MFRC

Midwest Forensics Resource Center

Research and Development Program Summary

November 2012

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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 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 Laboratory, Olathe, KS

Analytical data for synthetic cannabinoids and substituted cathinones 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 and substitued cathinones in emerging designer drugs.

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

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 analysis. 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 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) difficult.

TECHNOLOGY DESCRIPTION

Previous research demonstrated the success of Principal Component 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. This project builds upon these findings by investigating matrix interferences and weathering effects of ignitable liquids.

METHODOLOGY

The objective of this research is to develop a methodology to associate ILRs to the corresponding NILs, using multivariate statistical procedures. Specific goals are to:

- Investigate increased matrix interference effects from three household matrices.
- Investigate the effect of increased matrix interference effects on the association of evaporated liquids to the neat liquids using PCA and PPMC coefficients.
- Investigate the association of simulated ILRs to the corresponding neat liquid using PCA and PPMC coefficients.

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, and 120 seconds.

Aliquots (20 µl) of diluted liquid standards were spiked onto the burned matrix. Samples were placed in a nylon bag with an activated carbon strip and activated in an oven for 4 hours at 80º 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 burn times increased further, no additional matrix interference was observed. Hence, 60 seconds was selected as the optimal burn time.

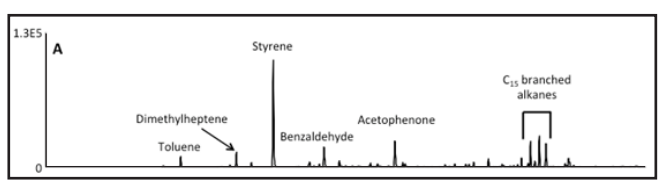

Figure 1. Matrix interferences present in nylon carpet after burning for 60 seconds.

During the course of the research, nine data sets were generated each consisting of total ion chromatograms of the neat and evaporated liquid standards, along with the chromatograms of the liquids extracted from the matrices.

Four different pretreatment procedures were applied to each data set: background subtraction, chromatogram smoothing, retention time alignment, and normalization. Pretreated data sets were then subjected to statistical analysis.

Using PCA, it was possible to differentiate not only the liquid standards to liquid type (gasoline, lighter fluid, and kerosene), but also to associate liquids extracted from the burned matrix and the simulated ILRs according to liquid type.

In some cases, association to the appropriate liquid was not possible, based on visual assessment of the scores plot. This was primarily due to the difference in abundance of the characteristic compounds in the extract and corresponding standard.

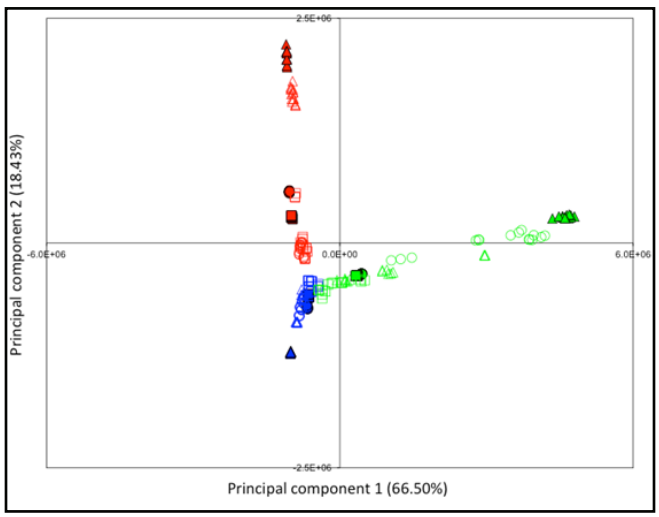

Figure 2. PCA Scores plot of gasoline (red), kerosene (blue), and lighter fluid (green) liquid standards (filled symbol) and simulants fire debris (open symbols). Squares indicate neat liquid, circles indicate 10% evaporated liquid, and triangles - 90% evaporated liquid for gasoline and lighter fluid and 70% evaporated for kerosene.

Matrix interferences in the high density polyethylene matrix consisted of normal alkanes, which are characteristic compounds in kerosene. Since these compounds were present in the liquid standards, their presence did affect positioning of extracts on the scores plot. This means that the gasoline and lighter fluid extracts shifted toward the kerosene standards in the scores plot, making association to the corresponding liquid more difficult.

PPMC coefficients were calculated to assess association of the extract or simulated ILR and corresponding standards. They were used to assess the similarity between two chromatograms, using all the data points in the chromatograms.

It was found that the presence of interferences in one chromatogram, but not the other, lowers the PPMC. It was also found that, despite this issue, it was possible to associate the extracts to the corresponding neat standard using PPMC coefficients.

Liquid extracted from simulated ILR for carpet padding matrix	Mean PPMC coefficient ± standard deviation			
	Corresponding standard	Neat lighter fluid standard	Neat gasoline standard	Neat kerosene standard
Neat gasoline	0.766 ± 0.055	0.316 ± 0.062	0.766 ± 0.055	0.360 ± 0.087
10% evaporated gasoline	0.747 ± 0.032	0.302 ± 0.033	0.733 ± 0.032	0.344 ± 0.053
90% evaporated gasoline	0.951 ± 0.024	0.281 ± 0.033	0.573 ± 0.033	0.419 ± 0.042
Neat kerosene	0.626 ± 0.097	0.447 ± 0.033	0.451 ± 0.054	0.626 ± 0.097
10% evaporated kerosene	0.696 ± 0.137	0.405 ± 0.057	0.408 ± 0.079	0.724 ± 0.092
70% evaporated kerosene	0.582 ± 0.066	0.394 ± 0.025	0.446 ± 0.059	0.672 ± 0.034
Neat lighter fluid	0.518 ± 0.140	0.518 ± 0.140	0.272 ± 0.060	0.319 ± 0.052
10% evaporated lighter fluid	0.478 ± 0.158	0.481 ± 0.155	0.280 ± 0.043	0.327 ± 0.064
90% evaporated lighter fluid	0.519 ± 0.125	0.498 ± 0.062	0.338 ± 0.055	0.448 ± 0.027

Table 1. Mean Pearson product moment correlation coefficients for replicates of each liquid extracted from the simulated ignitable liquid residue (n=225).

