.0 m × 0.25 mm i.d. × .25 DF). The column temperature was initially set to 40 ^{o}C for 2 min. Following this, the temperature was increased at a rate of 25 ^{o}C /min and ramped to a final temperature of 200 ^{o}C for 1 min at 25 ^{o}C/min. Both the injector and the detector were held at a constant temperature of 200 ^{o}C. The column flow rate for both instruments were maintained at 1 ml/min. The GC/MS was operated with H2 set at 100 psi.

Method Limitations

Optimum Sample Creation Method

During initial phases of method development to determine optimum procedure a variety of techniques were used. In one method Sodium Chloride was added to salt out the isopropyl alcohol from each sample into the headspace. It is acknowledged that salt disrupts the intramolecular forces between the water and the alcohol; this would have allowed the isopropyl

alcohol to release in gas form into headspace. However, this technique did not increase sensitivity of results. Additionally, thermastating was trialed by heating vials to 35 ^{o}C in a isobath. This was necessary because a cool surface would lead to condensation of the isopropyl alcohol in the gas phase and produce erroneous results. Just heat to room temperature otherwise if too warm it will heat up the fiber and evaporate the sample off the fiber. Sonication was another technique used in sample creation as a way of teasing out the isopropyl alcohol into the gas phase; however, again, there was no increase in sensitivity. Additionally, sonication can lead to dehydration of the isopropyl alcohol by removing the water molecule to form propene which would dilute the isopropyl alcohol in the headspace and skew the results.

Isopropyl Alcohol Extraction Techniques

In an attempt to obtain the largest amount of headspace from the samples, a 5 ml gas tight syringe was used to extract the headspace of a 20 ml vial containing 10 ml samples and inject it directly into the GC. Results were deemed not reproducible and inaccurate due to the gas tight syringes losing pressure and consequently headspace sample after a number of injections. Additionally, it was not possible to standardize the injection time or technique of the sample into the GC which created a confounding variable. Another technique was used by taking a 10 ml sample of headspace using the 5 ml gas tight syringe from a 20 ml sample and placing it in a 10 ml vial. An 85 micron polyacrylate SPME fiber would then be placed into the 10ml vial to sample the headspace. This proved not to be sensitive for results and time consuming. The PDMS SPME fiber was opted for over the 85 micron polyacrylate fiber as it proved more sensitive, the results continued to be not replicable or valid.

As peak heights on the GC and GC/MS were inconsistent, attempts were made to ensure the isopropyl alcohol concentration of each sample being tested, the SPME fiber was dipped in

each sample and then placed into GC. However, this technique proved futile as neither the GC nor GC/MS can tolerate direct liquid samples as they extinguished the instruments operational flames.

The initial stages of implementation, the GC instrument was used to analyze the isopropyl alcohol dilutions in an attempt to establish the calibration curve. The results were inconsistent and proved nonreproducible. The decision was made to direct further analysis to the GC/MS due to its greater sensitivity in detecting single ions as discussed in the instrumentation theory.

Project Timeline

After receiving IRB determination as nonhuman subject research in Fall 2021 and approval by both AHU's Scientific Review Committee (SRC) and Environmental Health and Safety Office (EHS) in Spring 2022, planning of the scholarly project, meeting with key players, and gathering of supplies began in Summer 2021. Implementation in the lab began in fall of 2021 and concluded in fall 2022 when results were collected. Data analysis and scholarly project paper were completed in Fall 2022 in preparation for dissemination of results and recommendations in Spring 2023.

Results/Findings

Calibration Curve

A typical calibration curve plots peak measurement against concentration and is used to determine an unknown concentration of a substance against an internal standard. See Appendix D. The calibration curve from this scholarly project shows that the response of isopropyl alcohol in the range tested was not linear, more importantly at the higher concentration the response decreased with time. Between 0.01 and 0.07 ppm concentration range, the curve seems to

increase exponentially, suggesting that a log plot might be warranted. However, when the log of the peak response versus the isopropyl alcohol concentration was plotted it improved, but the correlation coefficient was $R^2 = 0.8864$, which was significantly less than 0.1 goodness of fit. See Appendix E.

Epidural Cleansing and Bolusing

Epidural bolusing results showed isopropyl alcohol presence with all corresponding dry times (0, 5, 10, 15, and 20 seconds). Isopropyl alcohol presence results of 0 seconds dry time as well as a dry time of 20 seconds are presented. See Appendix F. Isopropyl alcohol breakthrough was seen, but the peak height was not always consistent for a given dry time interval. Blank baselines for GC/MS and SPME fiber analysis were established prior to the initial sampling of epidural bolusing to ensure calibration and no other analytes were present. See Appendix G. Next, retention times of isopropyl alcohol and 2-methyl-1-propanol were established for ion identification purposes and validated with the single ion monitoring method library. See Appendix C. This baseline was reestablished at the end of all epidural catheter runs to ensure the GC/MS and SPME fiber had no residual isopropyl alcohol or other analytes present. See Appendix H.

Discussion and Implications

Calibration Curve

It is believed that at higher concentrations when the vapor load is much more and the headspace is fully saturated with vapor, the vapor begins to extract itself from the headspace decreasing the overall concentration of isopropyl alcohol in the headspace. This causes self-extraction. The higher the concentration the more this will happen, ultimately altering the reliability of headspace concentration. Attempts to thermostat the vial at a higher temperature so

as to prevent premature condensation of the vapor did not lead to better outcomes because the higher temperature can also lead to vaporization of the sample from the SPME fiber. If the vial is heated, then the SPME fiber will also be heated when placed in the headspace.

As the peak heights fluctuate inconsistently instead of linearly from concentration to concentration, saturation may have been assumed. However, the data suggest that the fiber was not saturated with the sample at higher concentration levels. Had this been the case, the peak of the response curve would have plateaued rather than decreased; also, the GC/MS chromatogram would show evidence of saturation with a flat, broadened peak but this was not observed. See Appendix E.

To establish a reliable calibration curve, the experiment was repeated multiple times with different fibers and sampling techniques yielding similar results showing no linear response. Thus, a calibration curve was not established, preventing the determination of isopropyl alcohol concentration.

