Document Title:	**Characterization and Classification of Sexual Assault Lubricants using Direct Analysis in Real Time-Time of Flight Mass Spectrometry**
Author(s):	**Candice Bridge**
Document Number:	**254402**
Date Received:	**November 2019**
Award Number:	**2016-NE-BX-0001**

Final Summary Overview

PI: C. Bridge

Grant: 2016-NE-BX-0001

Purpose

This project intends to capitalize on the ease of use and the high mass resolution of the direct analysis in real time-high resoltuion mass spectrometry (DART-HRMS) instrument to create a rapid screening technique and a classification scheme for unknown lubricant samples. This method will be compared to current techniques such as gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FTIR). Upon analyzing more than 100 lubricants that could be used to facilitate sexual assaults, the spectra and metadata of each of the samples will be used to populate a database that will be available to the public and the forensic lubricant community domestically and internationally.

Project Design and Methods

Project Design:

This research project includes the comprehensive examination of lubricant samples to understand and identify similarities and differences between samples to create classification scheme(s) for unknown lubricant samples. Lubricant samples will be analyzed by DART-HRMS as a novel and rapid technique for screening and analysis. DART-HRMS provides a complimentary technique to current analytical methodologies for lubricant analysis and thus all samples will be compared to current techniques such as GC-MS and FTIR. The classifications schemes developed will be provided in the forensic lubricant database, the Sexual Assault Lubricant Database. The major goals of this project are listed below:

1. **Lubricant Method Development:** Develop reliable instrumental methods on DART-TOFMS, GC-MS and FTIR to analyze neat lubricants and their extracts.
2. **Conduct Sample Analysis and Characterization:** Each lubricant sample will be analyzed and characterized to identify ions/peaks that are similar between samples or are unique to a particular class of samples.
3. **Online Database Design and Development:** Develop and design an online database that will store the classified lubricant samples for public use by the forensic lubricant community.

Analytical Methods:

To ensure that we developed a comprehensive database that included samples from a wide variety of samples that are available in the consumer market were represented in this project. The condom samples were unique, since we were aware that there was a different lubricant inside the condom vs. the outside, the analysis of the outside of the condom was incorporated into this lubricant. The number of samples that were purchased for each group are listed in the table below:

Manufacturing Base	No Additives	Flavors	Edible	Sensatory	Anesthetics
Silicone Based	14	2		4	
Water Based	13	8		5	12
Oil/Natural Oil	15		15		
Condom	13	3		2	
PHP	10				1
Total	65	13	15	12	13

A total of 113 lubricant samples were purchased and analyzed via three different instruments: DART-HRMS, GC-MS and FTIR. The following analytical methods were used for each instrument:

- DART-HRMS: Lubricant samples were prepared in three different manners: neat (directly out of the bottle), a methanol extract, and a hexane extract. The extract samples were prepared to a 1000 ppm concentration. After sample preparation, the sample was analyzed at 350 ^{o}C 25 times.

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

The ionizing gas used was helium. A smaller study was done for the water-based lubricants, and in this study the ionization temperature was dropped to 150 ^{o}C. Considering that DART-HRMS is an ambient mass spectral technique, it was necessary to obtain more spectra to collect a better representation of the variance in the spectra collected. Therefore, the first 5 spectra were averaged to generate replicate #2 for the samples. The second 5 spectra were averaged to generate replicate #2 and so on, until there were 5 replicates for each lubricant sample.

- GC-MS: Lubricant samples were prepared in two different manners: a methanol extract a hexane extract. The extract samples were prepared to a 1000 ppm concentration. The career gas was helium. Both extracts were analyzed on the GC-MS in triplicate. Each of the three measurements were used as an individual replicate for subsequent chemometric analysis.
- FTIR: Lubricant samples were prepared in three different manners: neat (directly out of the bottle), a methanol extract, and a hexane extract. The extract samples were prepared to a 1000 ppm concentration. After analysis, the samples were deposited onto a microscope slide. With respect to the extracts, the solvent was allowed to evaporate before the IR spectra was obtained. Samples were analyzed in triplicate. Each of the three measurements were used as an individual replicate for subsequent chemometric analysis.