Hierarchical Cluster Analysis (HCA) was applied to determine the similarity of samples relative to the three liquids investigated. It was found that it is possible to distinguish the ILRs according to the liquid type but not to a specific evaporation level.

Due to evaporative losses and the effects of burning, the ILRs were generally clustered with the most evaporated standard of the corresponding liquid type. These findings suggest the importance of including evaporated standards in any reference collection used for comparison.

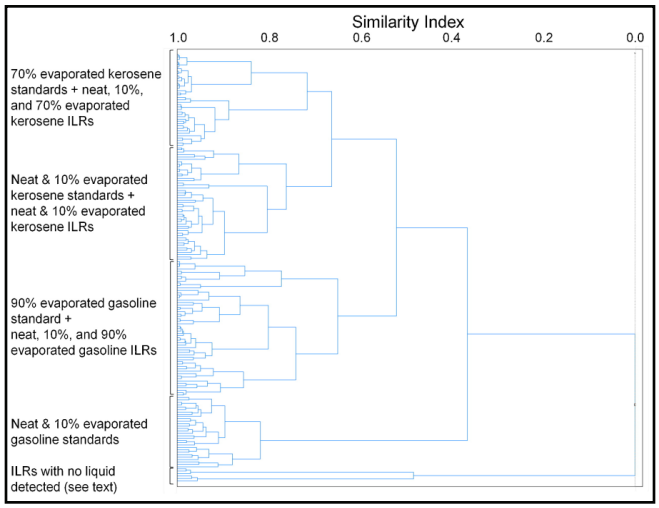

Figure 3. Dendogram generated for gasoline and kerosene standards and extracts from burned carpet using hierarchial cluster analysis, with Euclidean distance measurement.

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) in East Lansing, MI; and the Michigan State Police (MSP) at Grand Rapids, MI. The MSP served as consultants to ensure the practicality of the methodologies as well as the overall project approach.

DISSEMINATION

Project findings and results were represented at regional and national forensic and chemical meetings. A manuscript was submitted to the *Journal of Forensic Sciences* and *Forensic Science International*. A final project report was posted on the MFRC website, while a seminar was advertised via an e-mail announcement distributed to crime laboratories in the Midwest.

PUBLICATIONS AND PRESENTATIONS

- Prather, K., Tower, S., McGuffin, V., Waddell-Smith, R. "Effects of Matrix Interference, Weathering, and Thermal Degradation on the Association of Ignitable Liquid Residues to Neat Ignitable Liquids". Oral presentation at the 63rd Annual Meeting of the American Academy of Forensic Sciences, Chicago, IL. February 2011.

- Prather, K., McGuffin, V., Waddell-Smith, R. "Analysis of Fire Debris Using Gas Chromatography-Mass Spectrometry and Multivariable Statistical Procedures". Poster presentation at the 3rd Annual Graduate Academic Conference, Michigan State University, East Lansing, MI. March 2011.

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

- Tower, S., Prather, K., McGuffin, V., Waddell-Smith, R. "Effect of Evaporation, Matrix Interferences, and Thermal Degradation on the Association and Discrimination of Ignitable Liquids Extracted from High Density Polyethylene". Poster presentation at the 40th Annual Meeting of the Midwestern Association of Forensic Scientists, Chicago, IL. September, 2011.

- Prather, K., Tower, S., McGuffin, V., Waddell-Smith, R. "Investigating the Use of Multivariate Statistical Procedures in Fire Debris Analysis".

Oral presentation at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Orlando, FL. March 2012.

- Prather, K., McGuffin, V., Waddell-Smith, R. "Effect of Evaporation and Matrix Interferences on the Association of Simulated Ignitable Liquid Residues to the Corresponding Liquid Standard". Manuscript submitted to *Forensic Science International.*

- Prather, K., Tower, S., McGuffin, V., Waddell-Smith, R. "Effect of Interferences from High Density Polyethylene on Association of Simulated Ignitable Liquid Residues to the Corresponding Liquid". Manuscript submitted to the *Journal of Forensic Sciences*.

IMPLEMENTATION

Funding is being sought to develop and present a workshop to train analysts in the data analysis and interpretation of multivariate statistical procedures in fire debris analysis.

CONTACTS

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Chemical Characterization of Emerging Designer Drugs

FORENSIC TECHNOLOGY NEED

In 2009, products marketed as "herbal incense" started to appear in case submissions. Rather than legitimate incense materials, these products contained "synthetic cannabinoids". By 2010, the number of synthetic cannabinoids had exploded. At the same time, substituted cathinones started to appear as substitutes for cocaine and methamphetamine. This caused problems for forensic scientists as analytical data for these compounds was rarely available.

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 were 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 primary 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

Websites known to sell designer drugs were monitored and contacted. Eighty-three different samples were obtained from 16 different online vendors. The samples were pre-screened using GC-MS with aliquots of 70 samples shipped to collaborating laboratories for analysis by proton and carbon nuclear magnetic resonance spectroscopy (NMR).