Epidural Cleansing and Bolusing

Even though there was isopropyl alcohol noted in the epidural bolus samples, a quantitative concentration level cannot be determined until further studies establish a valid linear calibration curve. When cleansing the epidural catheter hub with isopropyl alcohol and bolusing through it, corresponding dry times yielded various results with no correlation or consistency. This suggested that the amount of isopropyl alcohol that was transferred from the pad to the hub was not always consistent. It is also recognized that as testing techniques for isopropyl alcohol presence and concentration were similar to those of calibration curve methodology there may be optimization concerns that led to inconsistency in the results of epidural catheter cleansing and bolusing at differing dry times.

Review of Problem/Purpose and Interpretation of Results

The concern for neurolysis/apoptosis in the epidural space when cleansing the epidural catheter hub has been debated in the anesthesia field. As a repeatable and reliable calibration curve to determine the isopropyl alcohol concentrations of samples were not established, co-investigators were not able to address the primary and secondary aims along with many of the objectives of this scholarly project. Since isopropyl alcohol concentrations could not be determined at the end of epidural catheters, co-investigators were unable to determine the effect of isopropyl alcohol in the epidural space. This prevented proceeding to testing rat astrocyte cells, observing for neurolysis/apoptosis effects, or making evidence based recommendations for 70% isopropyl alcohol as a cleansing agent for epidural catheter hubs prior to bolus administration.

Recommendations

As the results of this scholarly project are incomplete and the aims and objectives were not achieved, further research on this subject is recommended in order to interpret findings and establish evidence based recommendations for anesthesia practice. First concerns must be focused on establishing a valid calibration curve; thus another technique would be utilizing a liquid chromatographer which was not available to co-investigators during this experimentation. This would allow for direct analysis of the liquid samples created for the calibration curve and epidural catheter bolusing and avoid erroneous methods affecting the results. Additionally, in a more controlled experiment, it would be best to control the amount of alcohol per pad, as there is no way to guarantee the concentration of commercially available 70% isopropyl alcohol pad and thus the concentration transferred to the epidural hub upon cleansing. Potentially in the future pads could be made in the lab to ensure consistency with a predetermined mass but could prove

time consuming. Finally, co-investigators anticipate a future barrier to be related to administration of infusions and boluses onto the rat astrocytes, be it 18 Ohms deionized water, 0.2% ropivacaine, or 2% lidocaine with epinephrine 1:200,000. This concern exists over potential washout of the medium needed to ensure survival of rat astrocyte cells.

Applicability to Practice and Contribution to Professional Growth

Evidence-based practices must remain a high level of concern for CRNAs in order to provide the safest and most efficient care to patients. Standard of care must be consistent and proven, rather than developed from preference, tradition, and/or extrapolated misinformation. Thus, discovery of true arachnoiditis and neurolysis/apoptosis risk related to cleansing the epidural catheter hub must continue to be investigated. As anesthesia providers, CRNAs practice antiseptic techniques and proven infection prevention routinely with patient care. Monitoring patients, recognizing adverse effects, and providing intervention as necessary are skills only qualified anesthesia providers possess and are specific to CRNAs' scope of practice. Due to the degree of education and responsibility to the community and anesthesia field, CRNAs are able to implement evidence-based practice from collection, testing, evaluation, and dissemination of results. As access and management of epidurals are specialized, CRNAs are the providers to advocate for improved practices based on evidence and not inferred information or tradition. Unfortunately, due to the lack of results from this scholarly project recommendations cannot be made for nurse anesthesia practice at this time. However, this project and future research remain applicable to practice as there continues to be a gap in knowledge and need for evidence-based practice recommendations.

Conclusion

This scholarly project was unable to establish a valid calibration curve, and thus unable to determine concentration of isopropyl alcohol within a sample using SPME technique and GC and GC/MS instrumentation. Without a valid calibration curve, the concentration of isopropyl alcohol at the end of epidural catheters after cleansing the hub with 70% isopropyl alcohol pads and bolusing 18 Ohms deionized water after differing dry times (0, 5, 10, 15, and 20 seconds). Further, as isopropyl alcohol concentration was unknown, the presence/development of adhesive arachnoiditis and/or neurolysis/apoptosis of rat astrocyte cells could not be determined. The only conclusion possible from this scholarly project was that cleansing the epidural catheter hub for 30 seconds with a 70% isopropyl alcohol most likely results in the presence of isopropyl alcohol in the sample at the end of the epidural catheter. Further research is needed to establish the calibration curve using different methodology continuing the generational study. No recommendations can be established against or for the use of 70% isopropyl alcohol cleansing epidural catheter hubs in practice.

Dissemination

Plans for dissemination of scholarly project results will be done at AHU to stakeholders including AHU faculty, students, and possibly anesthesia providers. It is hoped that future generational studies will reveal promising and impactful results that can be disseminated to peer-reviewed publications and conferences related to nurse anesthesia practice.

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Appendix A

Matrix Tables

<table>
<tr><td colspan="6">De Cicco, M., Matovic, M., Castellani, G. T., Basaglia, G., Santini, G., Del Pup, C., Fantin, D., & Testa, V. (1995). Time-dependent efficacy of bacterial filters and infection risk in long-term epidural catherization. Anesthesiology, 82(3), 765 – 771. https://doi.org/10.1097/00000542-199503000-00019
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<tr><td>Purpose</td><td>Variables</td><td>Setting/Subjects</td><td>Measurement and Instruments</td><td>Results</td><td>Evidence Quality</td></tr>
<tr><td>Study One:
Catheter hub: 40-54% of potential routes of epidural microbial colonization.

