Midwest Forensics Resource Center

Research and Development Program Summary

October 2011

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Midwest Forensics Resource Center (MFRC)
Research and Development Program

Introduction

The mission of the MFRC Research and Development Program is to provide technological advances in forensic science for the benefit of our regional partners, as well as the forensic science community at large. Key areas of forensic science needs are identified through participation in national meetings in forensic science and guidance by national studies and reports. Under the sponsorship of the National Institute of Justice (NIJ), the MFRC solicits proposals for the development of practical and useful forensic science tools that require proof-of-concept experimentation and tools proven in other fields that require experimentation to demonstrate feasibility for addressing specific forensic science needs. The MFRC facilitates proposal development by working to establish partnerships between researchers and our regional partners. The MFRC administers a peer-review of the proposals and then funds the selected projects at the level of approximately $75,000 each, with a 12-month period of performance.

The process for selection of these projects includes the following steps: 1) Drafting of a call for proposals by MFRC staff; 2) Review of the draft call by members of the R&D Advisory Committee; 3) Review and approval of the call by NIJ; 4) Issuance of the call to Iowa State University (ISU), Ames Laboratory, regional partners, and various academic and non-academic research organizations; 5) Receipt of proposals; 6) Review of proposals by the R&D Advisory Committee; 7) Ranking and selection by MFRC staff using Advisory Committee reviews; 8) Concurrence by NIJ of selected proposals; 9) Notification of proposers and awards; 10) Receipt and review of progress reports by MFRC; 11) Receipt and review of final reports by MFRC, R&D Advisory Committee, and NIJ; and 12) Posting of final reports on the MFRC website.

The decision to fund any specific project is based upon a peer-reviewed call-for-proposal system administered by the MFRC. The reviewers are crime laboratory specialists and scientists who are asked to rate the proposals on four criteria including: 1) Relevance to the mission of the MFRC; 2) Technical approach and procedures; 3) Capabilities, teaming, and leveraging; and 4) Dissemination and implementation of research findings. A successful proposal demonstrates knowledge of the background for the research and includes a research methodology with a well-defined plan to transfer research findings into the hands of stakeholders to pursue further research or into the hands of users to facilitate application of the developed tools.

Program Summary Technical Sheets

The following project summaries, while not a complete summary of all research areas, are meant to demonstrate the range of research funded by the MFRC. The project summaries describe the forensic need the projects serve as well as the benefits derived from the technology. The summaries provide a brief description of the technology and the accomplishments to date. In addition, the collaboration with regional partners and the status of the dissemination of project results and implementation of the product are highlighted. These technical summaries represent the development and implementation of practical and useful technology for crime laboratories that the MFRC hopes to accomplish.

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Abstracts of Funded Projects

CHEMISTRY

Application of a Novel Mixed-Mode Reversed-Phase HPLC Column to the Rapid Confirmatory Analysis of Intoxicants and their Hydrophilic Metabolites by LC-MS/MS

Dwight Stoll, Department of Chemistry, Gustavus Adolphus College, St. Peter, MN

High Performance Liquid Chromatography (HPLC) has become the dominant analytical methodology in forensic drug analysis. Yet, low retention of highly hydrophilic compounds has historically been a significant weakness of reverse-phased HPLC. This project evaluates the application of reverse-phased HPLC methods for confirmatory analysis of common benzodiazepines and opiates and their major metabolites in blood and urine. Detection of intoxicants is achieved by liquid chromatography tandem mass spectrometry.

Application of Multivariate Statistical Procedures in Fire Debris Analysis: Investigating Matrix Interference Effects and Weathering of Ignitable Liquids on Association of Ignitable Liquid Residues to Neat Ignitable Liquids

Ruth Waddell-Smith and Victoria McGuffin, School of Criminal Justice and Department of Chemistry, Michigan State University, East Lansing, MI

Previous research demonstrated the successful use of Principal Component Analysis and Pearson Product Moment Correlation Coefficients for the association and discrimination of ignitable liquids from the same ASTM class. This project further develops the objective methodology by considering the effects of both matrix interferences and weathering on the association of ignitable liquid residues to the neat liquid.

Chemical Characterization of Emerging Designer Drugs

Jeremiah Morris, Johnson County Sheriff's Office Criminalistics Laboratory, Mission, KS

Analytical data for synthetic cannabinoids is rarely available. Without verified analytical data from known sources, structural elucidation of unknown compounds by mass spectral data alone is insufficient. This project elucidates the chemical structure of emerging designer drugs using four different analytical techniques and a presumptive color test. In doing so, it provides the forensic drug chemist with the analytical data to identify synthetic cannabinoids in emerging designer drugs.

Comparison of Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Mass Spectrometry for Discrimination of *Salvia divinorum* from Related *Salvia* Species Using Chemometric Procedures

Victoria McGuffin and Ruth Waddell-Smith, Department of Chemistry and School of Criminal Justice, Michigan State University, East Lansing, MI

Salvia divinorum is a hallucinogenic herb that is regulated in 24 states. The plant is one of nearly 1,000 species of *Salvia,* some of which are culinary herbs and ornamental shrubs. The goal of this project is to develop a method to definitively identify *S. divinorum.* This is accomplished by analyzing samples by both gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine which technique offers the greatest selectivity for the identification of *S. divinorum.* Chemometric procedures are used to identify the most characteristic chemical components that allow differentiation of *S. divinorum* from other *Salvia* species.

Fast Gas Chromatography Capabilities in Drug Identification

Charles Cornett, Department of Chemistry and Engineering Physics, University of Wisconsin-Platteville, Platteville, WI, and Leah Macans, Wisconsin State Crime Laboratory-Milwaukee, Milwaukee, WI

Gas chromatography-mass spectrometry (GC-MS) is the primary method used for the identification of drugs. While the technique has sufficient sensitivity and specificity, it can be slow, thus limiting the number of samples that can be analyzed. This project examines the capabilities of FastGC in drug analysis using hydrogen instead of helium as the carrier gas.

Random Probability Match Procedure for Statistical Comparison of Mass Spectral Data

Ruth Waddell-Smith and Victoria McGuffin, School of Criminal Justice and Department of Chemistry, Michigan State University, East Lansing, MI

To address the need for accessing the significance of associations in the comparison of evidence, a method is developed for statistical comparison of mass spectral data obtained using gas chromatography mass spectrometry (GC-MS). The procedure is based on classic probability theory: determining the random probability that a match between the mass spectrum of a questioned sample and that of a reference standard occurs by chance. The mass spectra used are those of controlled substances.

Rapid Arson Sample Analysis Using DART Mass Spectrometry

John McClelland, Ames Laboratory, U.S. Department of Energy, Iowa State University, Ames, IA

Gas chromatography/mass spectrometry (GC/MS) is commonly used to identify accelerants in fire debris. The method is not ideal, requiring substantial time for sample analysis and data interpretation. This project investigates the use of Direct Analysis in Real Time (DART) mass spectrometry as a faster, more efficient alternative to GC/MS. Specifically, the accuracy, reliability, and validity of DART-MS for arson analysis is investigated.

DNA

Degradation in Chromosomal DNA Assessed Using PCR Amplification and Capillary Electrophoresis

Robert Allen, Department of Forensic Sciences, Oklahoma State University, Tulsa, OK

DNA profiling methods are widely used in the forensic community. For highly degraded DNA samples, the number of methods is limited, and oftentimes require the use of new, expensive instrumentation. This project investigates the use of quantitative template amplification technology (Q-TAT) assay to provide information about DNA integrity. The overall objective of this project is to establish parameters enabling Q-TAT assay to reliably identify DNA samples that are sufficiently degraded to require specialized testing methods to produce a DNA profile.

PATTERN EVIDENCE

Application of Face Recognition Technology to Microstamped Cartridge Cases

Scott Chumbley, Department of Materials Science and Engineering - Ames Laboratory, U.S. Department of Energy, Ames, IA, and Song Zhang, Department of Mechanical Engineering, Iowa State University, Ames, IA

Microstamping identification has been suggested as a way of providing an objective means to relate guns to fired ammunition. While the method shows promise, questions remain with regard to the durability of the mark. The purpose of this project is to examine the transfer of identifiers as a function of firearm action and ammunition. To achieve this goal, three different handguns are tested using 10 different types of ammunition.

Discrimination of Dyed Cotton Fibers Based on UV-Visible Microspectrophotometry and Multivariate Statistical Analysis

John Goodpaster, Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis (IUPUI), Indianapolis, IN

Dyed cotton fibers are a common fiber type found in clothing. One of the most popular methods for their analysis is UV-visible microspectrophotometry (UV-MSP). However, sample association with UV-MSP can be problematic. To establish the validity of UV-MSP for fiber examinations, this project compares MSP data, and evaluates the use of multivariate statistical analysis to discriminante dyed cotton fibers.

Shape Measurement Tools in Impression Evidence: A Statistical Approach

Mary Bush, School of Dental Medicine, State University of New York (SUNY) at Buffalo, Buffalo, NY, and H. David Sheets, Department of Physics, Canisius College, Buffalo, NY

The need for robust statistical models has been noted for pattern evidence analysis. The project develops a multivariate approach using a shape analysis software based on a shape change measurement technique called geometric morphometric methods. The goal of the project is to explore the feasibility of applying the shape measurement tool to fingerprint evidence and footwear impressions.

The Development of a New Model to Study Firearms Related Blood Spatter

Michael Taylor, Institute of Environmental Science and Research, Christchurch, NZ., Kevin Winer, Kansas City Police Crime Laboratory, Kansas City, MO, and Jules Kieser, Sir John Walsh Research Institute, University of Otago, Dunedin, NZ.

The study of firearm-related blood spatter is a common and often critical task for investigators. Simulating the formation of the spatter to answer case-related questions is difficult, partly because of the lack of suitability models. This project develops a physical model to study cranial gunshot wounding and associated blood spatter formation. Construction of the model is accomplished using anatomically accurate dimensions and best available simulant materials.

Application of a Novel Mixed-Mode Reversed-Phase HPLC Column to the Rapid Confirmatory Analysis of Intoxicants and Their Hydrophilic Metabolites by LC-MS/MS

FORENSIC TECHNOLOGY NEED

Benzodiazepines are some of the most widely used prescribed and abused drugs in the Midwestern states. To detect parent drugs and hydrophilic metabolites of certain benzodiazepines, most forensic laboratories currently use Liquid Chromatography-Mass Spectrometry (LC-MS) technology to test for 25 drugs and metabolites in blood and urine samples. However, each run is time consuming (about 30 minutes) and many of the closely related drugs are not well separated.

TECHNOLOGY DESCRIPTION

Low retention of highly hydrophilic compounds has historically been a significant weakness of reversed-phase high performance liquid chromatography (HPLC): the most common mode of liquid chromatography in use today. Recently, new column technology for HPLC has been developed that provides different options and is sufficiently retentive for polar compounds to analyze both the parent drugs and their conjugate metabolites in a single, rapid analysis.

METHODOLOGY

This project evaluates the application of a novel mixed-mode reversed-phase HPLC column. Specific objectives are to:

- Compare the retention and selectivity of 16 benzodiazepines and nine opiate target compounds on a novel mixed mode stationary phase to that observed on a traditional C 18-bonded phase
- Compare the LC-MS/MS detection limits for the 25 target compounds on the two stationary phases
- Optimize separation conditions to increase throughput for both classes of compounds
- Use an opiate method that is capable of determining the parent drugs and metabolites in a single analysis
- Adopt a benzodiazepine method that improves throughput by a factor of two

ACCOMPLISHMENTS AND ONGOING WORK

Initally, the project called for the comparison of a conventional C18 phase and a novel Hyper-Crosslinked hydrophilic weak cation-exchange phase (HC-COOH). However, at the start of the project, a prototype perfluorinated phenyl phase (F5) became available which is built upon the increasingly popular shell particle architecture. Along with the C18 and HC-COOH phases, it was incorporated into the project.

A comparison of the retention of three of the most hydrophilic opiates and their metabolites on the three stationary phases showed an increase in retention of the hydrophilic opiates on the HC-COOH phase compared to the C18 phase. Yet, the increase was not as large as expected based on previous work and not nearly as large as the increase in retention observed on the F5 phase.

Similarly, a comparison of the retention of a select group of benzodiazepines and their hydrophilic metabolites on the C18 and F5 phases indicated that although significant differences in selectivity (elution order) could be seen, the absolute retention

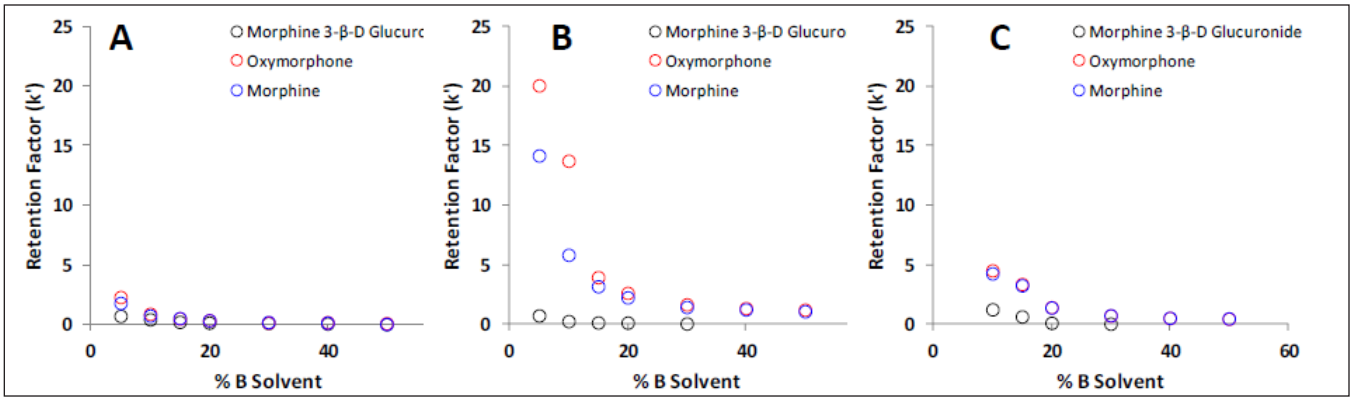

Figure 1. Comparison of retention of three of the most hydrophilic opiates on three different phases under isocratic conditions: A) Ascentis Express C18, B) Ascentis Express F5, and C) HC-COOH. The F5 phase shows dramatically increased retention over both of the other phases for the compounds.

values were quite similar. Given the observations, it was decided to continue the comparisons using the C18 and F5 phases only.