Data Analysis

To develop the classification schemes, once the spectra were collected for each sample, the samples were analyzed using the following chemometric techniques. The following process was used for all of the instrumental dataset using each of the sample preparation methods.

- Hierarchal Cluster Analysis (HCA) with the Ward's clustering method was used as an unsupervised

method for grouping the samples together. To determine which the number of unique groups observed was to use a heatmap associated to the HCA dendrogram. Seeing the actual spectra observed for each group, allowed us to observe why the samples grouped together and which groups had a significantly different instrumental spectra.

- Principle Component Analysis (PCA) was another unsupervised technique used to observe the clusters in a 3D principle component (PC) space to observe how the samples group together. This loading plots, obtained through this method, allowed us to identify which components were the most instrumental in separating the groups on each principle component. This was used to also generate the classification scheme, because by known which components are unique to each group, that allows us to classify unknown samples based on the presence, absence or relative ratio of components observe din the spectra.
- Linear Discriminant Analysis (LDA) was a supervised technique that classifies a test set of data against the model dataset, created by PCA, to determine how well this model can be used to accurately classify the unknown test sample. To perform this method, a 20% of the model dataset was separated into a separate test set that was then classified by the remaining model (80% of the original dataset). This provided a numerical estimation of how accurately we can classify an unknown sample according to the data that we have in the database created in this project.
- Pearson Correlation Coefficient (PCC) scores were calculated for each of the inter-class and intra-class pairwise comparisons for the DART-HRMS dataset alone. Samples were treated in two different ways: 1) were each lubricant sample was an individual class, and 2) were the HCA groups previously determined was used as the class. The inter-class and inter-class PCC scores, for each definition of class, were calculated and the average and confidence intervals were calculated to determine the potential overlap of true positives and true negatives based on the correlation score calculated for a pairwise comparison. **This was not included in the initial proposal, but we**

felt it was important in determining how a crime lab could compare a known and known samples and determine the potential of a false positive or false negative. We will do the same for the GC-MS data and the FTIR data.

- Likelihood Ratio Calculations was a measure of the strength of making a determination a determine of a true positive, true negative, false positive and false negative. Therefore, both the positive likelihood ratio (PLR) and the negative likelihood ratios (NLR) were calculated for the DART-HRMS dataset. Thus, providing the crime lab with a numerical value of the strength of the comparison based on the PCC score calculated between the known and unknown samples. **This was not included in the initial proposal, but we felt it was important in determining how a crime lab could compare a known and known samples and determine the potential of a false positive or false negative. We will do the same for the GC-MS data and the FTIR data.**

Database Design.

The database was designed by Tony Elminger (our contractor). This databse has the following parts:

- A home page that explains how the search engine works
- Lubricant sample pages that contain information metadata for each sample, ingredients, components analyzed from each method and each solvent preparation method, and the class for that sample as determined by DART-HRMS. The condoms has an added feature that we can analyze both the inside and outside of the lubricant since there are two different spectra were observed between the two sides.
- Classification schemes
- Database of all lubricant research published in literature (**not originally proposed)**
- The analytical methods used for analysis for each instrument: DART-HRMS, GC-MS and FTIR.
- A separate location for the lubricant community to approve new entries before going live to the

community.

Findings

We have developed validated instrumental methods to analyze lubricants of various bases (silicone-based, water-based, oil-based, edible, condom and personal hygiene products) using each instrument. We have determined which extraction methods extract the most components particularly for GC-MS that will best be able to discriminate samples. Unfortunately, many of the spectra for GC-MS did not yield any actual spectra, regardless of extraction method and that is simply due to the fact that a common GC column used in crime labs did not retain many of the components observed in the sample. This is where DART-HRMS was able to shine because it could detect many of the components that GC-MS was not able to detect. FTIR was very useful for determining the primarily lubricant base. This would be helpful in the operational scheme of the laboratory. Based on the FTIR data, if the sample was a water-based sample then that sample could be analyzed in both low and high temperatures, for the reasons listed below. Additionally, if the sample was silicone-based then the analyst could forgo GC-MS analysis and use DART-HRMS has both a screening and analytical method, so as not to degrade the GC silicone based column.