Study Two:
Bloodstream infections under control of anesthesiologists.</td><td rowspan="2">Study One:
<u>Primary outcome:</u>
micropore filter: hub contaminated during filter change
<u>Secondary outcome:</u>
micropore filter: prolonged use efficacy,

Study Two:
<u>Primary outcome</u>: Hand hygiene, workplace contamination., timely antibiotic administration, patient temp control.
<u>Secondary outcome</u>: Preferred agents for skin antisepsis.</td><td rowspan="2">Study One:
Clinical: 47 patients, tunneled epidural catheters outpatient
Lab: 96 micropore filters 0.2 um, 4 types

Study Two:
<u>Setting</u>: Seattle Children's Hospital, Seattle research Institute, University of Washington.
<u>Subject</u>: Infection prevention in pediatric anesthesia practice.</td><td rowspan="2">Study One:
Clinical: skin swab and culture of the filtrate
Lab: simulated condition and med administration. Tested with Strep

Study Two: None stated, broad explanations of comparison studies.</td><td>Study One:
Clinical: High correlation of positive filtrates and hubs
Lab: 2 showed growth immediately and 2 showed no growth
Study Two:
Chlorhexidine preparations reduce risk of catheter related infection by 49% compared to povidone-iodine.</td><td rowspan="2">Study One:
<u>Methodological flaws:</u>
lab cannot account for all clinical scenarios
<u>Inconsistency:</u>
Differences between filter types
<u>Indirectness:</u>
small sample
<u>Imprecision:</u>
Clinical filter types unknown
<u>Publication bias:</u>
Unknown
Study Two:
<u>Methodological flaws:</u>
Unsure of comparison articles.
<u>Inconsistency:</u> Prepping skin with chlorhexidine decreases infection rate by decreasing colonization, dressing dont.
<u>Indirectness:</u> Dressing changes are no benefit.</td></tr>
<tr><td>Design</td><td>Implications</td></tr>
<tr><td>Study One:
Quasi Experimental Study

Study Two:
Anesthesia’s role in preventing infection rates in the pediatric population related to bloodstream.</td><td>Study One:
Micropore = barrier, prevent particulate. Colonization = distant source, insertion site, or hub, during change and type
Study Two:
Infections from regional anesthesia is from skin pathogen penetration..</td></tr>
</table>

					Imprecision: Does Not state specific regional techniques. Publication bias: None

McKenzie, A. G. & Darragh, K. (2011). A national survey of prevention of infection in obstetric central neuraxial blockade in the uk. *Journal of the Association of Anaesthetists of Great Britain and Ireland, 66*(6), 497-502. https://doi.org/10.1111/j.1365-2044.2011.06705.x Poddar, K., Dasgupta, S., & Gulati, R. (2016). Epidural alcohol neurolysis–A good option for cancer pain management in developing countries. *Journal of Anesthesia & Critical Care, 6*(5), 00244. https://doi.org/10.15406/jaccoa.2016.06.00244					
Purpose	**Variables**	**Setting/Subjects**	**Measurement and Instruments**	**Results**	**Evidence Quality**
Study One: Assess the standard of aseptic technique used for neuraxial blocks and determine improvement needs. **Study Two:** Establish safety and efficacy of epidural alcohol neurolysis for severe cancer pain where other modalities have failed. **Design** **Study One:** Survey Research **Study Two:** Open-label design	**Study One:** Primary outcome: Assess compliance with current recommended good practice Secondary outcome: Facility and practice of setting up; management of epidural catheter disconnect; method of administering epidural drugs and their source; spinal precaution of drug administration; comment on identified cases of sepsis and neuraxial block **Study Two:** Primary outcome: Cancer pain relief Secondary outcome: Assess risk benefit ratio – neuritis, neurologic deficit, damage to non-neural tissue or non-targeted neural structures, and impermanent effects	**Study One:** Lead anesthesiologist of 221 maternity units in the UK **Study Two:** Setting: Unknown Subject: Lung (7) and breast (3) cancer patients with proven cancer pain. Males: 7 and Female: 3 Age: 30-78 years	**Study One:** Questionnaire approved by the Obstetric Anaesthetists' Association's Audit Subcommittee **Study Two:** Pain Intensity (VAS). Complications noted before and after administration (how, frequency, and what not addressed related to neurotoxicity).	**Study One:** 76% response rate. 128/164 clean disconnected epidural with antiseptic, dry, cut proximal; 12/25 wipe hub prior to pulling up med from bag. 29/138 wipe med vial with alcohol prior. **Study Two:** Pain decreased 3.5-8 points over 1-12 weeks. Increased quality of life. No serious adverse effects or complications **Implications** **Study One:** Alcohol to scrub ports of epidurals or medications for epidural administration is acceptable practice by some practitioners. Not using alcohol and adverse implications were not discussed. **Study Two:**	**Study One:** Methodological flaws: Practice assessed by lead anesthesiologists not individual providers. Inconsistency: Missing response. Good, recommended practice not followed or known. Indirectness: Reasoning for practice not known Imprecision: Responses and survey not verified Publication bias: None **Study Two:** Methodological flaws: Monitoring, evaluation, and results of neurolysis damage not reported. Inconsistency & Imprecision:

				Stated no serious adverse effects, but unclear, non-replicable, unreliable, and not applicable to practice.	No instrument or validity of neurolysis measurement reported Indirectness: Referenced safety of neurolysis/from other studies primarily Publication bias: Unknown

Kinirons, B., Mimoz, O., Lafendi, L., Naas, T., Meunier, J., & Nordmann, P. (2001). Chlorhexidine versus povidone iodine in preventing colonization of continuous epidural catheters in children: A randomized, controlled trial. *Anesthesiology*, *94*(2), 239-244. https://doi.org/10.1097/00000542-200102000-00012

Mohamed Iqbal, I., Morris, R. & Hersch, M.. (2018). Adhesive arachnoiditis following inadvertent epidural injection of 2% chlorhexidine in 70% alcohol—partial recovery over the ensuing eight years *Anaesth Intensive*, 46(6) 572-574 DOI: 10.1177/0310057X1804600606

Purpose	**Variables**	**Setting/Subjects**	**Measurement and Instruments**	**Results**	**Evidence Quality**
Study One To determine if an alcoholic solution of 0.5% chlorhexidine is more effective than an aqueous solution of 10% povidone iodine in reducing catheter colonization associated with short term epidural placement. **Study Two** A case study to determine the causative agent for a neurological consequence related to epidural injection.	**Study One** Primary outcome: Effectiveness cutaneous antisepsis before epidural catheter insertion. Secondary outcome: Culture of epidural insertion site, hub, catheter tip. **Study Two** Primary outcome: - Establishing 2% chlorhexidine in 70% alcohol caused Chronic Adhesive Arachnoiditis. Secondary outcome:	**Study one** **Setting:** 1,000 bed University affiliated hospital in France **Subjects:**. 100 randomly assigned patients younger than 15 who have epidural placement for abdominal or urological procedures. **Study Two** **Setting:** Minneapolis Veterans Affairs Medical Center **Subjects:** 32-Year-old at term in labor in mid-2010. Setting not stated.	**Study One** Standard microbiological methods and criteria recovered microorganisms. Dry swab culture tips. Tenover criteria **Study Two** Comparison of clinical course and radiological findings to previously reported cases.	**Study One** Chlorhexidine was 1/6 as likely and less quickly to be colonized as catheters inserted after skin preparation with povidone iodine (1 of 52 catheters [0.9 per 100 catheter days] vs. 5 of 44 catheters [5.6 per 100 catheter days]; relative risk, 0.2 [95% confidence interval, 0.1–1.0]; P 5 0.02). **Study Two** Case report establishes that Chronic Adhesive Arachnoiditis was caused by injecting 2% chlorhexidine and 70% alcohol into epidural space.	**Study One:** **Methodological flaws:** Lack of randomization, skin not cleaned before catheter removed. MRCS. Physicians were not blinded. **Inconsistency:** None **Indirectness:** None **Imprecision** Small sample size. **Publication bias** None **Study Two:** **Methodological flaws:** Does not discuss how many other case studies compared to. **Inconsistency:** None
Design				**Implications**	