Analytical data were collected for sixty different compounds, representing seven different classes of drugs: Adamantoylindoles, Tetramethylcyclopropanoylindoles, substituted cathinones, acrylcyclohexylamines, substituted tryptamines, hallucinogenic phenethylamines, and benzodiazepines. In addition, the study includes a number of samples analyzed did not fall into any of these classes. Collectively, the samples represent the most popular classes of drugs pursued by online vendors.

A review of the vendor websites revealed that some marketed a compound called 5-(2-aminopropyl) indole (5-IT) along with another known as alpha-methyltryptamine (aMT). 5-IT is a positional isomer of alpha-methyltryptamine (aMT) and N-methyltyptamine (NMT). The three compounds are close enough structurally that successful differentiation between the three was problematic using standard GC-MS runs.

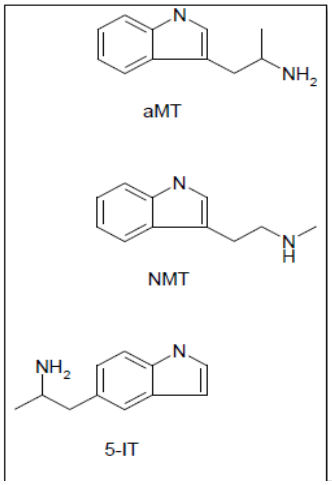

Figure 1. Chemical structures of compounds called 5-(2-aminopropyl) indole (5-IT) and alpha-methyltryptamine (aMT) and N-methyltyptamine (NMT).

Although the IR spectra were all different, the three compounds had virtually identical mass spectra and retention times. When acetylated, only NMT could be differentiated while 5-IT and aMT were indistinguishable. It was found that the easiest means of differentiation between 5-IT and aMT was not through instrumental techniques but through presumptive color tests.

Compound	Marquis	Liebermann's	Meche's	Froedhe's	Ehrlich's
aMT	yellow-brown	black	brown	yellow	purple
5-IT	dark red	dark brown	brown	red-brown	red

Table 2. Presumptive color test results for aMT and 5-IT

While the project is still ongoing, information as gained is being shared with drug chemistst thoughout the nation. One of these involved the potential instability of 4-acetoxy hypotamines. It appears that 4-acetoxy dimethyltryptamine gradually decomposes at room temperature as well as in acidic extractions into 4-hydroxy dimethyltryptamine. This is extremely problematic because the former compound is currently not controlled while the latter is also known as psilocin, which is specifically listed as a Schedule 1 controlled substance nationally.

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

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

DISSEMINATION

Research findings and results were and 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). Upon completion of the project, a final technical report will be posted on the MFRC website, with analytical data for compounds investigated published in the *Journal of the Clandestine Laboratory Investigating Chemists Association*.

PUBLICATIONS AND PRESENTATIONS

- Morris, J. "Review of a Research Project on Emerging Drugs of Abuse". Oral presentation at the 41st Annual Meeting of the Midwestern Association of Forensic Scientists, Milwaukee, WI. September, 2012.
- Poyner, B., Morris, J. "Differentiation Between Three Structurally Similar Indoles". Poster presentation at the 41st Annual Meeting of the Midwestern Association of Forensic Scientists, Milwaukee, WI. September, 2012.
- Stenzel, J. "Structural Characterization of Disgner Drugs Using 1-Dimensional Nuclear Magnetic Resonance (NMR) Techniques". Oral presentation at the Clandestine Laboratory Investigating Chemists Association Meeting, Indianopolis, IN. September, 2012.
- Stenzel, J. "Rapid Structural Elucidation of Designer Drugs". Oral presentation at the Clandestine Laboratory Investigating Chemists Association Meeting, Indianapolis, IN. September, 2012.
- Poyner, B., Morris, J. "Presumptive Color Test for Synthetic Cannabinoids Containing an Indole Substructure". Manuscript submitted to the *Journal of the Clandestine Laboratory Investigating Chemists Association.*
- Pyner, B., Spangler, M., Morris, J., Stenzel, J. "Analyticial Profile of the N-Benzyl-Ortho-Methoxy Derivative of 2C-1". Manuscript submitted to the *Journal of the Clandestine Laboratory Investigating Chemists Association.*
- Poyner, B., Spangler, M., Morris, J., and Stenzel, J. "Differentiation Between Three Structurally Similar Indoles: 5-(2-AminopropyL) Indole (5-IT), Alpha-MethylTriptamine (amT), and N-MethylTriptamine (NMT)". Manuscript submitted to the *Journal of the Clandestine Laboratory Investigating Chemists Association.*

IMPLEMENTATION

Analytical information was shared with forensic drug chemists using the CLIC List drug chemist e-mail list. Knowledge exchanged dealt with the difficulty in obtaining mass spectral information for JTE-907, a discussion about polymorphs in cathinones and tryptamines, and a discussion about the analytical difficulties in differentiating NMT, 5-IT, and aMT. Preliminary information about color test results was also shared, in particular results regarding cobalt thiocyanate and substituted cathinones. A mass spectral library containing spectra of all investigated compounds is currently being developed. Analytical data for over fifty compounds have been submitted to the database hosted by the SAFS Forendex website.

CONTACTS

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Table 1. List of 70 samples analyzed by WMR.