Combining theoretical factors with practical considerations, column dimensions of 7.5 cm X 2.1 mm i.d. were used for the work. Flow rates were set at 0.25 ml/min, and reflected the rate desired by the project collaborator. Using these dimensions and conditions, separation of the 9 opiates was accomplished and satisfied the method goals established by the forensic collaborator. The separation time for the analysis of 9 opiates was 7 minutes.

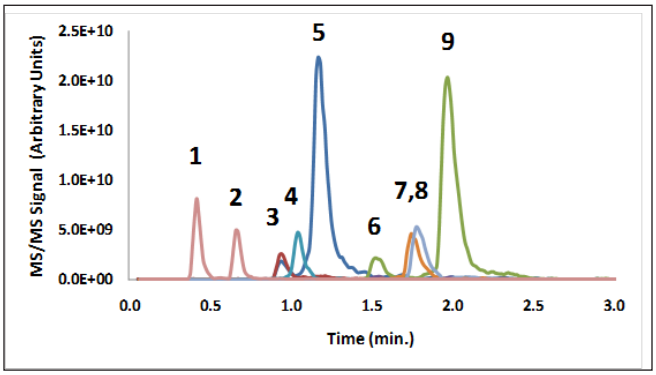

Figure 2. Separation of 9 opiates using a 7.5 cm X 1.0 mm i.d. Ascentis Express F5 column operated at 0.25 ml/min. (4.0 ml/min. total, 3.75 ml/min pre-column split to waste).

Additional gains in separation speed could be achieved by either reducing the column diameter while maintaining a flow rate of 0.25 ml/min, or by increasing the flow rate while maintaining the column diameter at 2.1 mm. For example, by reducing the column diameter to 1.0 mm i.d., analysis of the 9 opiate mixture could be attained in less than 3 minutes. Some of the resolution of the compounds was sacrificed in the interest of speed. However, none of the overlapping compounds were similar in mass, thus the mass spectrometer detector could be used to effectively resolve the compounds that were not resolved chromatographically.

For benzodiazepine analysis, using a column dimension of 7.5 X 2.1 mm i.d., and a flow rate of 0.25 ml/min, it was found that all 32 target compounds (parent and metabolite) could be resolved in at least one of the dimensions (mass; time), within a 10 minute timeframe. As with the opiate compounds, an improvement in throughput could be realized by using a flow rate through the column higher than 0.25 ml/min, and spliting some of that flow prior to the mass spectrometer. In doing so, the resolution of the 32 benzodiazepines was not compromised even though the analysis time was reduced to 4 minutes.

A number of experiments were then conducted to determine if the sensitivities observed under actual conditions were similar to those observed under flow-in conditions. One experiment had no column installed, while another experiment had a column installed under either reversed-phase (RP) or hydrophilic interaction (HILIC) conditions. It was found that, for the 3 opiate compounds

studied (morphine gluconoride, morphine, 6-acetyl morphine), there was a significant improvement in the detection sensitivity under HILIC conditions.

It was further found that the isobaric compounds hydromorphone and morphine were not separated under those conditions, as was the case with the other isobaric pair hydrocodone and codeine. Thus, the observed improvement in sensitivity is practically irrelevant because these conditions do not yield satisfactory separation for the critical pairs in this group of compounds.

TECHNOLOGY BENEFITS

The development of rapid HPLC methods for confirmatory analysis of benzodiazepines, opiates, and their metabolites, greatly improves the screening of drugs of abuse. Analyzing both the parent compounds and important polar metabolites in a single analysis will further increase the throughput of samples in forensic toxicology labs.

COLLABORATION

This project is a collaborative effort between Gustavus Adolphus College, St. Peter, MN, the Minnesota Bureau of Criminal Apprehension (BCA), St. Paul, MN, and the University of Minnesota (UM), Minneapolis, MN. The primary role of the Minnesota BCA is to provide counsel and extracts of blood and urine samples. The UM provides the HPLC columns and guidance for their use.

DISSEMINATION

Research findings and results were presented at annual meetings of the Society of Forensic Toxicologists (SOFT), the Minnesota Chromatography Forum (MCF), and the Midwestern Association of Forensic Scientists (MAFS). Depending on the outcome of a validation study conducted by the BCA, a manuscript will be submitted to the *Journal of Forensic Toxicology*. A technical report on the project and its findings has also been posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

- Bonnerup, S., Liskulin, T., Berry, J., Stoll, D. "Development of Rapid LC-MS/MS for Confirmatory Analysis of Opiates and Benzodiazepines" Poster presentation at the Pittsburg Conference, Pittsburg, PA. March 2011.

- Stoll, D., Bonnerup, S., Harmes, C., Liskulin, T., Berry, J. "Development of Rapid LC-MS/MS-based Methods for Confirmatory Analysis of Opiates and Benzodiazepines" Presentation at the Minnesota Chromatography Forum Spring Symposium, Minneapolis, MN. May 2011.

- Stoll, D., Bonnerup, S., Harmes, C., Liskulin, T., Berry, J. "Development of Rapid LC-MS/MS-based Methods for Confirmatory Analysis of Opiates and Bezodiazepines" Presentation at the annual meeting of the Midwestern Association of Forensic Scientists, Chicago, IL. September 2011.

- Stoll, D., Bonnerup, S., Harmes, C., Liskulin, T., Berry, J. "Development of Rapid LC-MS/MS-based Methods for Confirmatory Analysis of Opiates and Benzodiazepines" Presentation at the Annual Meeting of the Society of Forensic Toxicologists (SOFT), San Francisco, CA. September 2011.

IMPLEMENTATION

The method developed is currently being evaluated in accordance with the Minnesota BCA's validation plan for toxicology chromatographic methods. The outcome of the validation process will determine the feasibility of implementation of the F5 phase by the Minnesota BCA and other laboratories.

CONTACTS

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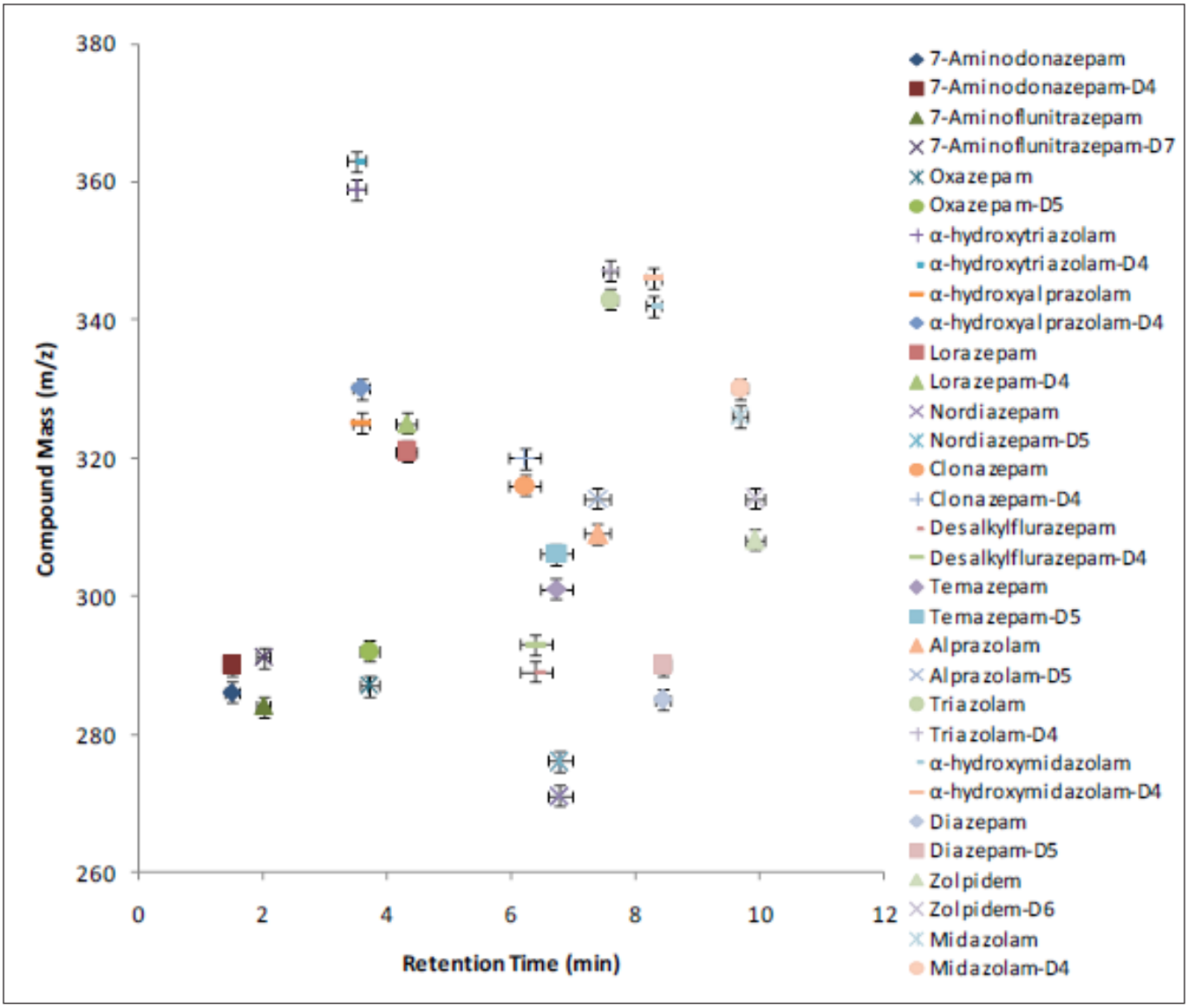

Figure 3. Resolution map for 16 benzodiazepines and metabolites under conditions involving flow splitting to improve analysis time. The flow rate through the column was 0.75 ml/min., with only 0.25 ml/min. directed to the mass spectrometer.

Application of Multivariate Statistical Procedures in Fire Debris Analysis: Investigating Matrix Interference Effects and Weathering of Ignitable Liquids on Association of Ignitable Liquid Residues to Neat Ignitable Liquids

FORENSIC TECHNOLOGY NEED

In arson investigations, fire debris is typically extracted and analyzed to determine the presence of Ignitable Liquid Residues (ILR). The identification of IRLs can be complicated by interference from substrates and weathering thus making the association of ILRs to Neat Ignitable Liquids (NILs) very difficult.

TECHNOLOGY DESCRIPTION

Previous research demonstrated the success of Principal Components Analysis (PCA) and Pearson Product Moment Correlation (PPMC) coefficients for the association of ignitable liquids (including evaporated liquids) from the same ASTM class with differentiation of ignitable liquids from different ASTM classes.

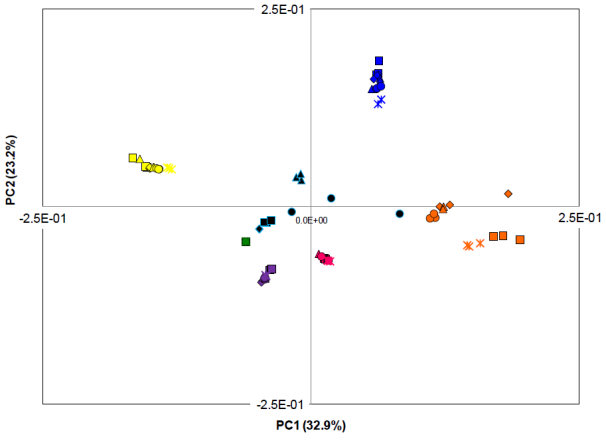

Figure 1. Scores plot of PC1 v PC2 based on the TIC (Total Ion Chromatograms) for six ignitable liquids with scores projected for evaporated liquids and burned substrates. Each color represents a different ASTM class.

This project further develops the objective methodology by considering the effect of both matrix interferences and weathering on the association of ILRs to the neat liquid. Additionally, a Soft Independent Modeling of Class Analogy (SIMCA) approach to classify an ILR to an ignitable liquid class is investigated, aiming to allow classification with statistical confidence.

METHODOLOGY

The objective of this research is to develop a methodology to associate ILRs to the corresponding NILs, even in the presence of matrix interferences and weathering effects. Specific goals are to:

- Investigate increased matrix interference effects from four household matrices
- Investigate the effect of increased matrix interference effects on the association of evaporated liquids to neat liquids using PCA and PPMC coefficients
- Investigate the association of simulated ILRs to the corresponding neat liquid using PCA and PPMC coefficients
- Investigate a SIMCA approach for the classification of a simulated ILR to an ignitable liquid class

ACCOMPLISHMENTS AND ONGOING WORK

To investigate matrix interference effects, three liquids (gasoline, kerosene, and lighter fluid) at three different evaporation levels (10%, 70%, 90%), and two different matrices (carpet and high density polyethylene) were targeted. Matrices were cut into 4 X 4 cm pieces that were burned

using a propane blowtorch for burn times of 10, 20, 30, 60, and 120 seconds.

Aliquots (20 µl) of diluted liquid standards were spiked on to the burned matrix. Samples were placed in a nylon bag with an activated carbon strip and activated in an oven for four hours at 80° C. After extraction, carbon strips were eluded with 200 µl of dichloromethane and analyzed by GC-MS.