Study One Randomized control trial. **Study Two** Qualitative research	Rates at which 2% chlorhexidine in 70% alcohol can cause Chronic Adhesive Arachnoiditis.			**Study one:** Infections can be prevented by chlorhexidine dressings. **Study Two:** Minute amounts that neuraxial chlorhexidine might contaminate the needle as it passes into the epidural space from the skin.	**Indirectness:** None **Imprecision:** Sample size of one case. **Publication bias:** None

American Society of Anesthesiologist. (2017). Practice advisory for the prevention, diagnosis, and management of infectious complications associated with neuraxial techniques: An updated report by the american society of anesthesiologists task force on infectious complications associated with neuraxial techniques and the american society of regional anesthesia and pain medicine*. *Anesthesiology, 126*(4), 585-601. https://doi.org/10.1097/ALN.0000000000001521 Campbell, J. P., Plaat, F., Checketts, M. R., Bogod, D., Tighe, S., Moriarty, A., & Koerner, R. (2014). Safety guideline: Skin antisepsis for central neuraxial blockade. *Anaesthesia, 69*(11), 1279-1286. https://doi.org/10.1111/anae.12844					
Purpose/Objectives	**Search Strategy**	**Number and Type of Studies in the Review Including Sample Sizes**	**Results**	**Conclusions/Implications**	**Evidence Quality**
Review One: Reduce risk of infection complications associated with neuraxial techniques. Identify patients at risk, and develop techniques to reduce risk and interventions to improve outcomes after infectious complication **Review Two:** Optimum and safe aseptic technique for central neuraxial blockade (CNB) and chlorhexidine in alcohol skin antisepsis preventing chlorhexidine in CSF neurotoxicity. 0.5% chlorhexidine and little amount as possible in children to provide antisepsis.	**Review One:** Peer-reviewed journals from health care databases, direct Internet searches, Task Force members, organization liaisons, and manual search of references located in reviewed articles. **Review Two:** Not stated	**Review One:** Random Control Trials, observational results of nonrandomized and RCT without pertinent comparison groups, retrospective comparative studies, observations, case reports, formal surveys, expert consult **Review Two:** Laboratory and clinical studies, published guidelines, case reports, and known properties of antiseptic agents.	**Review One:** Chlorhexidine v. povidone iodine and aseptic prep with/without alcohol: inconsistency in rate of positive bacterial cultures. Formal survey prefer chlorhexidine with alcohol for skin prep. ASA finds no clear preference. Bacterial colonization, infectious complications still occur with micropore filters. Not effective but still recommended. Inconsistent response to disconnected catheter. **Review Two:** Chlorhexidine superior to povidone iodine 5:1 studies. Alcohol increases antimicrobial properties of antiseptic. Chlorhexidine found to be neurotoxic but if allowed to dry, neuraxis very low risk base on deliverable amount. Alcohol also known to cause neurolysis. Infection benefit outweighs risk. Chlorhexidine plus alcohol vs isopropyl alcohol alone,	**Review One:** Limited literature and inconsistent in results of current and best practice related to antibacterial techniques and prep of epidural catheter. Additional studies must be done in order to direct practice. Use of aseptic techniques encouraged during prep and placement of neuraxial anesthesia **Review Two:** Isopropyl alcohol effective to provide antimicrobial effects alone, potential "apply-wipe-apply" will remove dead skin and other barriers allowing alcohol antisepsis to further penetrate and prolong effects. Research may show less antimicrobial time needed, thus allowing for only alcohol and one less neurotoxic agent used. Further research is needed to determine the effects or risk of neurological damage.	**Review One:** Methodological flaws: Unclear how many and of what type of research/data presented findings Inconsistency: Continue to recommend micropore filters despite infectious complication risk Indirectness: Aseptic is best but unclear type or alcohol Imprecision: Limited controlled studies, expert opinion Publication bias: none **Review Two:** Methodological flaws: Search methods unknown Inconsistency: best antimicrobial not known. Conflicting results Indirectness: Appears small study numbers in some of the studies. Imprecision: Number, type, and exact results of research examined is not known Publication bias: Some authors gave expert opinions on other similar works

			provided 4 times longer antimicrobial effects.		