Project lot#	Sample name	Drug class	Supplier
MFRC-2012-A1	MDAI	Stimulant/hallucinogen	mdairesearch.com
MFRC-2012-A2	4-fluoroamphetamine	Stimulant	mdairesearch.com
MFRC-2012-A3	UR-144	Cannabinoid	RCS Chemicals
MFRC-2012-A4	JTE-907	Cannabinoid	RCS Chemicals
MFRC-2012-A5	CB-13	Cannabinoid	RCS Chemicals
MFRC-2012-A6	AB-001	Cannabinoid	RCS Chemicals
MFRC-2012-A7	AM-1248	Cannabinoid	RCS Chemicals
MFRC-2012-A8	A-834,735	Cannabinoid	RCS Chemicals
MFRC-2012-A9	A-796,260	Cannabinoid	RCS Chemicals
MFRC-2012-A10	AB-034	Cannabinoid	RCS Chemicals
MFRC-2012-A11	URB597	FAAH Inhibitor	RCS Chemicals
MFRC-2012-A12	URB602	FAAH Inhibitor	RCS Chemicals
MFRC-2012-A13	URB754	FAAH Inhibitor	RCS Chemicals
MFRC-2012-A14	5-MeO-DALT	Hallucinogen	RCS Chemicals
MFRC-2012-A15	1,3-DMAA	Stimulant	RCS Chemicals
MFRC-2012-A16	5-APB	Stimulant/hallucinogen	Buckledbonzi
MFRC-2012-A17	6-APB	Stimulant/hallucinogen	Buckledbonzi
MFRC-2012-A18	Camfetamine	Stimulant	Buckledbonzi
MFRC-2012-A19	4MemAPB (4-methylbuphedrone)	Stimulant	SenseAromatic
MFRC-2012-A20	Pentedrone	Stimulant	SenseAromatic
MFRC-2012-A21	4-fluoroamphetamine	Stimulant	SenseAromatic
MFRC-2012-A22	3-fluoroamphetamine	Stimulant	SenseAromatic
MFRC-2012-A23	Methoxetamine	Dissociative	SenseAromatic
MFRC-2012-A24	Methiopropamine	Stimulant	SenseAromatic
MFRC-2012-A25	Buphedrone	Stimulant	SenseAromatic
MFRC-2012-A26	Butylone	Stimulant	SenseAromatic
MFRC-2012-A27	Etizolam	Benzodiazepine	BulkRC.net
MFRC-2012-A28	Methoxetamine	Dissociative	BulkRC.net
MFRC-2012-A29	Pentedrone	Stimulant	buy-jwh.com
MFRC-2012-A30	JWH-307	Cannabinoid	buy-jwh.com
MFRC-2012-A31	alpha-PVP	Stimulant	buy-jwh.com
MFRC-2012-A32	2C-I-NBOME	Hallucinogen	Biochem Distribution
MFRC-2012-A33	AB-23	Unknown	bpblabs.com
MFRC-2012-A34	PB-2	Unknown	bpblabs.com
MFRC-2012-A35	Phenazepam	Benzodiazepine	bpblabs.com
MFRC-2012-A36	BZ-6378	Unknown	bpblabs.com

MFRC-2012-A43	2C-P	Hallucinogen	Biochem Distribution
MFRC-2012-A44	4-Aco-DMT	Hallucinogen	Biochem Distribution
MFRC-2012-A45	4-Met-aET	Hallucinogen	Biochem Distribution
MFRC-2012-A46	5-MeO-DALT	Hallucinogen	bpblabs.com
MFRC-2012-A47	Naphyrone	Stimulant	bpblabs.com
MFRC-2012-A48	5-IAI	Stimulant/hallucinogen	bpblabs.com
MFRC-2012-A49	FUR-144	Cannabinoid	buy-jwh.com
MFRC-2012-A50	AKB48	Cannabinoid	buy-jwh.com
MFRC-2012-A51	Demoxepam	Tranquilizer	Composynth chemicals
MFRC-2012-A52	beta-methoxy-2C-D HCl	Hallucinogen	Composynth chemicals
MFRC-2012-A53	Etaqualone	Tranquilizer	Composynth chemicals
MFRC-2012-A54	Phenprobamate	Depressant	Composynth chemicals
MFRC-2012-A55	MDAI	Stimulant/hallucinogen	Composynth chemicals
MFRC-2012-A56	Dichloropane	Stimulant	Organic Home and Garden Supply
MFRC-2012-A57	4-MeO-PCP	Dissociative	Benzo Fury
MFRC-2012-A58	3-beta-FBT	Stimulant	Benzo Fury
MFRC-2012-A59	5-MeO-BFE	Hallucinogen	Benzo Fury
MFRC-2012-A60	alpha-methyltryptamine	Hallucinogen	Benzo Fury
MFRC-2012-A61	1-Aminomethyl-5-methoxyindane	Stimulant	Benzo Fury
MFRC-2012-A62	methoxy-methyl-aminoindane	Stimulant	Benzo Fury
MFRC-2012-A63	5-fluoro-AB-001	Cannabinoid	chemicals-trade.com
MFRC-2012-A64	STS-135	Cannabinoid	chemicals-trade.com
MFRC-2012-A65	4-methyl-N-benzylcathinone	Stimulant	chemicals-trade.com
MFRC-2012-A66	K-Mex	Dissociative	Wide Mouth Frogs
MFRC-2012-A67	C1C	Stimulant	Wide Mouth Frogs
MFRC-2012-A68	A3A Methano	Stimulant	Wide Mouth Frogs
MFRC-2012-A69	Methoxetamine (replacement)	Dissociative	Organic Home and Garden Supply
MFRC-2012-A70	4-MePPP	Stimulant	bpblabs.com
MFRC-2012-A71	Brephedrone	Stimulant	buybestrc.com
MFRC-2012-A72	4-ACO-DALT	Hallucinogen	buybestrc.com
MFRC-2012-A73	4-HO-MET	Hallucinogen	buybestrc.com
MFRC-2012-A74	4-HO-MIPT	Hallucinogen	buybestrc.com
MFRC-2012-A75	2-DPMP	Stimulant	Composynth chemicals
MFRC-2012-A76	3-HO-PCE	Dissociative	Composynth chemicals

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 by the principal investigator and others suggest 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 with hydrogen mobile phase (Fast GC-H_2) 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.