It was found that as burn times increased the number and abundance of matrix interferences also increased. At 60 seconds, there was a significant abundance of matrix interference. As the burn times increased further, there was no additional matrix interference. Hence, 60 seconds was selected as the optimal burn time.

For experiments with nylon carpet, PCA scores for liquids extracted from the burned matrix were calculated and projected onto the scores plot. In general, the extracts were positioned closely to the standard in the PCA scores plot although some shift in position was apparent. The shift was primarily due to differences in abundance of compounds between the standards and the extracts, rather than the presence of matrix interferences.

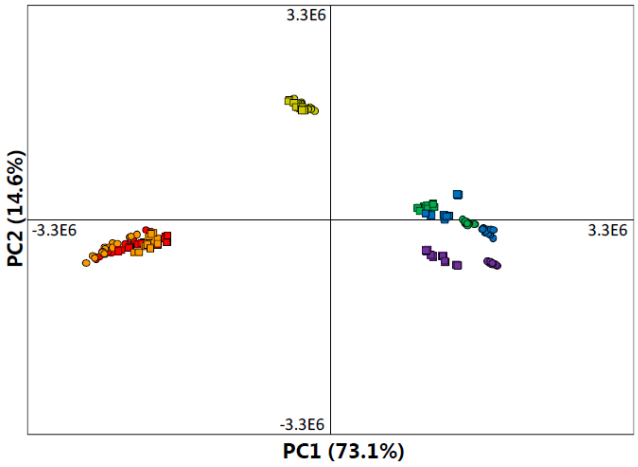

Figure 2. Scores plot of PC1 vs. PC2 for gasoline and kerosene standards (circles), with extracts from the burned carpet projected (squares): neat gasoline (red); 10% evaporated gasoline (orange); 90% evaporated gasoline (yellow); neat kerosene (green); 10% evaporated kerosene (blue); 70% evaporated kerosene (purple).

Experiments using high density polyethylene (HDPE) as the matrix yielded similar results albeit with some spread. The HDPE matrix contains alkadienes, alkenes, and normal alkanes as interferences. However, only the normal alkanes had any effect on positioning of the extracts as the compounds are present in kerosene. In addition, two of the alkene matrix interferences co-eluted with compounds present in gasoline and, hence, also contributed to the positioning of the extracts.

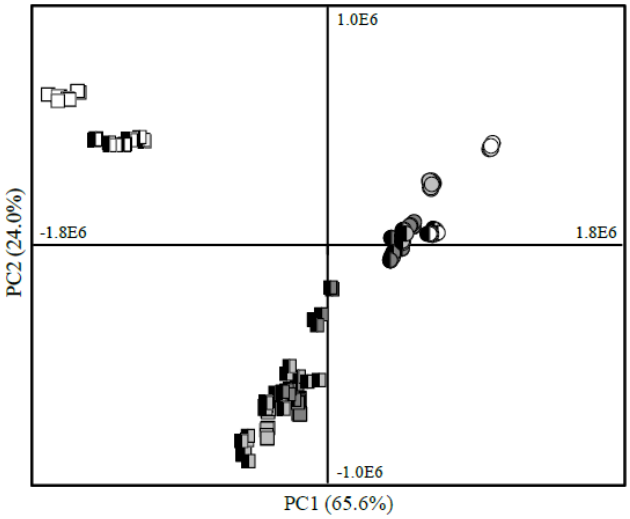

Figure 3. Scores plot of PC1 versus PC2 based on the TICs for the six ignitable liquid standards: neat gasoline (dark square), 10% evaporated gasoline (light gray square), 90% evaporated gasoline (white square), neat kerosene (dark circle), 10% evaporated kerosene (light gray circle), 70% evaporated kerosene (white circle). Burned HDPE extracts are indicated by half fill.

The research currently focuses on assessing the combined effects of thermal degradation and matrix interferences on the association of liquid extracts to the corresponding neat liquid.

TECHNOLOGY BENEFITS

The National Academy of Sciences report on the current state of forensic science questions the ability of forensic techniques to "demonstrate connection between evidence and a specific individual source." This project addresses these concerns with the development of an objective methodology for the association of an ILR to the corresponding neat liquid. As such, fire debris analysts will be

able to attribute statistical confidence in their ILR identifications, despite the presence of matrix interferences and weathering effects.

COLLABORATION

This study is a collaborative effort between Michigan State University (MSU), East Lansing, MI, and the Michigan State Police (MSP), Grand Rapids, MI. The MSP serves as consultants to ensure the practicality of the methodologies as well as the overall project approach.

DISSEMINATION

Project findings and results will be presented at regional and national forensic and chemical meetings. A manuscript is planned for submission to the *Journal of Forensic Sciences*. Upon completion of the project, a final project report will be posted on the MFRC website. An online seminar may be offered to discuss the methodology used to develop the methodology. If offered, the seminar will be advertised through the Midwestern Association of Forensic Scientists (MAFS) website.

PUBLICATIONS AND PRESENTATIONS

- Prather, K., McGuffin, V., Smith, R. "Using Multivariate Statistical Procedures to Identify Ignitable Liquid Residues in the Presence of Interferences" Presentation at the 42nd Meeting of the American Chemical Society Regional Meeting, Indianapolis, IN. June 2011.

IMPLEMENTATION

To reach as wide an audience as possible, an on-line seminar (accessible through MSU's on-line learning system) will be developed to outline the methodology developed. The seminar will be advertised via an e-mail announcement that will be distributed to crime labs in the Midwest. Additional funds may be sought to develop and present a workshop to train analysts more thoroughly in the data analysis and interpretation procedures.

CONTACTS

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Chemical Characterization of Emerging Designer Drugs

FORENSIC TECHNOLOGY NEED

In 2009, products marketed as "herbal incense" started to appear on the market. Rather than legitimate incense materials, these products contained "synthetic cannabinoids". By 2010, the number of synthetic cannabinoids detected in incense products increased to more than 20. Analytical data for these cannabinoids is rarely available. Substituted cathinones represent another rapidly evolving class of designer drugs. These compounds emerged as substitues for cocaine and methamphetamine.

Figure 1. MATRIX: a herbal incense containing cannabinoids.

TECHNOLOGY DESCRIPTION

In forensic drug chemistry, identification of unknown substances is difficult. Without verified analytical data from known sources, structural elucidation of unknown compounds by mass spectral data alone is insufficient. In this project, analytical data for emerging designer drugs of abuse will be obtained using gas chromatography-mass spectrometry (GC/MS), liquid chromatography-tandem mass spectrometry (LC/MS/MS), Fourier Transform Infrared Spectroscopy (FTIR), and presumptive color tests.

METHODOLOGY

The specific goal of this project is to provide forensic drug chemists with an awareness of emerging drugs of abuse along with the analytical data to identify the various compounds.

Specific objectives of the project are to:

- Monitor known sources of "research chemicals" and emerging drugs of abuse
- Obtain samples of new compounds as they become available
- Verify the chemical structure of new compounds
- Obtain various types of analytical data (instrumental and presumptive color tests)
- Publish the analytical data and specific methodologies utilized to obtain the data for forensic drug chemists
- Categorize the compounds into chemical classes to assist legislative initiatives

ACCOMPLISHMENTS AND ONGOING WORK

This is a new project that has not started work yet.

TECHNOLOGY BENEFITS

By creating a mechanism to obtain and characterize emerging designer drugs of abuse, the reaction time for analytical chemists is dramatically decreased.

Also, by providing analytical data on emerging drugs of abuse, forensic scientists can identify and confirm sample drugs as they appear in casework.

COLLABORATION

The project is a collaboration between the Johnson County Sheriff's Office Criminalistics Laboratory, Mission, KS; the Washington State Patrol Crime Laboratory (WSP-CL) in Spokane, WA; Gonzaga University (GU); and the Montgomery County Coroner's Office Laboratory (MCCOL) in Dayton, OH. The WSP-CL will cooperate with GU to perform the Nuclear Magnetic Resonance (NMR) analysis. The MCCOL will perform the LC-MS/MS analyses.

DISSEMINATION

Research findings and results will be presented at a number of regional forensic meetings, including the Midwestern Association of Forensic Scientists (MAFS), the Southern Association of Forensic Scientists (SAFS), and the Northwestern Association of Forensic Scientists (NWAFS). Analytical data for compounds will be published in the *Journal of the Clandestine Laboratory Investigating Chemists Association*. Upon completion of the project, a final technical report on the project will be posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

This is a new project with no publications or presentations to date.

IMPLEMENTATION

A mass spectral library containing spectra of all investigated compounds will be created. The results of this research will also be added to the database on the Forendex website which is offered through SAFS. Forensic drug chemists will be aware of updates through regular emails to the CLIC-List drug chemistry email list.

CONTACTS

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Comparison of Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Mass Spectrometry for Discrimination of *Salvia divinorum* from Related *Salvia* Species Using Chemometric Procedures

FORENSIC TECHNOLOGY NEED

Salvia divinorum is a hallucinogenic herb which is currently regulated in 24 states. With legislation pending in several other states, a need occurs to identify *S. divinorum* from other *Salvia* species. Currently there are about 1000 different species of *Salvia*, some of which are culinary herbs and ornamental shrubs. This project aims to develop a method to differentiate *S. divinorum* from other species of *Salvia* using chromatographic techniques and chemometric procedures.

Figure 1. *Salvia divinorum* (aka Maria Pastora).

TECHNOLOGY DESCRIPTION

Previous research investigated the selectivity of different organic solvents for extraction of salvinorin A (the active hallucinogenic component) from dried leaves of *S. divinorum* and *S. officinalis*. Methyl chloride appeared the most promising, extracting the greatest number of components and the highest abundance of salvinorum A.

METHODOLOGY

The objective of this project is to build upon the findings in previous work and to develop analytical methods for the definitive identification of *Salvia divinorum* with differentiation from other *Salvia* species.

This is accomplished by:

- Investigating and comparing different extraction procedures for components of interest
- Investigating the selectivity offered by Gas Chromatography-Mass Spectrometry (GC-MS) in creating a chemical fingerprint of *S. divinorum*
- Investigating the selectivity offered by Liquid Chromatography-Mass Spectrometry (LC-MS/MS) in creating a chemical fingerprint of *S. divinorum*
- Investigating the potential of discriminating *S. divinorum* from other *Salvia* species based on the chemical fingerprints using chemometric procedures.

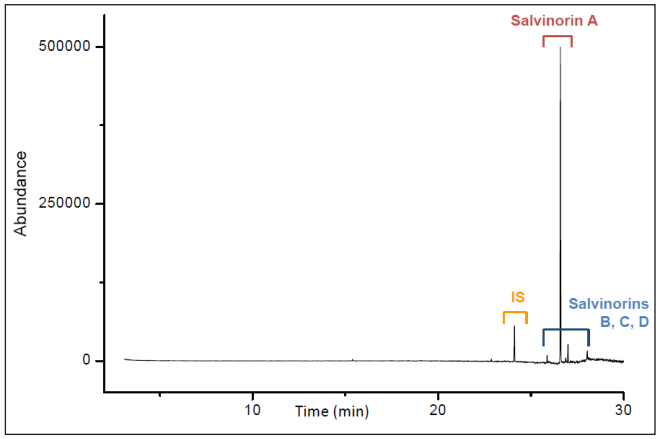

Figure 2. *Salvia divinorum* Total Ion Chromatogram (TIC), showing the abundance of salvinorum A.

ACCOMPLISHMENTS AND ONGOING WORK

Findings of previous research were used to optimize procedures to extract salvinorum A from *S. divinorum*. For manual agitation, the optimal extraction procedure consisted of a 5 minute extraction of the dried plant leaves in dichloromethane. While short extraction time is ideal for that purpose, longer extraction times and different extraction procedures were needed to generate a chemical fingerprint of *S. divinorum*.

Based on the number and abundance of compounds extracted, as well as the precision of the extraction, the rotary agitation procedure was deemed to generate the most informative chemical fingerprint for *S. divinorum*.

Using a 16 hour rotary agitation procedure with dichloromethane, four other *Salvia* species were extracted in addition to *S. divinorum*. They were: *Salvia officinalis, Salvia nemorosa, Salvia guaranitica,* and *Salvia splendens*.

Salvia officinalis

Salvia splendens

Salvia nemerosa

Salvia guaranitica

Figure 3. Four other *Salvia* species used in the analysis

Three extractions were performed for each species, using three different samples of leaf material. Each extract was then analyzed in triplicate. Before the analyses were conducted, the data collected was pretreated to remove non-chemical sources. Pre-treatment consisted of background subtraction, smoothing, retention time alignment, and normalization.

To differentiate *S. divinorum* from the other *Salvia* species, and using GC-MS and chemometric procedures, different combinations of user defined parameters, segment size and warp, were investigated. Pearson Product Moment Correlation (PPMC) coefficients were calculated for all pair-wise combinations of each species to determine the optimal warp and segment size for the alignment. To overcome the subjectivity in interpreting scores plots, student's t-test were applied to determine if there were statistically significant differences in the mean score for each species.

For volatile compounds, it was found that strong correlation occurred among extracts within each species. Also, that PPMC coefficients were highest using a segment size of 120 with a warp of 6, indicating inproved alignment among replicates of each species.

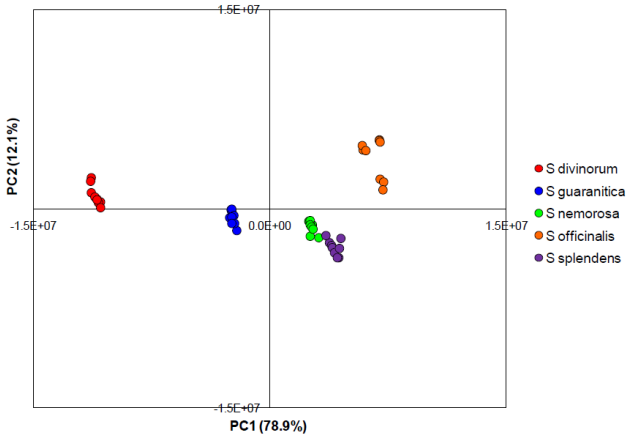

Figure 4. Scores plot showing PC1 vs. PC2 for five *Salvia* species, based on chemical fingerprints obtained by GC-MS.