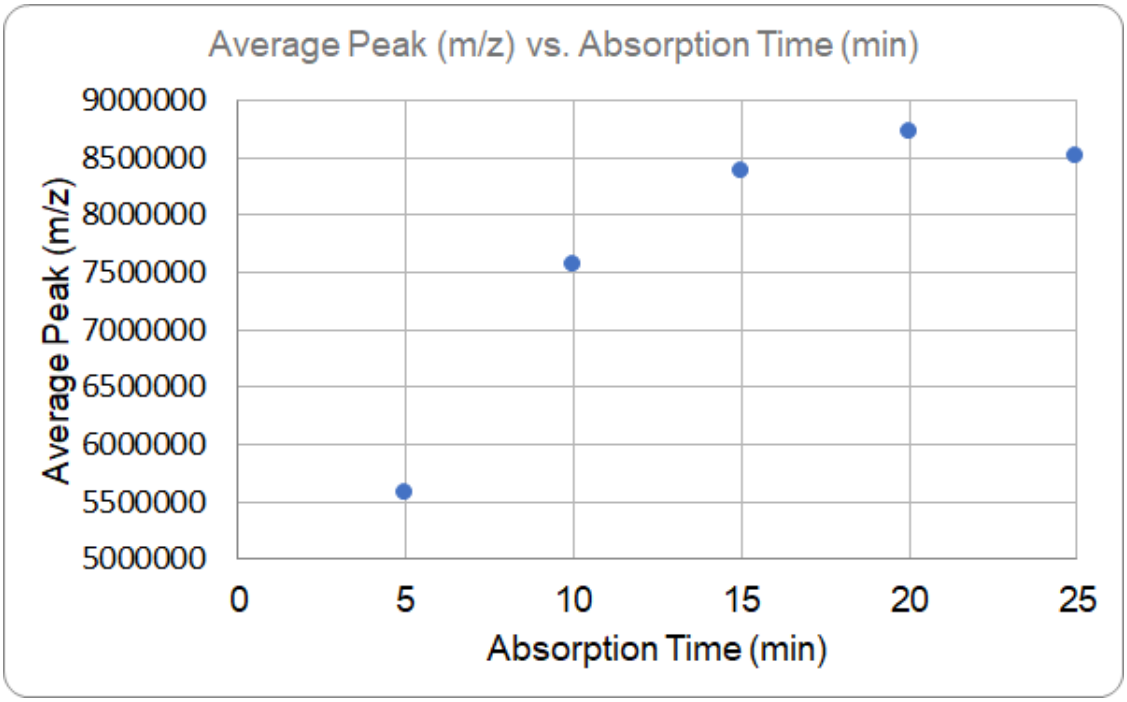
Tôrres de Araújo Azi, L. M., Martins Fonseca, N., & Gurgel Linard, L. (2020). SBA 2020: Regional anesthesia safety recommendations update. *Brazilian Journal of Anesthesiology (Elsevier), 70*(4), 398-418. https://doi.org10.1016/j.bjan.2020.02.005

Straube, S., Derry, S., Moore, R. A., & Cole, P. (2013). Cervico-thoracic or lumbar sympathectomy for neuropathic pain and complex regional pain syndrome. *The Cochrane Database of Systematic Reviews*, (9), https://doi.org10.1002/14651858.CD002918.pub3

Purpose/Objectives	Search Strategy	Number and Type of Studies in the Review Including Sample Sizes	Results	Conclusions/Implications	Evidence Quality
Review One: To provide a broad overview of the current knowledge regarding pre-procedure asepsis and antisepsis, risk factors, diagnosis and treatment of infectious complications resulting from anesthetic techniques. **Review Two:** To review efficacy and safety of chemical and surgical sympathectomy for neuropathic pain.	**Review One:** Databases: PubMed, Cochrane Library, and LILACS. Cross-references with the collected material were also used to identify articles with better methodological designs. The search was later limited to studies performed in humans Published in English, French, German, Portuguese, or Spanish. **Review Two:** Databases MEDLINE, EMBASE, and The Cochrane Library to May 2010. Screened references in the retrieved articles and literature reviews, contacted experts in the field.	**Review One:** Studies published between January 1, 2011, and September 31, 2019, in addition to articles published between 1965 and 2011, already considered in the previous review for n= 82 articles. **Review Two:** Randomized, double blind, placebo, or active controlled studies. One study satisfied this inclusion criteria in 20 participants.	**Review One:** Adequate hand hygiene should always be prepared before region anesthesia. Sterile gloves, and mask are recommended. No data on sterile gowns to prevent infection for region anesthesia. Use 0.5% chlorhexidine in 70% alcohol for skin antisepsis but avoid splashing. Patients with infection can have prophylactic antibiotics and have epidural catheter placement. Clean ampoule neks with alcohol before withdrawing or injecting. **Review Two:** Average baseline scores of 8-9/10 on several pain scales fell to about 4/10 initially (1 day) and remained at 3-5/10 over four months. One participant experienced post sympathectomy neuralgia, while two in the radiofrequency group and one in the phenol group had paresthesia. All participants had soreness at injection site.	**Review One:** Multi antiseptic techniques should be performed prior to epidural catheter placement. Infection complications tend to be due to prolonged epidural catheter length, use of catheter sites, and dressing change frequency. **Review Two:** The practice of surgical and chemical sympathectomy for neuropathic pain is based on little high-quality evidence. Sympathectomy should only be used in qualified patients and after all other treatments failed. Double blind random control trials with placebo comparators is needed to determine weather sympathectomy can relieve neuropathic pain.	**Review One:** Methodological flaws: Does not state why type of articles were researched with each recommendation. Inconsistency: States cleansing the skin twice with chlorhexidine and alcohol is the standard but lacks support with evidence. Indirectness: Chlorhexidine in alcohol is the solution of choice for skin prep Imprecision: Lacks efficient data in each recommendation. Publication bias: none **Review Two:** Methodological flaws: Only on inclusion article and 20 participants in that. Inconsistency: Meta analysis of another study. Conflicting results Indirectness: Grades other studies as low-quality evidence. Imprecision: Noted other large studies with success of treatment. Publication bias: One study did not report on method allocation concealment. Not a high risk of bias.