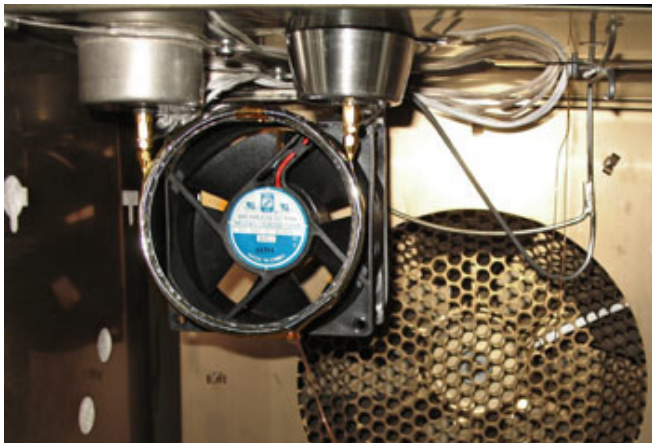

Figure 1. Fast GC conversion kit available through Valco Instruments (used with permission).

METHODOLOGY

The research builds upon the findings of a previously funded project that examined the capabilities of Fast GC in ignitable liquid identification 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 theoretical gain in resolution and sample throughput for a drug identification unit using a combination of Fast GC and hydrogen carrier gasses.
- 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 mass spectrometry applications.
- The dissemination of findings through the use of an asynchronous webinar and a continuous discussion board.

Three different experiments were designed to achieve these goals. The first experiment compared the effects of hydrogen and helium as carrier gasses in the analysis of scheduled compounds with respect to species resolution, peak shape and retention time. The second experiment examined the effects of column diameter and sample loading, and the third experiment examined the Fast GC effects on mass spectra by assessing peak width and processing as a function of heating ramp rate.

Analytical protocols of the Wisconsin State Crime Laboratory-Milwaukee (WSCL-M) were followed where appropriate. WSCL-M “short run” criteria were used following positive color tests, while

"long run" criteria were used following negative or inconclusive color tests. For each experiment, 0.2 to 1.0 ml was injected at 210° C with the hydrogen flow at less than 4.0 ml/min. Oven starts were 50° to 100° C with ramp rates from 50° C/min to 35° C/min. Columns were 20 mm long with an inner bore of 0.18 mm and DB-5.

ACCOMPLISHMENTS AND ONGOING WORK

Initially, the project centered on the concern that pushing an analyte through the mass spectrometer too quickly may yield a potential loss of signal relative to noise, resulting in a decrease in sensitivity. Discussions with Agilent instrument representatives and calculations by the research team 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 20 illicit drugs. The compounds selected incorporated all of the functional groups and retention times encountered in crime laboratory drug identification and analysis work, and also targeted common separation issues encountered in heroin and 6-MAM.

Compound	He@1.0 mL/min	H_2@3.0 mL/min	Compound	He@1.0 mL/min	H_2@3.0 mL/min
Methamphetamine	4.117	2.335	BTCP	10.867	6.791
BZP	6.053	3.609	THC	10.889	6.860
TFMPP-basified	6.248	3.744	Oxycodone	11.047	6.919
TFMPP	6.250	3.774	6-MAM	11.039	6.925
MDMA-basified	6.300	3.797	Heroin	11.438	7.214
MDMA	6.302	3.900	Clonazepam	12.120	7.881
Ketamine	8.087	4.973	Alprazolam	12.518	7.961
MDPV	9.412	5.843	LSD	13.284	8.358
Cocaine	9.734	6.097	Buprenorphine	14.875	9.112
Diazapem	10.727	6.741	Testosterone	16.549	9.889

Table 1. Scheduled compound library for experiments using methanolic standards.