The retention time aligned and normalized data was then subjected to Principal Component Analysis (PCA) and scores and loadings plots

were generated. It was found that 91% of the variance in the data set can be explained by the first two principal components, with some degree of spread observed among replicates of each of the five species. *S. officinalis* was positioned most positively on PC1, *S. divinorum* was positioned most negatively on PC1, and the remaining species were closer to zero.

For non-volatile compounds, the optimal alignment was agian based on PPMC coefficients calculated between pair-wise comparisons of each species. Since no general trend was apparent, the optimal alignment parameters were chosen as those that offered best alignment of the majority of the *Salvia* species. Hence, a warp of 6 and segment size of 120 were deemed the optimal alignment parameters of those investigated.

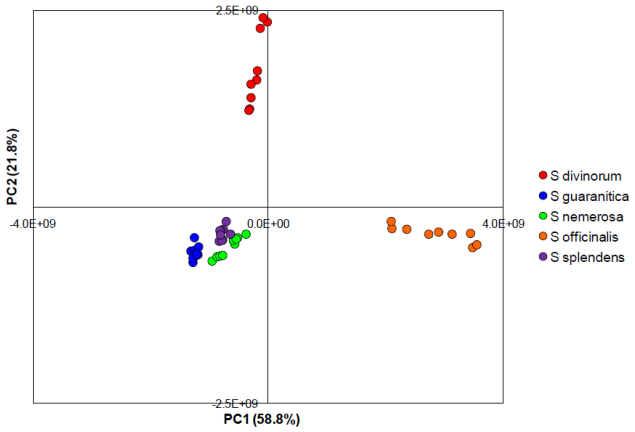

Figure 5. Scores plot showing PC1 vs. PC2 for five *Salvia* species, based on chemical fingerprints obtained by LC-MS.

Using PCA, it was found that 67.5% of the total variance in the data set can be explained by the first two principal components. Despite some spread among the replicates of the five species, three main groupings of *Salvia* species can be observed. *S. officinalis* is the only species positioned positively on PC1, with *S. divinorum* positioned positively on PC2. The remaining three species are all positioned slightly negatively on PC1 and PC2 and are more difficult to distinguish based only on visual assessment of the scores plot.

It was found that for each comparison, using both volatile and non-volatile compounds present, calculated t-statistic (at the 95% confidence level) was greater than the critical t-value. Hence, the null hypothesis was rejected. That is, based on chemical fingerprints of volatile and non-volatile compounds, *S. divinorum* can be discriminated from the other four *Salvia* species.

The data set turned out to be too small to test a suitable statistical model to classify samples of species. With such a small data set, the developed (SIMCA) model would not be truly representative and be prone to misclassifications.

TECHNOLOGY BENEFITS

The project establishes proof-of-concept and can help in the development of optimized and validated procedures for the analysis and identification of *Salvia divinorum.* The method can be implemented in states that have already regulated the plant and be made available to forensic laboratories should *S. divinorum* or its active ingredient, salvinorin A, become federally controlled.

COLLABORATION

The project is a collaborative effort between Michigan State University (MSU), East Lansing, MI, and the Michigan State Police (MSP), Bridgeport, MI. The MSP provided counsel for analysis.

DISSEMINATION

Aspects of this research were presented at a number of forensic science conferences and at the MFRC Annual Meeting. A manuscript has been submitted for publication in *Analytical and Bioanalytical Chemistry*, and a manuscript is also being prepared for submission to the *Journal of Forensic Sciences*. A final technical report has been posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

- Bodnar, W., McGuffin, V., Waddell-Smith, R. "Forensic Analysis of *Salvia divinorum* and Related *Salvia* Species Using Chemometric Procedures" Oral presentation at the 62nd Annual Meeting of the American Academy of Forensic Sciences, Seattle, WA. February 2010.

- McGuffin, V., Waddell-Smith, R., Bodnar, W., Dugeja, M. "Gas Chromatography Mass Spectrometry for Discrimination of *Salvia divinorum* from related *Salvia* species" Presentation at the MFRC Annual Meeting, Mackinac Island, MI. May 2010.

- Bugeja, M.L., Bodnar, W., McGuffin, V., and Waddell-Smith, R. "Comparison of Methods for the Extraction of Volatile Compounds from *Salvia divinorum*" Poster presentation at the 39th Annual Meeting of the Midwestern Association of Forensic Scientists, Kansas City, MO. October 2010.

- Bugeja, M., Bodnar W., McGuffin, V., Waddell-Smith, R. "Development of a Chemical Fingerprint for *Salvia divinorum* using Liquid Chromatography-Mass Spectrometry for Association and Discrimination from Related *Salvia* Species" Poster presentation at the 63rd Annual Meeting of the American Academy of Forensic Sciences, Chicago, IL. February 2011.

- Bugeja, M., Bodnar, W., McGuffin, V., Waddell-Smith, R. "Discrimination of *Salvia divinorum* from Related *Salvia* Species Using Chromatographic Techniques and Chemometric Procedures" Oral presentation at the 42nd Central Regional meeting of the American Chemical Society, Indianapolis, IN. June 2011.

IMPLEMENTATION

As the method develops, graduate students from the MSU Forensic Science Program will help train MSP drug analysts in implementing the method in MSP laboratories.

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Fast Gas Chromatography Capabilities in Drug Identification

FORENSIC TECHNOLOGY NEED

Gas Chromatography (GC), coupled with Mass Spectrometry (MS), is the primary method used at crime laboratories for the identification of drugs. While the technique has sufficient sensitivity and specificity, it can be slow thus limiting the number of samples that can be analyzed. Recent studies indicate that Fast Gas Chromatography (Fast GC) provides a more rapid analysis than conventional GC especially when combined with hydrogen as a carrier gas. This project examines the capabilities of Fast GC-Hydrogen in drug identification.

TECHNOLOGY DESCRIPTION

Fast GC is a separation technique that couples the stable, rapid heating cycles of GC with narrower capillary columns and high phase ratio. The combination creates more theoretical plates per meter, thereby enabling fast separations with potentially superior resolution and budget savings if hydrogen is used in the mobile phase.

METHODOLOGY

The research builds upon the findings of a previously funded project that examined the capabilities of Fast GC in drug analysis. The goal of this project is to assess the Fast GC-Hydrogen combination in the identification of drugs.

Specifically, the project assesses:

- The expected gain in resolution and sample throughput for a drug identification unit using a combination of Fast GC and hydrogen
- The effect of hydrogen use in GC on mass spectrometry data
- The feasibility of implementing and using the Fast GC-Hydrogen combination at the crime laboratory in light of hydrogen safety with both Mass Spectrometry and Flame Ionization Detection applications.

ACCOMPLISHMENTS AND ONGOING WORK

The investigation initially centered on the concern that pushing an analyte through the MS too quickly may yield a potential loss of signal relative to noise, resulting in a decrease in sensitivity. Discussions with instrument representatives and calculations demonstrated that the current (post 5972) MS units will not be a limiting factor in the process.

The investigation then focused on assessing the limits of Fast GC in drug identification using standard reference samples of 16 illicit drugs. It was found that the use of Fast GC does produce shorter retention times in the analysis of all compounds tested. The use of hydrogen as a carrier gas also improved resolution. The combination of Fast GC and hydrogen produced a shorter retention time and increased resolution in a mixed sample.

	Cocaine – 10ºC ramp	Cocaine – 20ºC ramp	Cocaine – 35ºC ramp
Retention Time and Peak Width (Conventional GC)	24.1 min; 0.038 min	N/A	N/A
Retention Time and Peak Width (Fast GC)	22.2 min; 0.032 min	13.6 min; 0.021 min	9.7 min; 0.020 min

Table 1. Comparison of oven heating ramps on cocaine chromatography.

It was also found that, using cocaine, the peak widths decrease with oven temperature ramp increases (0.032 min @ 10 degrees Celsius). This decrease is in sufficient proportion to retention time decrease and permits relatively high oven ramp temperatures to be used in Fast GC without compromising the resolution.

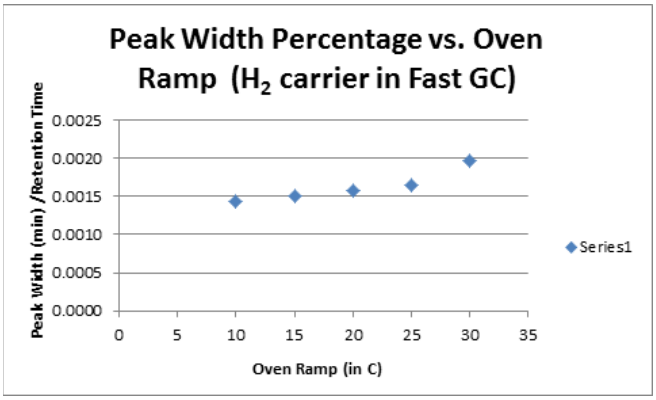

Figure 1. Analyte peak width/retention time as a function of GC oven ramp.

In assessing the effect of hydrogen use in GC on mass spectrometry data, it was found that the physical properties of hydrogen produced optimal separations at flow rates much higher than helium. This enabled the analysis of Alprazolam, one of the longest retained species, to be shortened from 27 minutes using a rate of 10 centimeters per minute and a symmetry of 0.889 to 11 minutes at a rate of 30 centimeters per minute and a symmetry of 0.963 in a mixed standard.

Given the positive Fast GC results and findings, work to convert a conventional GC to Fast GC is currently underway at the Wisconsin State Crime Lab in Milwaukee. When installed, the feasibility of implementing the Fast GC-Hydrogen combination will be investigated and the use of the combination will be assessed in light of hydrogen safety with both mass spectrometry and flame ionization detection applications.

TECHNOLOGY BENEFITS

Fast GC-Hydrogen is a relatively easy, simple to implement approach that yields great benefits in terms of improving the detection of suspected drugs through better resolution and increasing the instrumental capacity of drug chemistry units. The use of hydrogen as a carrier gas further results in sufficient cost savings over the use of helium mobile phase.

Fast Gas Chromatography Method		Columns	
		1) Conventional GC	
Injection Volume	1.0 µL	-Phase	DB-5
		-Length	30 m
Injection Inlet Temp	250 °C	-Film thickness	0.25 µm
FID Detector / MS Temp	300 °C		
		2) Fast GC	
Injection Mode	Splitless	-Phase	DB-5
		-Length	20 m
Column Inlet Pressure	7.34 psi	-Film thickness	0.18 µm
Hydrogen Linear Flow	3.2 mL/min		
		Heating Ramps	
Linear Velocity	57 cm/sec	Conventional	5 – 15 °C
		Fast GC	10 – 35 °C
Total Flow	169 mL/min		

Table 2. Instrumental parameters and methods for Fast GC.

COLLABORATION

The project is a collaborative effort between the University of Wisconsin-Platteville and the Wisconsin State Crime Laboratory at Milwaukee (WSCL-Milwaukee). The WSCL-Milwaukee serves as co-principal investigator in the research project and provides student intern oversight along with controlled substance analysis and research results review.

DISSEMINATION

Research results and findings were presented at annual meetings of the Midwestern Association of Forensic Scientists (MAFS) and the American Academy of Forensic Sciences (AAFS). Additional dissemination includes manuscript submission of meritorious results to a peer-reviewed journal, (*Journal of Forensic Sciences)*. Upon completion of the project, a technical report on the project and its findings will be posted on the MFRC website. Dissemination of research findings and results through the online Microgram posting of the U.S. Department of Justice Drug Enforcement Administration is also investigated. The Microgram assists and serves forensic scientists concerned with the detection and analysis of suspected controlled and other abused substances for forensic/law enforcement purposes.

PUBLICATIONS AND PRESENTATIONS

- Cornett, C., Hansen, R., Macans, L. "Evaluation of Fast Gas Chromatography Coupled with Hydrogen Mobile Phases in Drug Identification" Oral presentation at the Midwestern Association of Forensic Scientists, Chicago, IL. September 2011.

- Hansen, R., Cornett, C., Macans, L. "Evaluation of Fast Gas Chromatography Coupled with Hydrogen Mobile Phases in Drug Identification" Poster presentation at the Midwestern Association of Forensic Scientists, Chicago, IL. September 2011.

- Cornett, C., Hansen, R., Macans, L. "Evaluation of Fast Gas Chromatography Coupled with Hydrogen Mobile Phases in Drug Identification" Oral presentation at the American Academy of Forensic Scientists, Atlanta, GA. February 2012.

- Cornett, C., Hansen, R., Halligan, A., Macans, L., Wermeling, J. "Evaluation of Fast Gas Chromatography Coupled with Hydrogen Mobile Phases in Forensic Sciences" Poster presentation at the American Academy of Forensic Sciences, Atlanta, GA. February 2012.

IMPLEMENTATION

If the results of the research warrant further investigation, the WSCL-Milwaukee plans to conduct an in-house examination of Fast GC-Hydrogen prior to making a switch. Switching from a conventional GC to a Fast GC is a relatively easy process. It involves upgrading the power source to either 220 V or 240 V (preferred) service, and installing a high-heat oven shroud, power cord, and narrower capillary column. The cost of switching is about $4,000.

Through a series of asynchronous "webinars", the UWP will provide training on the use of Fast GC-Hydrogen. Videos will be made available through the UWP Media Technology Services Department for analysts to watch at their convenience. The PI will offer the opportunity for crime laboratory analysts to travel to UWP to have their samples analyzed using the Fast GC-Hydrogen method. Questions on the use of the technology and consultation on implementation of the approach will also be made available through UWP.