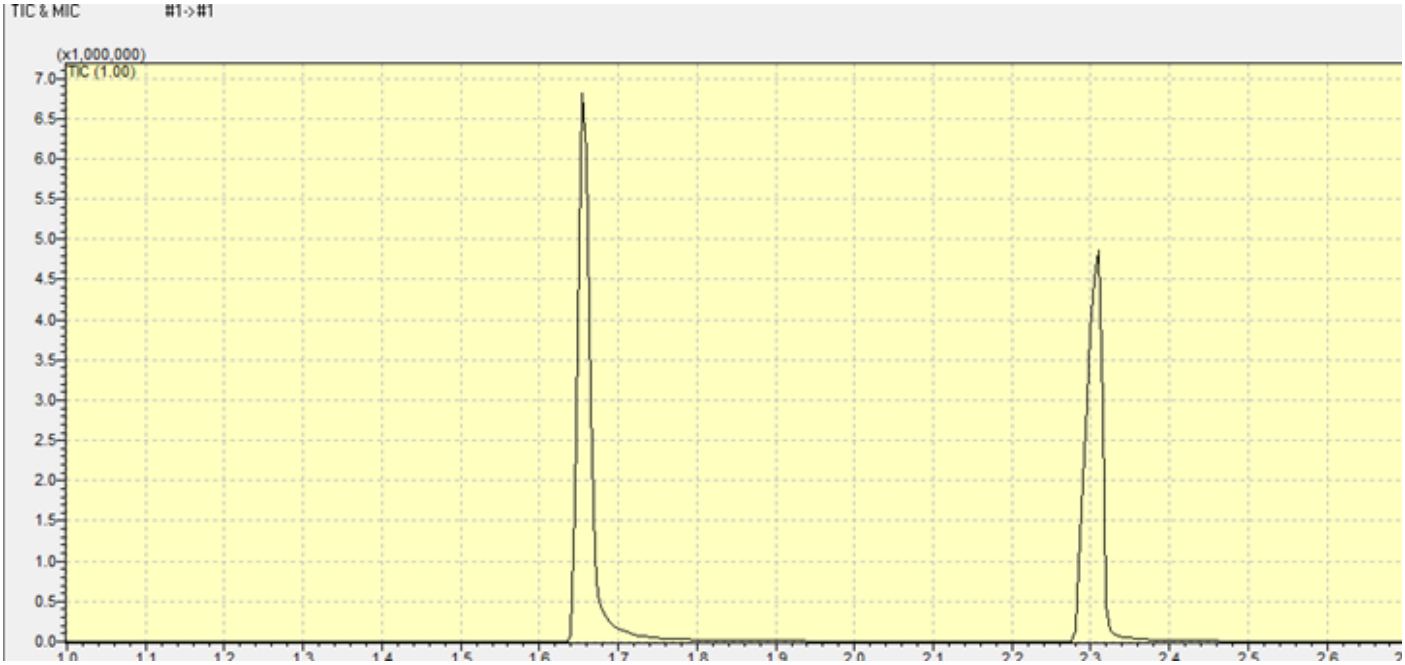
Appendix B

SPME Fiber Absorption Optimization Time

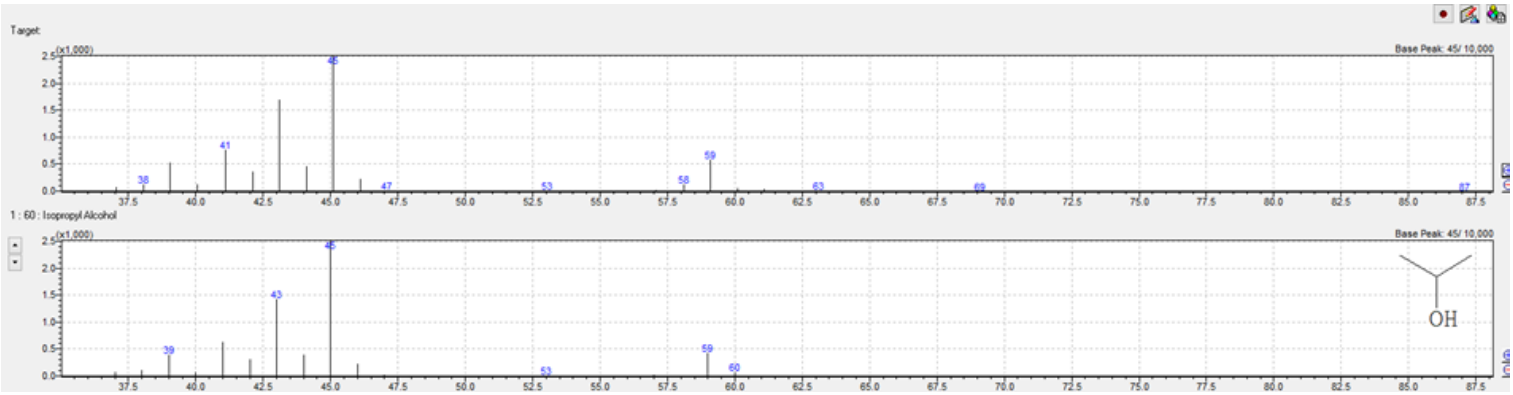


Appendix C

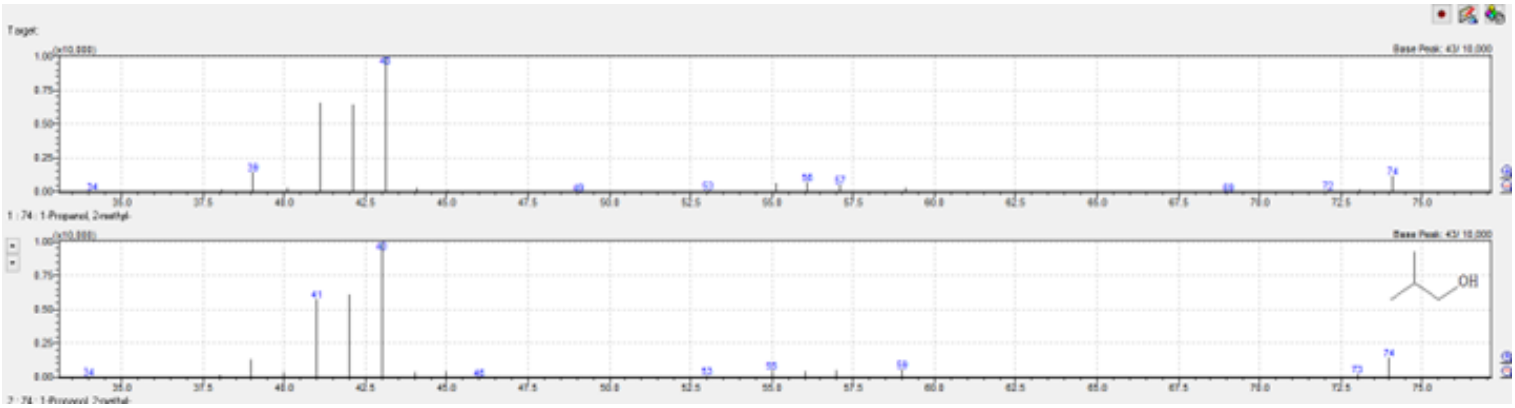
Identification of Isopropyl Alcohol and 2-Methyl-1-Propanol