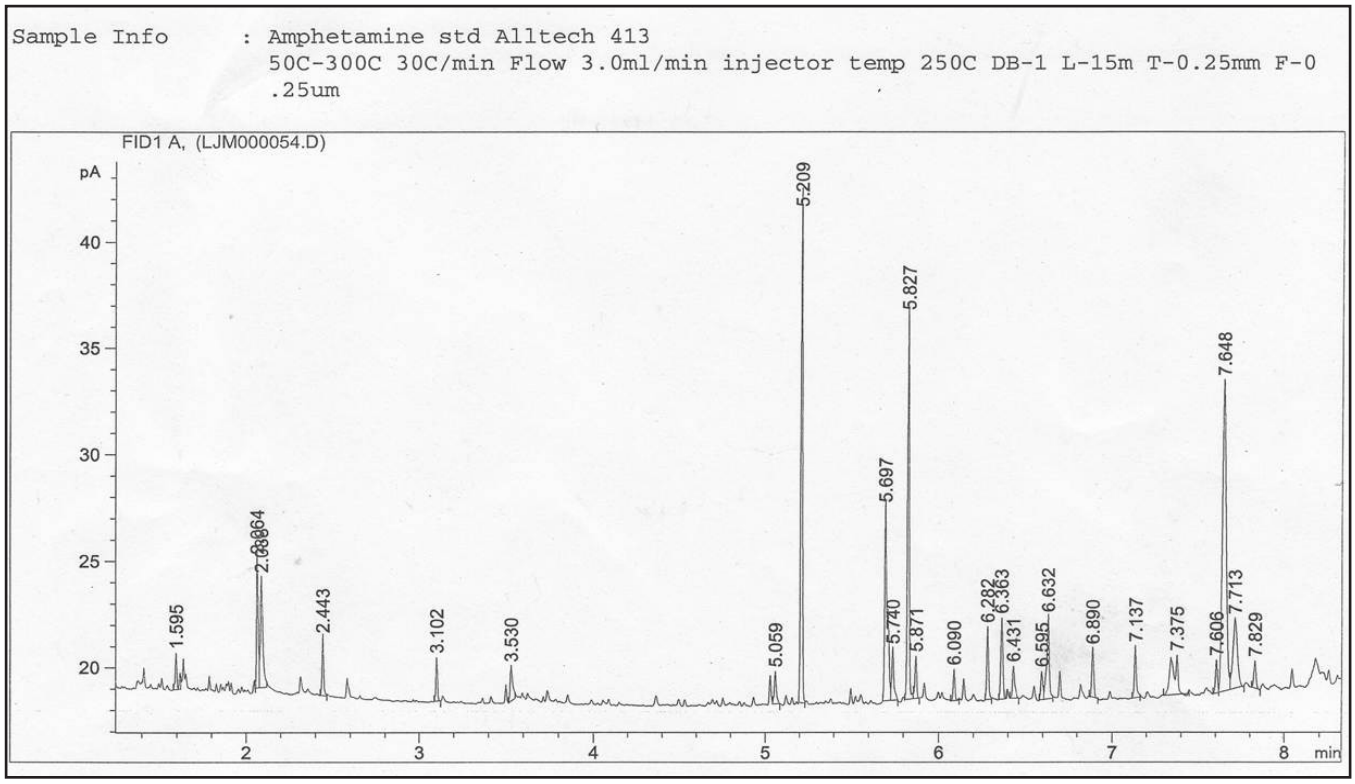

Figure 2. Amphetamine analysis using the Fast GC-hydrogen combination standard.

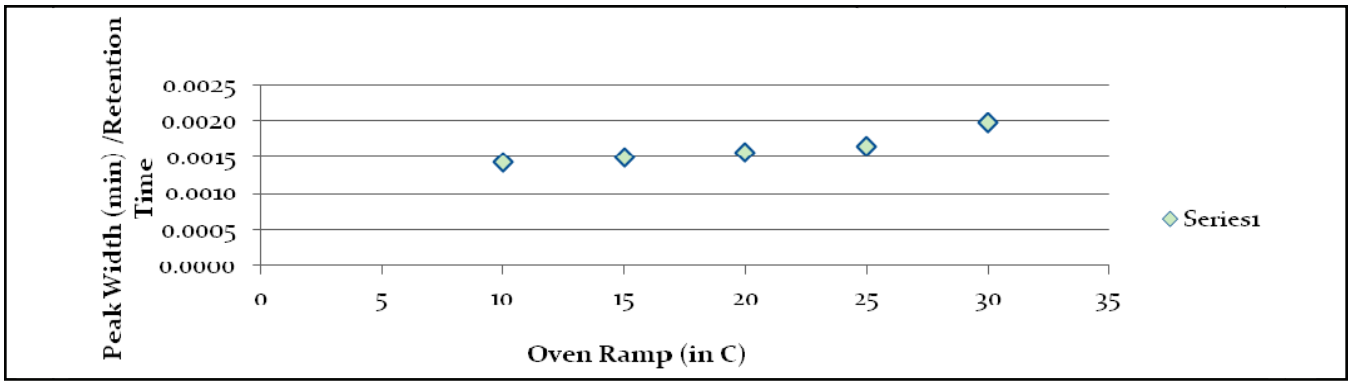

Figure 2. Short run peak widths relative to retention time as a function of GC oven ramp rate.

It was found that the use of Fast GC does produce shorter retention times in the analysis of all compounds tested. It was also 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 to 11 minutes in a rate of 10 centimeters per minute and a symmetry of 0.889 to a rate of 30 centimeters per minute and a symmetry of 0.963 in a mixed standard.

Methanolic standards of all scheduled compounds were then used to create mixes to assess the changes in resolution because of the relative simplicity of drug matrices. It was found that the use of hydrogen instead of helium resulted in resolution gains ranging from 30-60%.

In Fast GC applications with minibore columns (0.18-mm ID), column overloading occurred with traditional 1.0 ml injections. While manual injections provided a continuum of responses, the Agilent software controlled autosamplers used in the project were limited to 1.0 and 0.2 ml injection volumes. Column overloading must therefore be addressed when transitioning to minibore columns.

Finally, the effect of Fast GC oven ramp rates on peak width normalized to compound retention time was measured. It was found that peak width decrease is linear with respect to retention time up to 35° C but tends to bottom out at that ramp temperature. The quadrupole (Agilent 5972 MSD) was quite capable of reliably detecting the compounds of interest at ramps of 35° and 40° C/min.

Validation studies were conducted both at the UWP lab and at the WSCL-Milwaukee. Tests were run to establish instrument protocols (temperature programs) necessary to use Fast GC. No noticeable effects with hydrogenation were found using a standard configuration of Agilent 5890 GC- 5972 MSD. This finding supports literature discussions which do not suggest hydrogenation issues. However, there was some indication that contact with steel (i.e. purge and trap device) may catalyze this process if present.