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Random Probability Match Procedure for Statistical Comparison of Mass Spectral Data

FORENSIC TECHNOLOGY NEED

The National Academy of Sciences (NAS) in its recent report "*Strengthening Forensic Science in the United States: A Path Forward,*" draws attention to several limitations in the current state of forensic science. Among these is the need to statistically access the significance of associations in the comparison of evidence. To address this concern, this project targets the development of a procedure for the statistical comparison of mass spectral data obtained using gas chromatography-mass spectrometry (GC-MS).

TECHNOLOGY DESCRIPTION

The procedure is based on classic probability theory: determining the random probability that a match between the mass spectrum of a questioned sample and that of a reference standard occurs by chance. As such, the procedure is similar to the one currently used to evaluate DNA profile comparisons. Yet, method development in this project will use mass spectra of controlled substances.

METHODOLOGY

The objective of this project is to develop a probability-based method for the comparison of mass spectral data. The method will be developed using a data set of normal n-alkanes analyzed in replicate over several days. N-alkanes are selected because they have simple mass spectra with similar fragmentation patterns and are ideal compounds for method development purposes. To demonstrate practical application in forensic laboratories, the procedure will be applied to complex data sets containing mass spectral data of controlled substances.

Specific tasks to achieve the objectives are to:

- Define an optimal threshold to remove spectral noise present in low abundance
- Develop the random probability match method
- Investigate the amplication of method to complex GC-MS data sets from *Salvia* species and amphetamine-type stimulants
- Investigate improvement and refinement to the random probability match procedure

ACCOMPLISHMENTS AND ONGOING WORK

This is a new project that has not started work yet.

TECHNOLOGY BENEFITS

This research will impact the forensic community by developing a probability-based approach for the comparison of mass spectral data. While method development will use mass spectra of controlled substances, the procedure can also be applied to mass spectral data of other forensic evidence.

COLLABORATION

The project is a collaborative effort between Michigan State University (MSU), East Lansing, MI, the Alaska Scientific Crime Detection Laboratory (SCDL), Anchorage, AK, and the Northeastern Illinois Regional Crime Laboratory (NIRCL), Vernon Hills, IL. The SCDL and NIRCL provide access to data sets of regulated substances necessary for optimization, validation, and refinement of the procedure.

DISSEMINATION

The results of this project will be presented at annual meetings of the Midwestern Association of Forensic Scientists (MAFS) and the American Academy of Forensic Sciences (AAFS). If warranted, a manuscript will be submitted for publication in the *Journal of Forensic Sciences*. A presentation may also be made to the scientific working group for the analysis of seized drugs. Upon completion of the project, a technical report on the project and its findings will be posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

This is a new research project with no publications or presentations to date.

IMPLEMENTATION

An on-line seminar will be developed to demonstrate the probability-based approach for the comparison of mass spectral data. The webinar will be accessible through MSU's on-line learning center. Excel spreadsheets containing templates for the developed method will be distributed at the AAFS and MAFS meetings. Interested analysts will then have the opportunity to evaluate the current version of the method and, based on comments and suggestions from this community, the method will be enhanced appropriately.

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Rapid Arson Sample Analysis Using DART Mass Spectrometry

FORENSIC TECHNOLOGY NEED

Gas Chromatography-Mass Spectrometry (GC/MS) is the primary analytical method used in arson investigations. The technology, however, is not ideal requiring substantial time for sample analysis and data interpretation. This limits the number of samples that can be tested leading to an increase in the backlog of cases. Consequently, there is a need for a technology which maintains sensitivity and specificity, yet significantly reduces sample time analysis and data analysis complexity.

TECHNOLOGY DESCRIPTION

The goal of this project is to develop Direct Analysis in Real Time (DART) Mass Spectrometry (MS) as a faster, more efficient alternative to GC/MS. DART is a new ionization method for the rapid detection of analytes. By using mass spectrometry, the ionization source enables near instantaneous determination of sample composition without sample preparation and extraction steps. The technology is versatile and potentially can be applied to a wide range of chemicals.

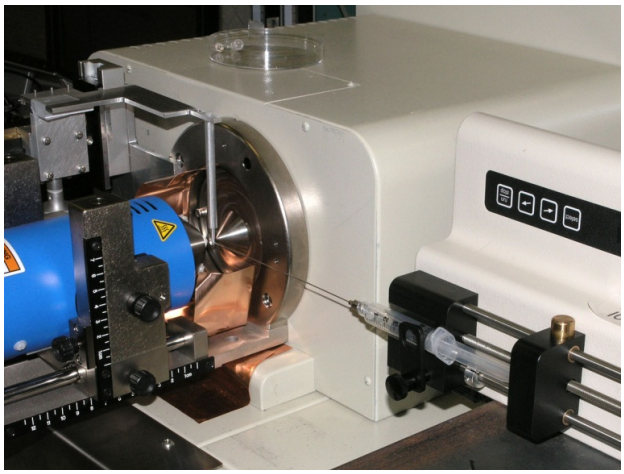

Figure 1. Headspace vapors injected into the DART-MS sample gap for analysis. The blue cylinder on the left is the DART ion source. The sample syringe and syringe pump are on the right.

METHODOLOGY

In previous work, the ability of DART-MS to detect accelerants in arson investigations was established. In this project, the accuracy, reliability, and validity of DART-MS for a wide range of accelerants and substrates are demonstrated. To achieve these objectives, the project:

- Collects DART-MS spectra of vapors from the major classes of ignitable liquids to build a small, proof-of-principle library
- Collects DART-MS spectra of common fire-debris matrices after being burned with and without accelerants. Spectra will be added to the library
- Uses the library of data generated to demonstrate the ability of DART-MS to identify accelerants on burned materials

ACCOMPLISHMENTS AND ONGOING WORK

A data library was created for 11 different accelerants and 2 substrates. The accelerants included: charcoal starter, Coleman camp fuel, diesel fuel, gasoline, 2-stroke engine gasoline, kerosene, mineral spirits, paint thinner, turpenoid, turpentine, and WD-40. Substrates tested were: Shaw Comfortouch carpet (88% polypropylene, 12% nylon), and Mohawk #P81A padding.

For each burn test, one-inch diameter circles of carpet and padding were cut out and stacked in an aluminum foil cup. Five milliliters of accelerant was poured slowly onto the substrate so that the accelerant was completely absorbed by the sample. The sample was ignited and the burn time was recorded. At the end of the burn, the fire was extinguished by placing a glass jar over the foil cup. The burned sample was sealed in a 2 oz. glass jar with rubber in the lid for extracting vapor samples.

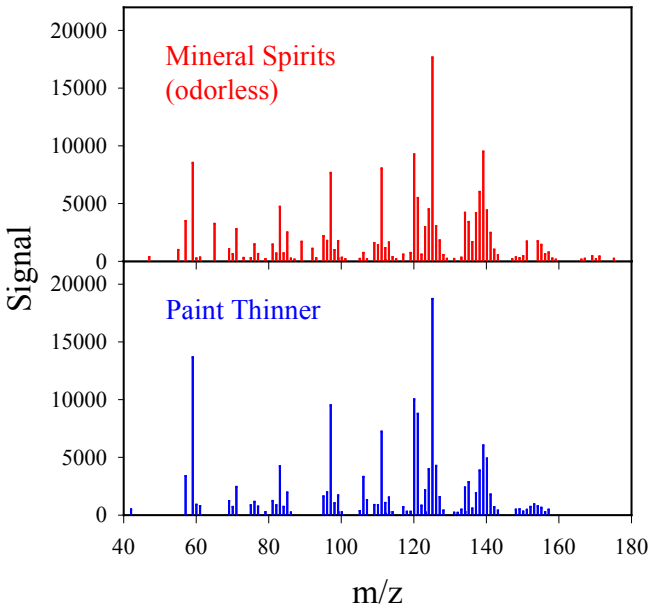

Figure 2. Similar vapor spectra for similar accelerants sold under different names.

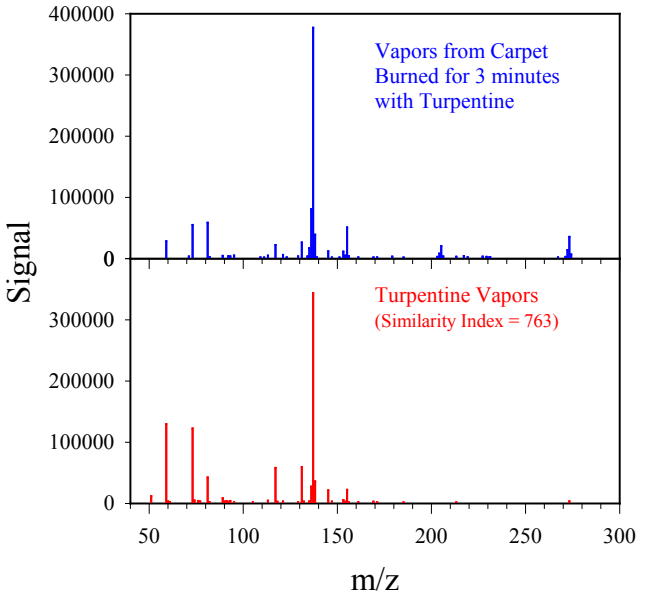

Figure 3. Headspace vapors of carpet burned with turpentine.

Experiments with accelerants showed that the mass spectra of accelerant vapors generally differ substantially from one another. Yet, a number of materials sold under different names had similar or even identical compositions thereby producing very similar spectra.

It was also found that spectra of headspace vapors for burned carpet and padding samples were generally similar to the vapor spectra of the accelerant used in the burn. Because the burned sample spectra resemble those of the accelerant vapors, the search of a burned sample spectrum against the library of accelerant-vapor spectra generally matched with the correct accelerant. In only one case did the library search of the headspace vapors from a burned sample produce an incorrect result.

To date, only burned samples have been examined that were sealed in containers shortly after the flames were extinguished. In the future, to determine the effects of aging, containers of some of the already examined samples will be opened to allow evaporation and aging to occur and then re-analyze the samples. In addition, since aged samples and samples allowed to burn themselves out produce substantially less vapor, the use of collection media (i.e. activated carbon and solid phase microextraction fibers) will be examined to enhance the analysis.

TECHNOLOGY BENEFITS

GC/MS is commonly used to identify accelerants in fire debris. It is, however, not ideal because GC is slow and generates a very large data set, thus demanding substantial analyst time. Dropping the GC step while maintaining specificity and sensitivity will greatly speed up the analysis and substantially reduce data-analysis complexity.

COLLABORATION

This project is a collaborative effort between the U.S. Department of Energy's Ames Laboratory, Ames, IA, the Minnesota Bureau of Criminal Apprehension (BCA), St. Paul, MN, and the Iowa Department of Public Safety State Fire Department (DPS-SFD), Des Moines, IA. The Minnesota BCA and the Iowa DPS-SFD will provide advice on the proper methods for production and handling of test samples and on the data required for successful

investigations. The Iowa DPS-SFD will also provide samples for analysis from test burns at the Iowa Fire Service Training Bureau.

DISSEMINATION

Research findings and results were presented at the annual meeting of the MFRC and at the Fall meeting of the Midwestern Association of Forensic Scientists (MAFS). Depending on the outcome of the project, a manuscript will be submitted for publication in the *Journal of Forensic Sciences*. Upon completion of the project, a technical report on the project and its findings will be posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

- Jones, R., McClelland, J., Reinot, T. "Rapid Arson Sample Analysis Using DART Mass Spectrometry" Presentation at the annual meeting of the Midwest Forensics Resource Center, St. Louis, MO. May 2011.

- Jones, R., McClelland, J., Reinot, T. "Rapid Arson Sample Analysis Using DART Mass Spectrometry." Presentation at the Midwestern Association of Forensic Scientists, Chicago, IL. September 2011.

IMPLEMENTATION

The project establishes proof-of-concept for rapid arson sample analysis using DART mass spectrometry. Depending on the findings in this project, the PI may apply for federal funding to continue the work. At the completion of this project, the investigator plans to host a workshop on DART-MS applications in forensic arson analysis.

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Degradation in Chromosomal DNA Assessed Using PCR Amplification and Capillary Electrophoresis

FORENSIC TECHNOLOGY NEED

DNA profiling methods are widely used in the forensic community. For highly degraded samples the number of methods is limited and oftentimes differ in discriminating power and tolerance level. A cost effective method is needed for the efficient identification of usable samples for DNA profiling. If an assay can be developed for both nDNA and mtDNA that provides information on the quantity and quality of DNA recovered, a complete picture of the testing plan can emerge.

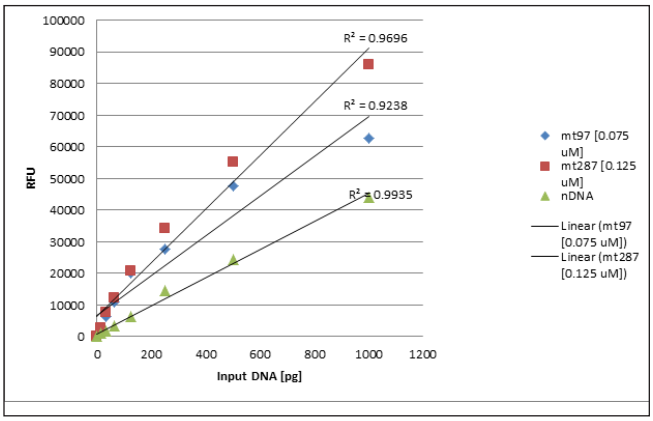

Figure 1. Standard curves produced from known amounts of input male DNA. The RFU values shown represent total area under the different amplicon peaks in the electropherograms which were averaged (total of 10 curves included). Fluorescence in mt97 and mt287 are shown as individual products whereas the line labeled nDNA consists of the sum of fluorescence in Amel-X + Amel-y.