Isopropyl Alcohol

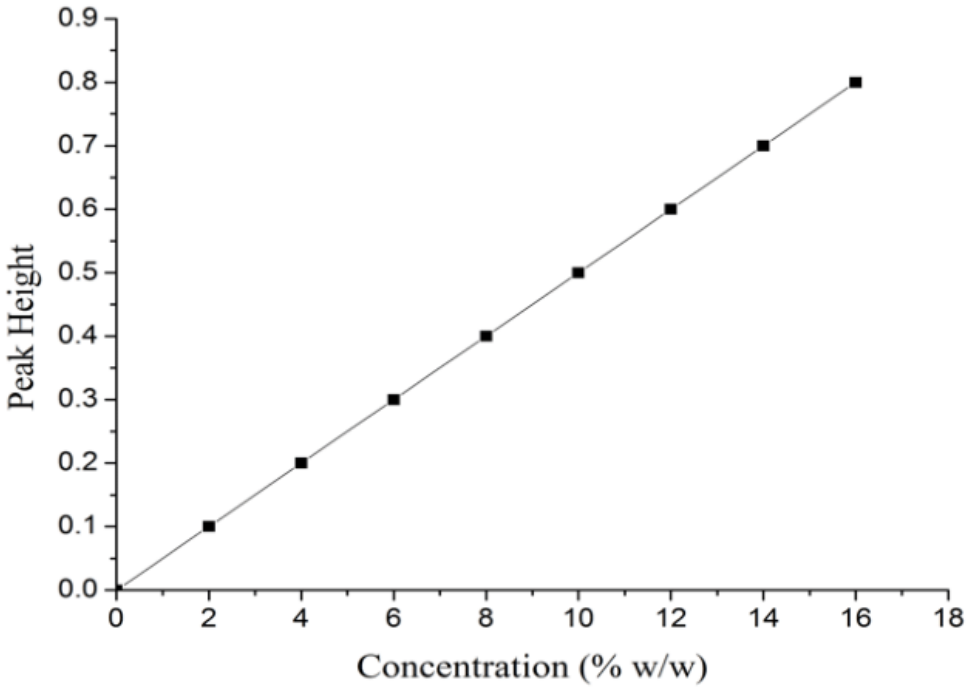


2-Methyl-1-Propranol



Appendix D

Typical Calibration Curve (Haile et al., 2018)



Appendix E

Figure E1: Scholarly Project Calibration Curve

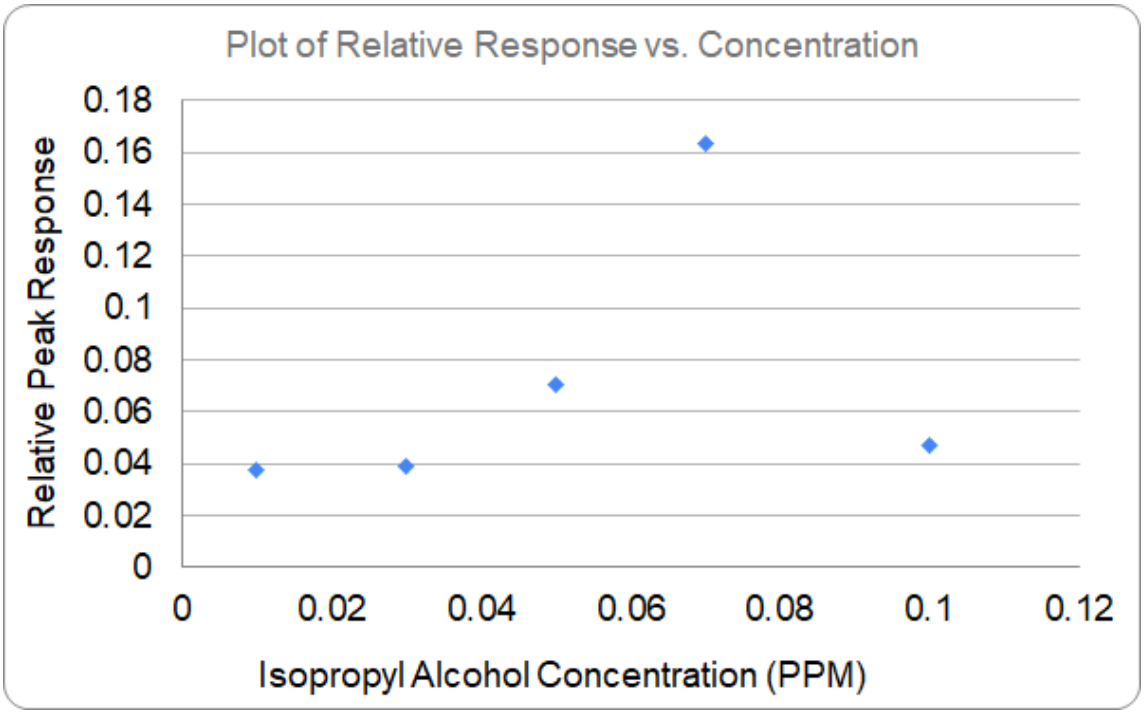
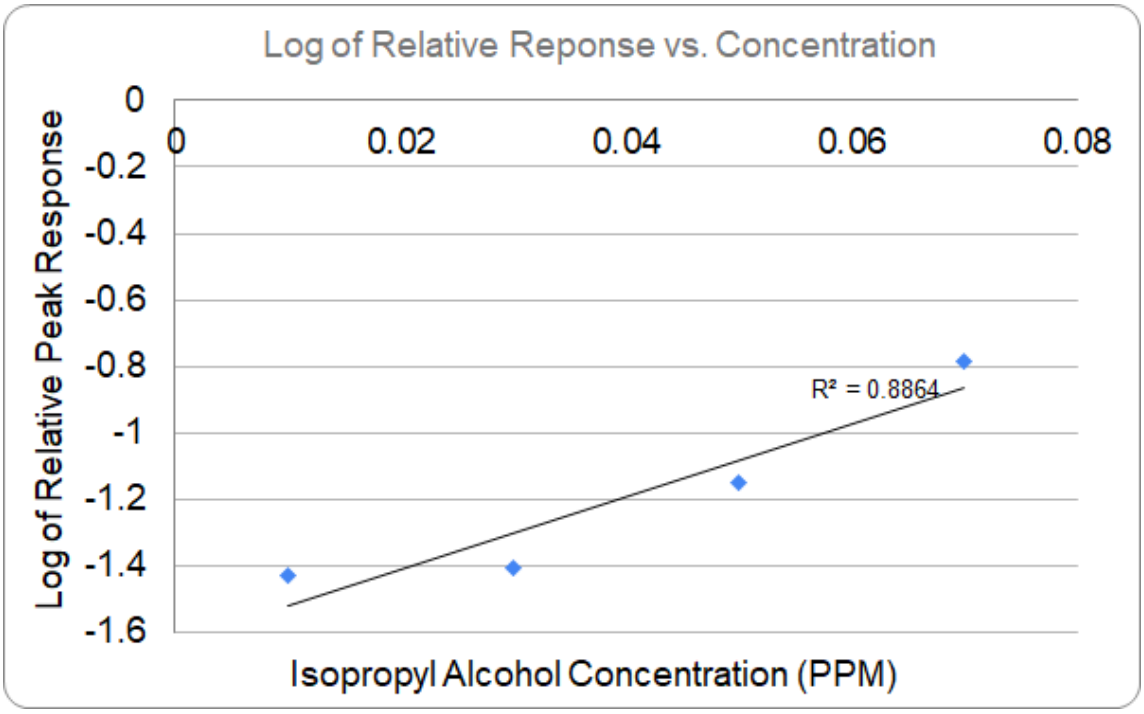