TECHNOLOGY BENEFITS

Fast GC is a relatively easy, simple to implement approach that yields great benefits to the forensic scientist in terms of improving the separation and detection of schedule compounds. When combined with hydrogen as a carrier gas, resolution improves and retention times are shortened, thus increasing both the quality of the sample analysis and throughout. The use of hydrogen as a carrier gas also results in significant cost savings as hydrogen costs are approximately 60% of the cost of helium. As the supply of hydrogen is far more stable, concerns over suppliers refusing to guarantee annual helium contracts are eliminated.

COLLABORATION

The project is a collaborative effort between the University of Wisconsin-Platteville in Platteville, WI, and the Wisconsin State Crime Laboratory at Milwaukee (WSCL-Milwaukee), WI. The WSCL-Milwaukee served as co-principal investigator and provided controlled substance analysis and research results review, along with student intern oversight during crime lab validation studies.

DISSEMINATION

Research results and project findings were presented at annual meetings of the Midwestern Association of Forensic Scientists (MAFS), the American Academy of Forensic Sciences (AAFS), and the Midwest Forensics Resource Center (MFRC). A technical report on the project and its findings has been posted on the MFRC website.

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 40th Annual Meeting of 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 40th Annual Meeting of 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 64th Annual Meeting of 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 64th Annual Meeting of the American Academy of Forensic Sciences, Atlanta, GA. February 2012.

- Cornett, C., Hansen, R., Macans, L. "Evaluation of Fast Gas Chromatography Coupled with Hydrogen Mobile Phases in Drug Identification". Oral presentation at the annual meeting of the Midwest Forensics Resource Center, Columbus, OH. May 2012.

- Cornett, C., Macans L., Johnson, C., Hansen, R., Jannssen, D. "Implementation of Fast GC Coupled with Hydrogen Mobile Phases in Drug Identification". Oral presentation at the 41st Annual Meeting of the Midwestern Association of Forensic Scientists, Milwaukee, WI. September 2012.

- Johnson, C., Macans, L., Hansen, R., Janssen, D., Cornett, C. "Validation of Fast Gas Chromatography and Hydrogen Mobile Phase in Drug Identification". Poster presentation at the 41st Annual Meeting of the Midwestern Association of Forensic Scientists, Milwaukee, WI. September 2012.

IMPLEMENTATION

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. Standard operating procedures need to be modified to safely use hydrogen in MSD systems. Conversion kits are commercially available and cost as little as $1,000.

Through a series of asynchronous webinars, the UWP provides training on the use of Fast GC-Hydrogen with videos made available through the UWP Media Technology Services Department. The PI also offers the opportunity for crime lab 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 are available through a Fast GC discussion board (http://fastgc-hydrogen.proboards.com/index.cgi).

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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 assess the significance of associations in the comparison of evidence.

TECHNOLOGY DESCRIPTION

Although many forensic disciplines utilize mass spectrometry for the analysis of evidence, there is currently no widely accepted method for the statistical comparison of the resulting spectra. To address this concern, this project targets the development of a procedure to statistically compare 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.

METHODOLOGY

The objective of the project is to develop a probability-based method for the comparison of mass spectral data. The method is developed using a data set of normal n-alkanes and alkylbenzenes analyzed in replicate over several days.

The compounds were selected because they have simple mass spectra with similar fragmentation patterns and are ideal for method development purposes. The procedure was applied to complex data sets containing mass spectral data of controlled substances to demonstrate the practical application in forensic laboratories.

Specific tasks to achieve the objectives are:

- Define a threshold level to remove mass spectral noise present at low abundance.
- Develop the random probability match method using alkane standards analyzed under a variety of different conditions.
- Apply the developed method to more complex GC-MS mass spectral data obtained from the *Salvia* plant species.
- Apply the developed method to case samples obtained from a controlled substances laboratory.

ACCOMPLISHMENTS AND ONGOING WORK

The normal alkanes decane (C_{10}), undecane (C_{11}), dodecane (C_{12}), tridecane (C_{13}), tetradecane (C_{14}), and hexadecane (C_{16}), along with four alkylbenzenes (propylbenzene, butylbenzene, amylbenzene, and hexylbenzene) were purchased from Sigma-Aldrich (St. Louis, MO). A standard solution of the alkanes at concentrations of 0.05, 0.1, 0.5, 1, and 5 mM, and a standard solution of the alkylbenzenes at a concentration of 1 mM, was prepared in methylene chloride (purchased at 99.9% purity from Honeywell Burdick and Jackson, Morristown, NJ). Each standard contained all compounds in equal concentration.

Standards were analyzed in triplicate by gas GC-MS with the analysis repeated 10-15 hours later using the same standard solution again analyzed in triplicate. Two sets of standards were generated for comparison.

Mass spectra were generated and the normalized abundance, relative to the base peak, was calculated at all m/z. For each standard, the mean abundance

and corresponding standard deviation for each m/z were calculated, based on the triplicate mass spectra generated.

Comparisons were made between the same compounds in Sets 1 and Set 2, as well as between each compound in Set 1 and the remaining compounds in Set 2, using an unequal variance Student's t-test. Spectra were compared at each m/z.

It was found that all comparisons of the same alkane and alkylbenzene were statistically indistinguishable at the 99.9% confidence level, indicating association. A random-match probability was calculated based on the frequency of ion occurrence at each m/z value. In each case, the calculated probabilities were infinitely small indicating that the probability of the associated m/z pattern occurring by chance is infinitely small.