TECHNOLOGY DESCRIPTION

Previous work has resulted in a multiplex PCR (polymerase chain reaction) assay Q-TAT (Quantitative Template Amplification Technology) that provides quantitative and qualitative information about DNA samples. Characteristics of the Q-TAT assay are: mtDNA is expressed in cell equivalents based on DNA extracted from white cells in blood; male DNA can be quantified using the SRY amplicon, and the dynamic range for nDNA is from ~20-1000 pg. This project builds upon previous work and investigates the use of Q-TAT assay to provide information about DNA integrity.

METHODOLOGY

The overall objective of this project is to establish parameters enabling Q-TAT assay to reliably identify DNA samples that are sufficiently degraded to require specialized testing methods to produce a DNA profile.

Specific goals of the project are to:

- Identify suitable conditions for the random degradation of both nDNA and mtDNA in a controlled fashion using chemical, physical, and/or enzymatic means in an attempt to mimic DNA degraded by natural processes

- Identify a "threshold ratio" of fluorescence in low molecular weight Q-TAT amplicons (i.e. HPRT and SRY) versus high molecular weight products (i.e. Amel-X and Amel-Y) that is diagnostic for an analyst in assessing the extent of degradation of nuclear DNA recovered from a sample

- Apply the analysis criteria developed for the Q-TAT assay with idealized DNA samples produced in the laboratory to real world forensic samples provided as non-probative DNA samples from the Tulsa Police Lab

ACCOMPLISHMENTS AND ONGOING WORK

Five micrograms of genomic DNA, extracted from a freshly procured blood sample, were suspended in 25 µl of ultrapure water and subjected to heating to 95°C in a thermal cycler. Samples were removed from the thermal cycler at 10 minute intervals (to

90 minutes total) and quickly chilled by adding 175 µl of ice cold TE-4. The sample was then placed on ice. After all samples had been chilled, 1 µl of each was diluted further five-fold with TE-4, with 1 µl amplified in the Q-TAT assay.

Using an agarose gel to determine the ratio fluorescence in small versus large amplicons, it was found that DNA degraded by heat exposure and that the heat exposure leads to a predominance of fragments in the 300 base pair size range. Using the ABI Identifier multiplex kit, amplification of STR loci confirmed that in heated samples, higher molecular weight loci disappeared from STR profiles faster than low molecular weight loci.

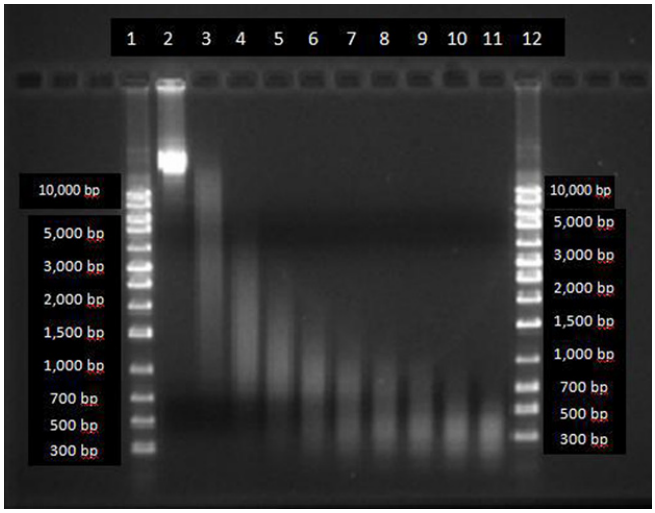

Figure 2. Detection of template degradation (each lane represents 10 minutes of increased exposure (left to right) of DNA to 95°C. Lanes 1 and 12 are size standards, lane 2 is intact genomic DNA.)

It was also found that the templates for the small amplicons produced in the Q-TAT assay are not appreciably degraded during 80 minutes of heating at 95°C. Only after 90 minutes does the fluorescence in the small Q-TAT amplicons begin to fall. This finding is important because it indicates that even in seriously degraded DNA, the DNA targets for available mini-STR typing kits may provide probative results.

Current studies focus on performing detailed statistical analyses on LMW/HMW ratios of fluorescence in genomic DNA samples heated to varying degrees. The goal of these studies is to determine the limits of variability for the different LMW/HMW ratios in Q-TAT amplicons produced from genomic DNA templates exhibiting different degrees of degradation as determined by STR typing with Identifier and Powerdex 16 multiplex kits.

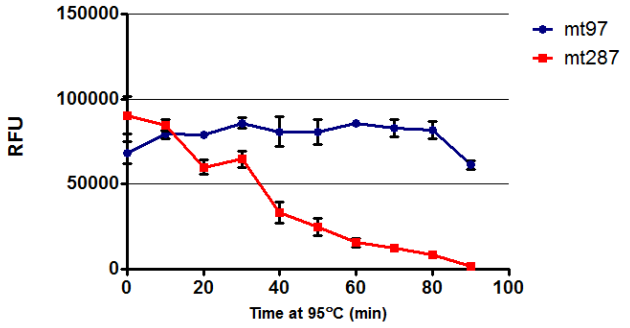

Figure 3. Aliquots of heated genomic DNA were used as templates for the amplification of the mt97 and mt287 amplicons. Fluorescence incorporated into these products is plotted as a function of heating time at 95°C.

TECHNOLOGY BENEFITS

The benefit of the Q-TAT assay to the forensic community is two fold. First, the method provides a rather complete picture on the quantity and quality of DNA recovered from evidence. Second, a cost effective method for efficient identification of samples for DNA profiling enhances the efficiency of casework processing and help reduce the number of backlogged cases.

COLLABORATION

The project is a collaborative effort between the DNA laboratory of the Oklahoma State University's (OSU) Department of Forensic Sciences, Tusla, OK and the Tulsa Police Department (TPD), Tulsa, OK. TPD provides adjudicated samples for re-testing with Q-TAT as well as DNA data from the samples for re-analysis of Q-TAT amplicon rfu ratios. Additionally, TPD provides validation and casework experience as the TPD laboratory routinely utilizes the assay for DNA quantification.

DISSEMINATION

Project results were presented at annual meetings of the MFRC, the Midwestern Association of Forensic Scientists (MAFS), and the American Academy of Forensic Sciences (AAFS). A manuscript is currently being prepared for submission to the *Journal of Forensic Sciences*.

Upon completion of the project, a technical report on the project and its results will be posted on the MFRC website. The possibility of posting research findings and results using OSU distance learning capabilities is also explored.

PUBLICATIONS AND PRESENTATIONS

- Allen, R. "Assessment of the Quantity and Quality of DNA in Evidentiary Samples Using the Q-TAT Multiplex Assay" Presentation at the MFRC Annual Meeting, St. Louis, MO. May 2011.

IMPLEMENTATION

The Q-TAT assay is routinely used by the Tulsa Police Laboratory resulting in savings of $50,000-$100,000 per year on reagents, service contracts, etc. The assay is also being used to quantify DNA in new labs around the world. To support the needs of these new labs, Maven Analytical, Inc., purchased the licensing rights for the assay from OSU and makes kits available commercially.

To demonstrate the assay, the partners will host two training sessions at the Forensic Sciences and Biomedical Research Facility on the OSU campus in Tulsa, OK. Interested and/or potential users will be given an opportunity for hands-on experience using the Q-TAT method. Training sessions will be offered free of charge.

CONTACTS

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Application of Face Recognition Technology to Microstamped Cartridge Cases

FORENSIC TECHNOLOGY NEED

The National Research Council (NRC), in its 2008 "Ballistic Imaging Report", states that the fundamental assumption underlying forensic firearm identification (i.e. that every gun leaves unique microscopic marks on bullets and cartridge cases) has not been fully demonstrated. In an effort to reduce the subjectivity of tool mark examination, the NRC recommends that "microstamping" be researched to relate guns to fired ammunition, and to track the sources of illegally trafficked firearms.

TECHNOLOGY DESCRIPTION

Microstamping is a technique used to etch individual serial numbers onto critical parts of a firearm. When the weapon is fired, the etchings are transferred to the cartridge cases to be related to the fired weapon.

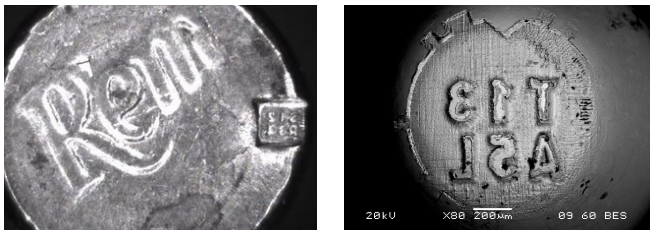

Figure 1. Identifiers placed on firing pins and breech faces.

METHODOLOGY

The purpose of this project is to examine the transfer of identifiers as a function of firearm action and ammunition. The primary objectives of this project are to:

- Determine the durability of microstamped identifiers on three handguns covering a range of manufactured quality (low quality, medium, high) using optical/computer means
- Determine the ability of advanced imaging and recognition software to discern microstamped identifiers as a potential solution to problems associated with this technology
- Determine whether a microstamped identifier can be recovered using metallurgical etching techniques in the same manner serial numbers can be restored after they have been removed

ACCOMPLISHMENTS AND ONGOING WORK

Ten different types of ammunition were selected for study. Ammunition was chosen based on primer material (nickel, brass), type, presence of sealant and pre-stamped letters. Cartridges were scribed before firing.

Firing Order	Ammunition Brand	Primer Type	Cartridge Material	Description
1	Brown Bear	Berdan	Lacquered Steel	115 gr., full metal jacket, brass primer
2	DAG	Boxer	Brass	224 gr., full metal jacket, brass primer
3	Federal - American Eagle	Boxer	Brass	115 gr., full metal jacket, nickel primer
4	Remington - UMC	Boxer	Brass	115 gr., Flat Nose Enclosed Base, nickel primer, letters "H F" stamped into the primer
5	PMC	Boxer	Brass	115 gr., full metal jacket, brass primer
6	Silver Bear	Berdan	Zinc-plated steel	115 gr., full metal jacket, brass primer
7	CCI Blazer	Boxer	Aluminum	115 gr., full metal jacket, nickel primer
8	Cor-Bon	Boxer	Brass	147 gr., full metal jacket, nickel primer
9	Independence	Boxer	Brass	115 gr., full metal jacket, nickel primer
10	Sellier & Bellot	Boxer	Brass	115 gr., full metal jacket, brass primer, covered with red lacquer sealant

Table 1. Ammunition used in the project to determine the transfer of identifiers.

One hundred rounds of each type of ammunition were fired from each of the three handguns selected (Hi-Point, Taurus, and Sig Sauer), for a total of 3000 cartridge cases. Handguns were chosen based on price point and ejection styles. Firearms were outfitted with a matrix of characters of different geometrics and resolutions. Test character sets

were cycled through the firearm to determine the resulting quality of the marks with characters and geometries tested for repeatability of transfer.

The transfer of identifiers was graded based on the numbering of distinguishable characters (C1 through C6, with C6 being best). Two evaluations were performed: one by an experienced examiner and one by a novice. All optical examinations were conducted with a stereo microscope using polarized light. A noise reduction software algorithm was developed to reduce the noise in the scanned data.

Strike Grade Summary						
Sig Sauer						
C6	**C5**	**C4**	**C3**	**C2**	**C1**	**C0**
948 *968*	30 *19*	14 *7*	5 *2*	1 *1*	0 *1*	2 *2*
Taurus						
C6	**C5**	**C4**	**C3**	**C2**	**C1**	**C0**
848 *854*	43 *35*	3 *5*	1 *3*	3 *2*	2 *1*	0 *0*
Hi-Point						
C6	**C5**	**C4**	**C3**	**C2**	**C1**	**C0**
663 *684*	139 *113*	47 *65*	26 *25*	15 *7*	5 *4*	4 *1*

Table 2. Results for the three semi-automatic handguns. Italicized numbers are for the novice evaluator.

It was found that the type of firearm used had the greatest effect on identifier transfer, with Sig Sauer having the greatest number of C6 transfers and Hi-Point the lowest. Primer hardness had little effect on the quality of transfer. Yet, the presence of primer lacquer interfered with the transfer.

It was also found that auto-identification of alpha-numeric identifiers and gearcode is promising but needs more work. For example, when the surface of the microstamped cartridge was scanned using a profilometer, and virtual lighting was applied to the photograph to reduce shiny areas, the virtually enhanced 2D textured image could be combined with the color-encoded enhanced 3D structures resulting in enhanced visualization of both gear code and characters. Yet, more work is needed especially since non-ideal transfers pose more difficulty than ideal transfers.

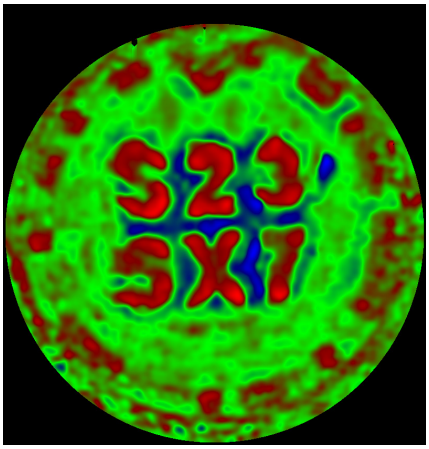

Figure 3. Color-coded enhanced 3D structures.

TECHNOLOGY BENEFITS

On January 1, 2010 California signed into law a bill requiring microstamping. Other states are also investigating use of the technology. The findings of this project may help states determine if microstamping is indeed a viable technology to link a firearm to a cartridge. The results may also aid in firearm investigations by providing a quantitative measure of the degree of match.

COLLABORATION

This project is a collaborative effort between the U.S. Department of Energy's Ames Laboratory, Ames, IA, Iowa State University, Ames, IA, the Illinois State Police (Jim Kreiser, ret.), and the Microstamping Technology Center, a division of Pivotal Development, LLC of Londondeery, NH.