Classification methods were generated for each instrument alone and for each sample preparation method for each instrument. We tried to conduct generate a fused dataset to develop a classification scheme that would encompass the data from all three instruments. However, this method did not work as was hoped due to the fact that the DART-HRMS dominated the clustering of the samples. This was determined because the same clustering observed with the DART-HRMS data was the same as the fused dataset. It is believed that the reason DART-HRMS dominated the unsupervised clustering method because of the sheer number of datapoints for individual components observed for DART-HRMS over GC-MS and FTIR. Additionally, since different extraction solvents will extract different components from the

lubricants (i.e. methanol should extract most of the polar components, and hexane will extract most of the non-polar components), we thought it best to generate a different classification scheme for each solvent at this time, until we can make the weighted fused dataset work. Therefore, on the website there were will before 8 classification schemes that a lab can chose from based on how they analyze their data: 1) DART-HRMS neat, 2) DART-HRMS methanol, 3) DART-HRMS hexane, 4) GC-MS methanol, 5) GC-MS hexane, 6) FTIR neat, 7) FTIR methanol, and 8) FTIR hexane.

A smaller study was conducted on the water based lubricants, where the samples were analyzed at 350 ^{o}C and 150 ^{o}C. This was done because most of the water based lubricants contained most of the flavors and anesthetics. At higher temperatures, most of the lubricant base was observed, even among lubricants from the same manufacturer but different flavors. At lower temperatures, it was expected that more differentiation would be achieved because you could get the flavor or fragrance profile that would evaporate off rapidly at higher temperatures. It was determined that the lower temperature does actually increase the discrimination power between samples from the same manufacturer just different flavors/fragrances. Additionally, the PLR for the 150 ^{o}C provided stronger likelihood ratios when the PCC score increased compared to the 350 ^{o}C data. The NLR was comparable between the two datasets and were moderately strong in their support of the evidence depending on the PCC score chose as the threshold.

The database is operational and available to the public. It includes all of the information that was proposed in the project.

Implications for Criminal Justice Policy and Practice in the United States.

This research project has produced a new database and associated classification schemes that can be used

in the analysis of unknown sexual lubricant samples collected in sexual assault cases. This is the first of its kind database domestically or even internationally. We have become a new group that others look toward for forensic lubricant research that supports the work that crime laboratory current do or to support crime laboratories to incorporate the analysis of forensic lubricants in their daily operations. We have already be sought out by several crime laboratories for collaborations or interest in supporting this work, (e.g. 2 in America, 1 in New Zealand, and 1 in Canada).

Scholarly Products.

Websites:

The Sexual Assault Lubricant database is currently available to the public. It can be found at https://ncfs.ucf.edu/sal/.

Publications:

1. C. **Bridge**, M. Maric, "Temperature Dependent DART-MS Analysis of Sexual Lubricants to Increase Accurate Associations". *Journal of the American Society for Mass Spectrometry.* **2019.** *In Press.* DOI: 10.1007/s13361-019-02158-x.
2. B. Baumgarten, M. Marić, L. Harvey, C. **Bridge**, "Preliminary Characterization Scheme of Silicone Based Lubricants using DART-TOFMS". *Forensic Chemistry*, **2018,** 8, 28-39. DOI: 10.1016/j.forc.2017.12.005.
3. M. Maric, L. Harvey, M. Tomcsak, A. Solano, C. **Bridge**, "Chemical Discrimination of Lubricant M.eting Types Using Direct Analysis in Real Time Time-of-Flight Mass Spectrometry", *Rapid Communications in Mass Spectrometry*, **2017**. 31(12), 1014-1022. DOI: 10.1002/rcm.7876.