Figure E2: Log of Scholarly Project Calibration



Appendix F

Figure F1: 0 Second Dry Time

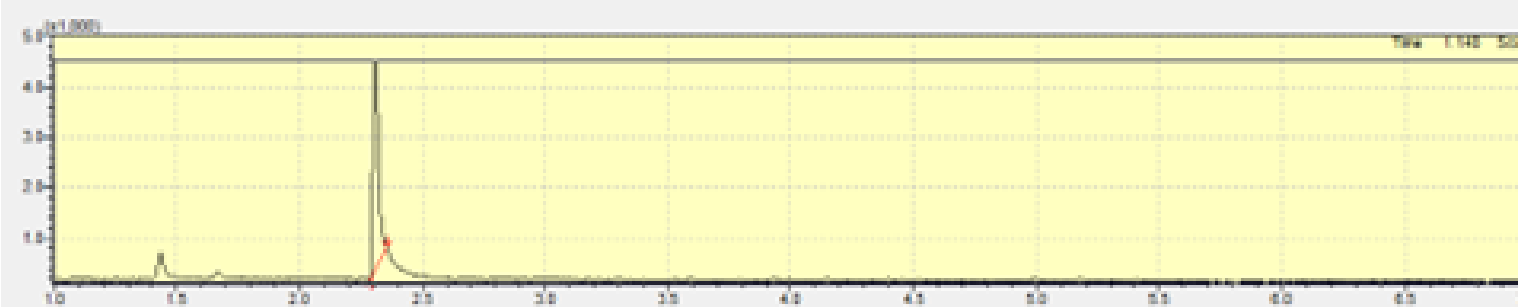
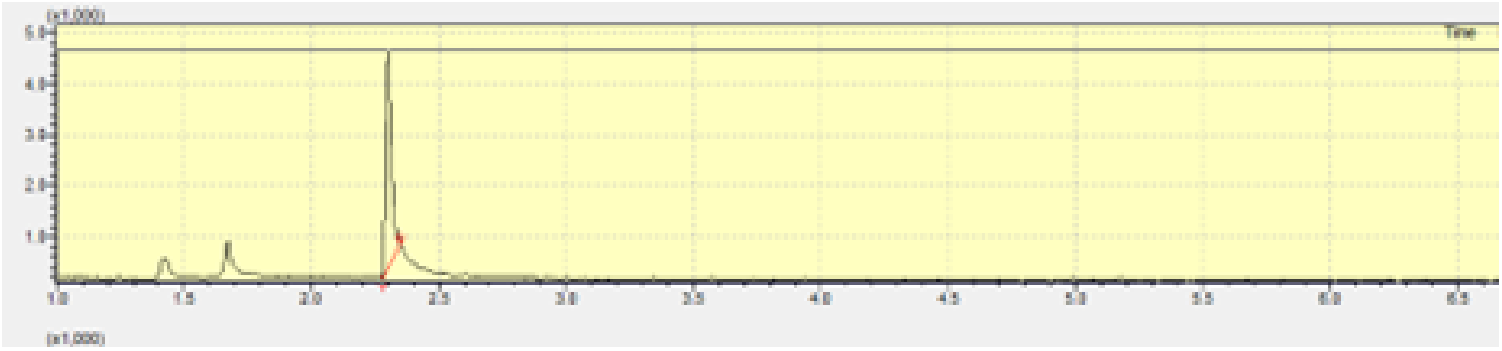
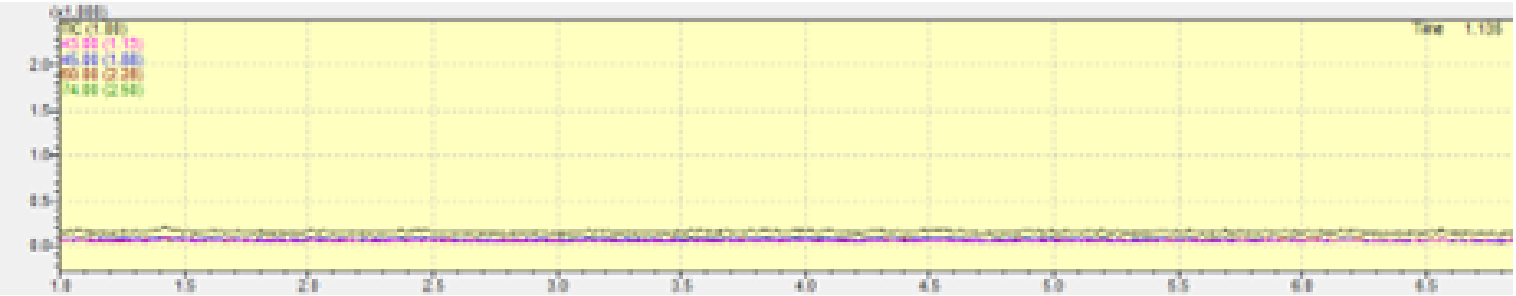


Figure F2: 20 Seconds Dry Time



Appendix G

Initial GC/MS and SPME Blank Baseline



Appendix H

Final GC/MS and SPME Blank Baseline