Mr. Kreiser assisted in obtaining the required firearms and ammunition and in overseeing the test firings and cleaning of the weapons. The Microstamping Technology Center marked the firing pins of the study weapons using the latest methods under development at Pivotal Development, LLC.

DISSEMINATION

Project findings and results have been disseminated at the annual Association of Firearm and Tool Mark Examiners conference and at the annual MFRC meeting. A manuscript based on the research has also been submitted to the *Association of Firearm and Tool Mark Examiners (AFTE) Journal*. Upon completion of the project, a final report on the project and its findings will be posted on the MFRC's website.

PUBLICATIONS AND PRESENTATIONS

- Grieve, T., Chumbley, S., Eisenmann, D., Morris, M., Zhang, S., Kreiser, J., Lizette, T., Ohar, O. "Microstamped Identifiers: Evaluation and Analysis" Presentation at the MFRC Annual Meeting, St. Louis, MO. May 2011.

IMPLEMENTATION

Should the technology be mandated by legislatures, it is anticipated that wide-scale implementation will be less costly than the current National Integrated Ballistics Information Network (NIBIN) system.

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Discrimination of Dyed Cotton Fibers Based on UV-Visible Microspectrophotometry and Multivariate Statistical Analysis

FORENSIC TECHNOLOGY NEED

UV-visible microspectrophotometry (UV-MSP) is a popular technique used by trace evidence examiners for characterizing fiber color. While fast and generally considered to be highly discriminating, sample association with UV-MSP can be problematic due to sample heterogeneity and a lack of quantitative criteria for comparing spectra. To establish the validity of UV-MSP for fiber examinations, this project compares MSP data and evaluates the use of multivariate statistical analysis to discriminate dyed cotton fibers.

TECHNOLOGY DESCRIPTION

UV-Visible microspectrophotometers are instruments designed to measure the UV-visible spectra of microscopic samples. With specialized software, UV-MSP's can be used to measure thin film thickness, colorimetry and more. Preliminary results demonstrate that replicate spectra from fibers treated with different dyes form distinct clusters with spectra showing systematic differences. Statistical analysis further reveals that classes of dyes can be identified, leading to possible development of a dye classification scheme.

METHODOLOGY

The objectives of the project are to:

- acquire a collection of dyed cotton fibers
- acquire spectra from all samples using UV-MSP
- perform multivariate statistical analysis on the resultant spectra at two different laboratories

This approach is chosen to test the null hypothesis that the mean of a group of spectra from one laboratory is significantly different from the mean of a group of spectra from another laboratory. An additional test of consistency among data sets is employed by comparing the classification accuracy upon merging the two data sets to the classification performance of the separate tests.

Two popular data treatments are evaluated for the collection of spectra. The first is a calculation of first derivatives and the second is a transformation of the spectra into chromaticity coordinates. In each case, the multivariate calculations are repeated on the modified spectra and any differences in classification are noted to determine the extent to which spectra can be differentiated and shared between laboratories.

ACCOMPLISHMENTS AND ONGOING WORK

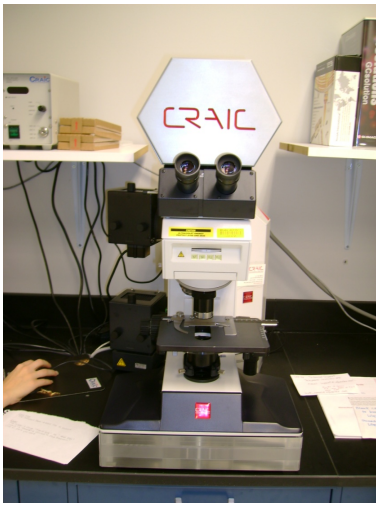

Figure 1. CRAIC QDI 2000 microspectrophotometer.

A set of twelve exemplars of various red dyed cotton fibers was acquired from a commercial

source (Testfabrics, Inc.) and from Stephen Morgan (University of South Carolina). Following standard MSP protocols, ten fibers were removed from each exemplar. The fibers were mounted on a glass slide with a glass coverslip with glycerin used as the mounting medium, and analyzed using a CRAIC QDI microspectrophotometer (magnification 35x).

Fiber ID	Dyed Exemplar	Image of Fiber from MSP
685	Reactive Red 180	
686	Reactive Red 198	
695	Reactive Red 239/241	
713	Direct Red 84	
721	Vat Red 10	
722	Vat Red 15	

Table 1. Dyed cotton exemplars provided by Stephen Morgan, University of South Carolina.

Samples were taken as absorbance values with ten scans taken at different locations for each fiber. All spectra were truncated to a wavelength range of 350-800 nm. They were then background subtracted by subtracting the minimum absorbance value from each data point in a sample. Following this, the data sets were normalized by dividing each absorbance value by the square root of the sum of the squares of all absorbance values. This eliminated variability in the data due to sample thickness and dye uptake.

Four chemometric techniques were applied to the data: Agglomerative Hiearchial Clustering (AHC), Principal Components Analysis (PCA), Discriminant Analysis (DA) and Analysis of Variance (ANOVA). Statistical evaluation of the data was performed using Microsoft Excel XLSTAT 2009 software. For the purposes of AHC, replicate spectra for each fiber were averaged together. In PCA, DA, and ANOVA, every scan was used instead of utilizing only the averages. AHC was performed to show the potential grouping of the data. Inspection of the AHC dendograms revealed that replicate spectra from fibers treated with different dyes formed three distinct clusters for the twelve exemplars analyzed (Figure 4). Class 3 represents spectra for dyes 721 (Vat Red 10) and 722 (Vat Red 15), whereas Class 2 contains spectra for dyes 713 (Direct Red 84) and C (Reactive Red 123). Class 1 contains the spectra for the remaining dyes. These do not cluster well and tend to co-mingle.

PCA confirmed the clustering of samples and further showed that 713 (Direct Red 84), 722 (Vat Red 15), and C (Reactive Red 123) are easily distinguishable, but that there is substantial variation and co-mingling within the other dyes. A somewhat similar result was obtained when performing a DA on the PCA data. However, instead of three dyes, five dyes can be distinguished (i.e., dyes A, C, 713, 721, and 722). All others were in classes that overlap.

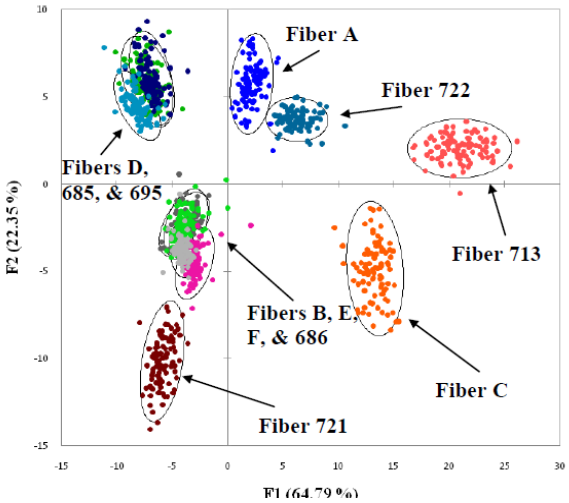

Figure 2. Observations plot from Discriminant Analysis using 12 classes (each dye is its own class).

DA was also performed to determine the extent to which the classes are truly distinguishable. Using leave-one-out cross validation techniques, it was found that groups listed with 100% accuracy (fibers A, C, 713, 721, and 722) had no errors in re-

classification. However, groups with low accuracy were easily confused with other dyes. Overall 85% of the samples were correctly classified.

Fiber	% Correct
A	100.00
B	67.00
C	100.00
D	72.00
E	50.00
F	83.00
713	100.00
685	89.00
686	92.00
695	67.00
721	100.00
722	100.00
Total	**85.00**

Table 2. DA classification results using leave-one-out validation techniques.

Univariate Fisher ratios were then calculated to identify the wavelengths that are the most discriminating. This was achieved by performing an ANOVA one wavelength at a time to determine the ratio of between class variance to within class variance. It was found that, for the spectral range observed, the most discriminating regions are around 481 nm and 568 nm. This was surprising given that the wavelengths that are the most discriminating do not include the wavelengths of maximum absorbance established by PCA.

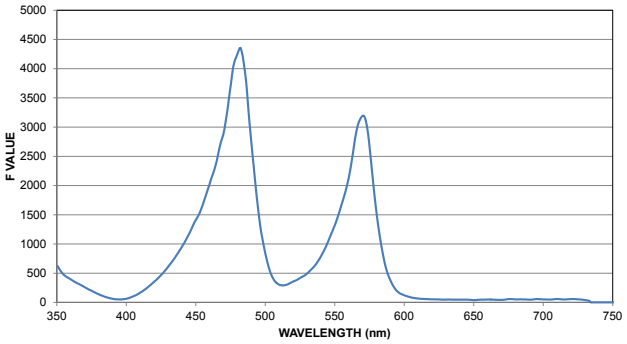

Figure 3. Univariate Fisher ratios (F values) showing the two most discriminating regions for the spectral range.

Finally, an inter-laboratory study was performed between IUPUI and the ISP using the same set of exemplars. Statistical procedures were performed on spectra obtained from a CRAIC QDI 2000 (IUPUI) and CRAIC QDI 1000 (ISP) microspectrophotometer. Results indicate that different instruments can generate subtle yet reproducible differences in spectra. These differences can affect classification schemes that are set up in different laboratories as well as the potential for spectral databases.

TECHNOLOGY BENEFITS

Trace evidence examiners generally do not have any quantitative basis for determining the probative value of an evidence type in question, which analytical method is the most discriminating for that evidence type, the effect of intro-sample variance on drawing their conclusion, or an objective criterion for comparing two samples. By applying multivariate statistical approaches to the data gathered from dyed cotton fibers, the understanding of trace evidence and the evaluation of the match of questioned and known samples is greatly improved.

COLLABORATION

This project is a collaborative effort between Indiana-University-Purdue-University-Indianapolis (IUPUI), the Indiana State Police (ISP) Laboratory Division, Indianapolis, IN, and the University of South Carolina (USC), Columbia, SC. The ISP provides guidance to the project and participates in the analysis of cotton fibers using their CRAIC QDU 1000 instrument. USC provides cotton fibers as well as software written for multivariate analysis of fiber spectra.

DISSEMINATION

The results of this research project have been presented at a number of forensic conferences, including the NIJ Trace Evidence Symposium and

the Midwestern Association of Forensic Scientists (MAFS). A review article on the analysis of dyed fibers appeared in *Analytical and Bioanalytical Chemistry* and a manuscript has been submitted for publication in the *Journal of Forensic Sciences.* A technical report on the project and its findings has also been posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

- Goodpaster, J. "Chemometric Analysis as a Means to Differentiate Class Evidence" Presentation at the Federation of Analytical Chemistry and Spectroscopic Society (FACSS), Reno, NV. October 2008.

- Goodpaster, J. "Applications of Multivariate Statistics to Forensic Science" Presentation at the Central Region Meeting of the American Chemical Society (CERMACS), Cleveland, OH. May 2009.

- Goodpaster, J. "Analysis of Trace Evidence Using Microspectrophotometry and Multivariate Statistics" Presentation at the NIJ Trace Evidence Symposium, Clearwater Beach, FL. August 2009.

- Goodpaster, J., Liszewski, E. "Forensic Analysis of Dyed Textile Fibers" *Anal. Bioanal. Chem,* 2009, Vol. 394: 2009-2018.

- Goodpaster, J., Morgan, S., Liszewski, E. "Discrimination of Dyed Cotton Fibers Based on UV-Visible Microspectrophotometry and Multivariate Statistical Analysis" Presentation at the Fall meeting of the Midwestern Association of Forensic Scientists, Kansas City, KS. October 2010.

- Szkudlarek, C., Liszewski, E., Goodpaster, J. 2011. "Inter-Laboratory Study with Red Cotton Fibers Based on Microspectrophotometry" Presentation at the Central Regional Meeting of the American Chemical Society, Indianapolis, IN. June 2011.

IMPLEMENTATION

Project findings and results are currently being evaluated to serve as guidelines for fiber examiners at the ISP Lab and potentially also the Scientific Working Group on Materials Analysis.