Presentations:

1. S. Dale, B. Baumgarten, M. Marić, C. **Bridge**. "Introducing the lubricant database for the analysis of sexual assault evidence". Showcase of Undergraduate Research Excellence. Orlando, FL. Apr **2019**.
2. M. Maric, C. **Bridge**. "Development of a Classification Scheme and Sexual Lubricant Database for the Forensic Analysis of Lubricant Evidence". Analyticon-2019. San Francisco, CA. Apr **2019**.
3. C. **Bridge**, M. Maric. "Temperature Controlled Ambient Ionization of Lubricants for Increased Match Determination". 257th ACS National Meeting. Orlando, FL. Mar **2019**.
4. C. **Bridge**, M. Maric, "Forensic Chemistry: Providing Actionable Intelligence in Criminal Investigations", 257th ACS National Meeting. Orlando, FL. Mar **2019**.
5. B. Baumgarten, C. Vadell-Orsini, M. Marić, C. **Bridge**. "Characterization of sexual assault lubricants: lubricant database use in an operational setting". National American Chemical Society Conference. Orlando, FL. Mar **2019**.
6. B. Baumgarten, C. Vadell-Orsini, M. Marić, C. **Bridge**. "Characterization of sexual assault lubricants: lubricant database use in an operational setting". Annual Pittsburg Conference on Analytical Chemistry and Applied Spectroscopy. Philadelphia, PA. Mar **2019**.
7. B. Baumgarten, C. Vadell-Orsini, M. Marić, C. **Bridge**. "Characterization of sexual assault lubricants: lubricant database use in an operational setting". National American Academy of Forensic Sciences. Baltimore, MD. Feb **2019**.
8. M. Maric, C. **Bridge**. "Developing of a Sexual Assault Analytical Protocol using DART-HRMS". National Organization of Black Chemists and Chemical Engineers, Orlando, FL.

This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

September 17-20, **2018**.

9. C. Vadell-Orsisi, B. Baumgarten, M. Marić, C. **Bridge**. "Preliminary Characterization of Sexual Assault Lubricants: Comparison between DART-TOFMS, GC-MS, and FT-IR". American Chemical Society FAME. Tampa, FL. Apr **2018**.
10. C. Vadell-Orsisi, B. Baumgarten, M. Marić, C. **Bridge**. "Preliminary Characterization and Classification of Sexual Lubricants and Condoms using Fourier Transform Infrared (FTIR) Spectrometry". Showcase of Undergraduate Research Excellence. Orlando, FL. Apr **2018**.
11. B. Baumgarten, C. Vadell-Orsini, M. Marić, C. **Bridge**. "Preliminary characterization of sexual assault lubricants: comparison between DART-TOFMS, GC-MS, and FT-IR". Annual Pittsburg Conference on Analytical Chemistry and Applied Spectroscopy. Orlando, FL. Mar **2018**.
12. B. Baumgarten, C. Vadell-Orsini, M. Marić, C. **Bridge**. "Preliminary characterization of sexual assault lubricants: comparison between DART-TOFMS, GC-MS, and FT-IR". National American Academy of Forensic Sciences. Seattle, WA. Feb **2018**.
13. B. Baumgarten, M. Marić, C. **Bridge**. "Characterization and Classification of Silicone Lubricants with Statistics". Florida Forensic Science Conference. Orlando, FL. Oct **2017**.
14. C. Vadell-Orsisi, B. Baumgarten, M. Marić, C. **Bridge**. "Characterization of Sexual Lubricants and Condoms using Fourier Transform Infrared Spectrometry (FTIR)". Florida Forensic Science Conference. Orlando, FL. Oct **2017**.
15. M. Maric, L. Harvey, M. Tomcsak, A. Solano, C. **Bridge**. "Development and validation of an analytical protocol for the characterization of lubricant evidence", AAFS 69th Annual Scientific Meeting. New Orleans, LA. Feb **2017**.