CONTACTS

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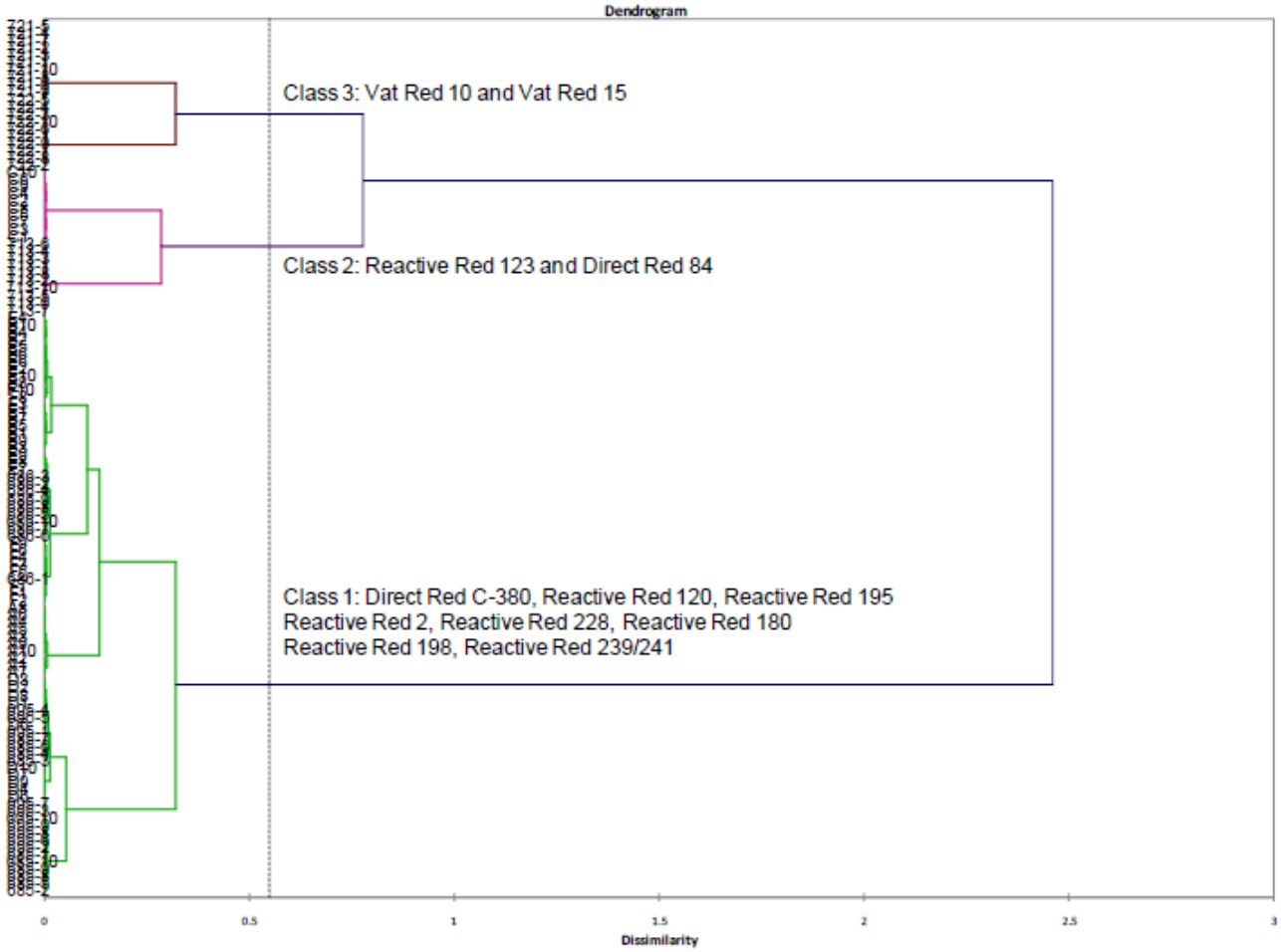

Figure 3. AHC dendogram showing that several dyes (Vat Red 10, Vat Red 15, Reactive Red 123, Direct Red 84) are consistently differentiated from the others. Other dyes are less reproducible and tend to co-mingle.

Shape Measurement Tools in Impression Evidence: A Statistical Approach

FORENSIC TECHNOLOGY NEED

The National Academy of Science (NAS), in its 2009 report "Strengthening Forensic Science in the United States: A Path Forward," notes the lack of a robust statistical framework for pattern evidence analysis. This project targets the development of a multivariate statistical approach to explore the principle tenants of impression evidence: unique features of an object (acquired characteristics) with transfer of the unique features to a substrate.

TECHNOLOGY DESCRIPTION

In an earlier study on quantitative analysis of bitemarks, a shape analysis software was developed based on a shape change measurement technique called geometric morphometric methods. The technique was developed to study patterns of change and variation in biological structures. This project builds upon that work.

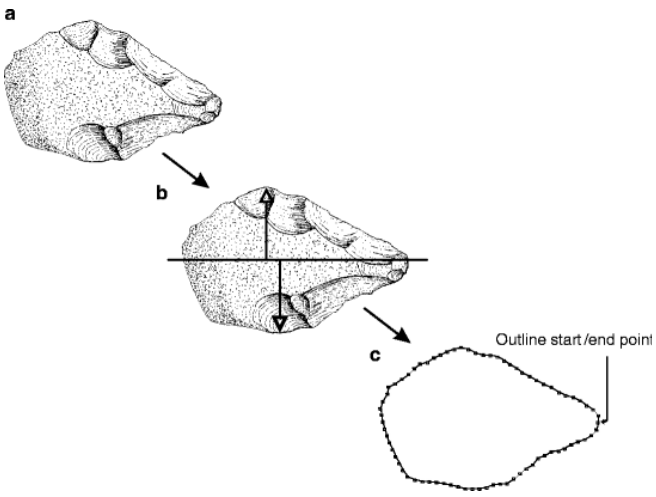

Figure 1. Bitmap data transformed from scans of biface illustrations (a) into Cartesian XY coordinates (b) using geometric morphometric assessment tools and 75 equidistant points (c).

METHODOLOGY

Specifically, expansion of the geometric morphometric measurement software to impression evidence is investigated.

The goal of the project is to explore the feasibility of applying shape measurement tools to fingerprint evidence and footwear impressions, and to examine the repeatability and similarity of these types of pattern evidence.

To accomplish this goal:

- Specimens are acquired from the crime laboratory partner. If needed, they are also created experimentally
- Landmarks are placed on digital images of the specimens using TPSdig software. Landmark coordinates are saved in data files
- Shape information is visualized by plotting landmark positions in superimposition
- Integrated Morphometrics Package (IMP) freeware is used to statistically analyze shape information
- Canonical Variate Analysis (CVA) is performed to determine relationships between groups of variables
- Principal Component Analysis (PCA) is conducted to plot and visualize the principal variations of shape
- Procrustes distances are determined to measure closeness in shape and specimen similarity

- Parameters and limits of similarity and dissimilarity (measurement error) are determined

- Measurement error rates are determined using Root Mean Square statistics, and Procrustes distances

- Inter- and intra-operator error are determined along with match rates based on the effective resolution limit determined by repeated measures

ACCOMPLISHMENTS AND ONGOING WORK

Samples were acquired for 210 fingerprints and 84 footwear impressions using three different pressure techniques (light, normal, heavy), with varying materials (dry, lotion, inkless) and four substrates (10 print cards, soft gloss paper, retabs, and computer paper). Extra light body Polyvinylsiloxane (PVS) was used to impress and capture the fingerprint. Scanning electron microscopy (SEM) was performed on all fingerprints to visualize ridge detail/pore structure with high resolution.

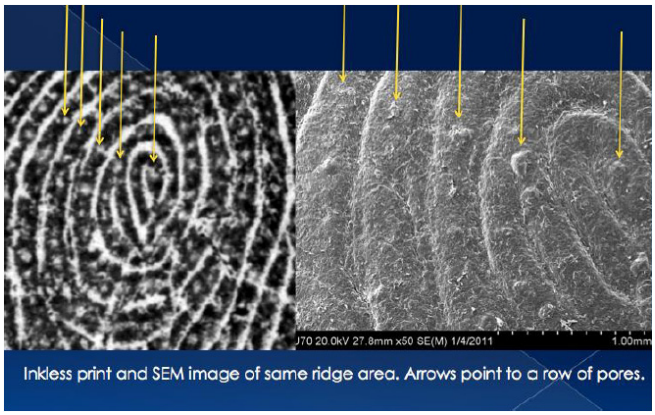

Figure 2. Fingerprint and SEM image of the print.

Landmarks were placed on 18 minutiae points of digital fingerprint images using TPSdig software. Coordinates were saved in data files. The coordinates constitute the measured set of information about a single specimen.

Using Procrustes distances and multivariate statistical techniques, it was found that fingerprints transferred in a reliable manner. Yet, altering the pressure systematically alters the fingerprint. Normal pressure has the lowest variance, more or less pressure increases the variance. It was further found that substrates do alter the fingerprint with effects depending on pressure. 10 print cards appear to be less susceptible to pressure changes then other media.

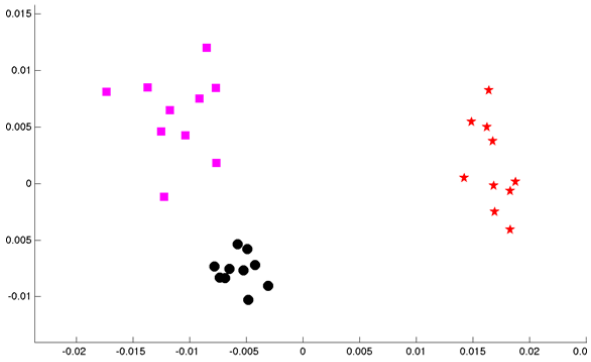

Figure 3. Plot of PCA scores of Repeated Digitizations of three images-Red-3-light, Magenta-2-heavy, and Black-1-normal. 10 measures each. Scores show variation within group vs. among group. First axis 56% of variance, second 16%.

For the analysis of footwear impressions, three brands of shoes were used: Nike Lunar, Converse All Stars, and Sperry Topsiders. The brands were chosen due to differences in sole patterns. The Nike pattern was very complex, the Sperry pattern very simple, and the Converse pattern was mid-range. A run of sizes was obtained for each brand: Nike 9-13; Converse 8-11; and Sperry 8-12. Right and left shoes were scanned separately.

Soles were scanned on a flatbed scanner. Landmarks were placed on the digital images of the soles at ending patterns at defined portions of the shoe. Root Mean Square (RMS) statistics were used to determine the Procrustes scatter of points about the mean shape. For Nike shoes, it was found that the scatter was very small (RMS=0.0028) meaning that the maximum error by chance alone is 0.0056.

The landmark placement method for footwear impressions needed to be re-evaluated and restructured. It was found that the inking system produced much variation and it became necessary to determine if effects seen were due to true distortion or simply the inking system.

TECHNOLOGY BENEFITS

The research provides crime laboratories with a tool that addresses fundamental issues of pattern evidence: individuality and transferability. It builds a framework for statistical models to assign match probabilities based on population distributions.

COLLABORATION

This project is a collaborative effort between the State University of New York (SUNY) at Buffalo, NY, Canisius College in Buffalo, NY, and the Bureau of Criminal Apprehension in St. Paul, MN. Canisius College developed the necessary shape measurement software, while the BCA serves as a consultant to the project and performs software testing and evaluation.

DISSEMINATION

Project findings will be presented at the 2012 meeting of the America Academy of Forensic Sciences (AAFS). A manuscript is being prepared for submission to the *Journal of Forensic Sciences*. Upon completion of the project, a final report will be posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

- Bush, M., Torres, A., Langenburg, G., Gross, S., Bush, P., Sheets, D. "Shape Measurement Tools in Fingerprint Analysis: A Statistical Investigation of Distortion" Presentation at the 64th Annual Meeting of the American Academy of Forensic Sciences, Atlanta, GA. February, 2012.
- Sheets, D., Gross, S., Langenburg, G., Torres, A., Bush, P., Bush, M. "Shape Measurement Tools in Footwear Evidence: An Investigation to Determine Size Without Scale" Presentation at the 64th Annual Meeting of the American Academy of Forensic Sciences, Atlanta, GA. February, 2012.

IMPLEMENTATION

The project will be highlighted in the Forensic Science Foundation Award Workshop titled "Applications of Geometric Morphometrics in 2D and 3D for Forensic Comparisons" at the Annual Meeting of the American Academy of Forensic Sciences (AAFS) in February 2012. Interest in the project has also been expressed by SWGTREAT.

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The Development of a New Model to Study Firearms Related Blood Spatter

FORENSIC TECHNOLOGY NEED

Relatively few studies have examined the dynamics of firearms-related blood spatter. Part of the reason for this is the difficulty of mimicking bloodstain pattern formation, which in turn is a function of the availability of suitable models. In order for a model to be appropriate, it needs to be realistic in both its materials and in its design.

TECHNOLOGY DESCRIPTION

In this project, a novel physical model is designed and constructed to study cranial gunshot wounding and associated blood spatter formation. In doing so, the project: simulates the formation of the gunshot-related bloodspatter to answer case-related questions, and provides insight for the mechanism of spatter projection.

Figure 1. Preliminary human head model as it collapses in on itself. Large bone fragments can be seen on the left of the image and blood droplets, traveling in a backwards direction can be seen in the middle of the image.

METHODOLOGY

The goal of the project is to design and develop a physical model of the human head that can be used to study cranial gunshot wounding and associated formation spatter. Construction of the model is accomplished by using anatomically accurate dimensions and best available simulant materials.

Specific objectives of the project are to:

- find materials that adequately simulate the relevant anatomical features of the human head
- construct a physical model that permits the visualization of intra-cranial dynamics and external spatter formation
- demonstrate the use of the model for case-specific reconstruction experiments
- compile a set of gunshot-related video clips to be added to the widely used BPA video library
- demonstrate the use of the model for studying critical cranial mechanistic components such as tail splashing and intra-cranial cavitation effects

ACCOMPLISHMENTS AND ONGOING WORK

This is a new project that has not started work yet.

TECHNOLOGY BENEFITS

The constructed model is suitable for the scientific simulation of cranial gunshot wounding and associated splatter formation. It gives investigators and researchers the opportunity to control the variables of interest to give reliable results from inflicted gunshot trauma. In addition, a comprehensive set of high-speed videos provide a valuable new teaching resource.

COLLABORATION

This project is a collaborative effort between the Institute of Environmental Science and Research (ESR), Christchurch, NZ, the University of Otago (UO), Dunedin, NZ, and the Kansas City Police Crime Lab (KCPCL) in Missouri. The project brings together forensic scientists from New Zealand and the U.S.A.

The ESR and UO will design, develop and construct the physical model. ESR will also provide the high speed digital camera and backlighting system for high speed video imaging. The KCPCL will provide the test site and conduct model testing.

DISSEMINATION

Project findings and results will be presented at national and international forensic meetings. A number of manuscripts will be drafted for submittal to a peer-reviewed journal. Upon completion of the project, a technical report, along with a set of high speed videos, will be posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

This is a new project with no presentations or publications to date.

IMPLEMENTATION

Materials from the project will be incorporated into a course titled "Fluid Dynamics: Advanced Bloodstain Pattern Analysis" offered worldwide by the ESR. Additionally, BPA instructors can take advantage of the model and the videos posted on the MFRC website for use in their courses.

Upon completion of the project, the KCPCL will host a demonstration of the model with invitations to participate sent to regional crime laboratories. Detailed step-by-step instructions on how to construct and use the model will be provided.

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