Alkane	Confidence Level (%)	Random Match Probability*
C_{10}	99.9	5.2×10^{-37}
C_{11}	99.9	1.9×10^{-44}
C_{12}	98.0	5.8×10^{-49}
C_{13}	99.9	1.1×10^{-50}
C_{14}	99.0	1.5×10^{-55}
C_{16}	98.0	1.1×10^{-60}

Table 1. Random match probability for normal alkanes. *Calculated for pairwise comparison of each alkane (*e.g.,* C_{10} vs C_{10}, C_{11} vs C_{11}, *etc.*) in Set 1 and Set 2.

It was also found that comparisons of the different alkanes and alkylbenzenes were statistically distinguishable at the 99.9% confidence level, with 1-24 ions responsible for discrimination in the alkanes, and 8-18 ions in the alkylbenzenes. Of these, more than half were low abundance ions (defined as <5% of the base peak).

Calculation of the t-statistic required replicas for each spectrum to be compared. Initially, the replicas were collected sequentially. However, the standard deviations associated with such spectra were found not to be representative of instrumental variance leading to false negatives. An alternative method to predict standard deviation associated with abundance was therefore investigated.

A log-log graph of standard deviation versus mean abundance for all alkane spectra at each concentration (n=90) was created to predict standard deviation. The plot indicated that the standard deviation was approximately linearly proportional to abundance.

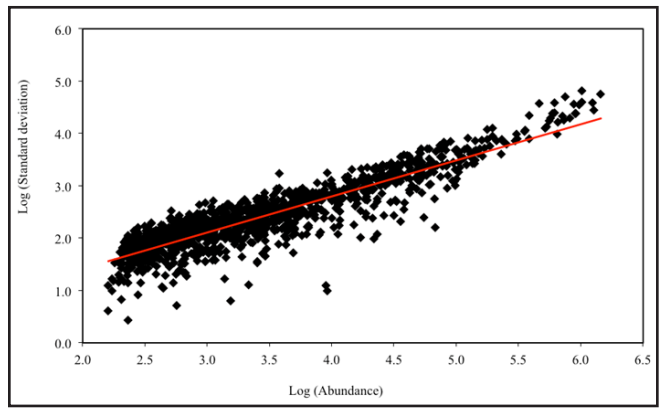

Figure 1. Log-log graph of standard deviation versus mean abundance based on mass spectra of alkane standards (n=90). Using linear least squares regression, the best-fit line had a slope of 0.6900 ± 0.0332 and an intercept of 0.0440 ± 0.0093.

Using this relationship, a standard deviation was predicted for any abundance at or below the instrument threshold (150 counts), based on a mean abundance of 150. The mean abundance at each m/z and the predicted standard deviation were then normalized to the base peak prior to comparison of spectra.

To validate the informed chemical probability approach the method was used for comparing mass spectra to investigate the association and discrimination of salvinorum A. This compound is the active hallucinogen found in plant material *Salvia divinorum*, which although not federally regulated is currently regulated in 25 states in the United States.

It was found that mass spectra of extracts of salvinorum A from *Salvia divinorum* were

statistically associated to salvinorum A standards at six different concentrations at the 99.9% confidence level. It was also found, that mass spectra of salvinorums B, C, and D in these extraxts were statistically distinguishable from salvinorum A at the 99.9% confidence level.

The method was also validated for the identification of Amphetamine-type stimulants. For each case sample, association to the appropriate reference standard was possible at the 99.9% confidence level. Similar findings were obtained for the case samples that were statistically discriminated from other reference standards.

The implications of the findings can be profound for the analysis of evidence where mass spectra are compared. By using the informed chemical probability method, forensic scientists may have access to a simple method that assigns statistical confidence and calculates the random match probability for the comparison of mass spectra of questioned samples and reference standards.

Comparison of Extract 1 to:	Random Match Probability ± Standard Deviation	Lowest Confidence Level (%) at which Association is Maintained
Extract 2[a]	$3.2 \pm 3.7 \times 10^{-126}$	95
Extract 3[a]	$4.1 \pm 6.4 \times 10^{-123}$	95
Extract 4[a]	$1.8 \pm 1.8 \times 10^{-118}$	98
Extract 5[a]	$2.2 \pm 3.9 \times 10^{-108}$	98
Extract 6[b]	$1.6 \pm 3.4 \times 10^{-122}$	98
Extract 7[b]	$3.2 \pm 5.9 \times 10^{-112}$	95
Extract 8[a]	$2.0 \pm 1.1 \times 10^{-125}$	98
Extract 9[c]	$2.1 \pm 3.0 \times 10^{-126}$	95
Extract 10[d]	3.3×10^{-100}	95
Extract 11[d]	2.1×10^{-103}	95
Extract 12[d]	2.0×10^{-40}	95
Extract 13[d]	1.1×10^{-102}	95

Table 2. Random match probabilities calculated for mass spectra of salvinorin A from Extract 1 compared to each of the other extracts, obtained from eight different geographical locations.

Alkane Comparison		Even Mass Ions (%)	Low Abundance Ions (%)*	M+ Ions
Set 1	Set 2			
C_{10}	C_{11}	67	33	C_{10}, C_{11}
	C_{12}	38	63	C_{10}, C_{12}
	C_{13}	53	67	C_{10}, C_{13}
	C_{14}	61	67	C_{10}, C_{14}
	C_{16}	56	50	C_{10}
C_{11}	C_{10}	0	0	---
	C_{12}	67	33	C_{12}
	C_{13}	50	50	C_{13}
	C_{14}	18	64	C_{14}
	C_{16}	0	67	---
C_{12}	C_{10}	66	50	---
	C_{11}	33	67	C_{11}
	C_{13}	100	50	C_{12}
	C_{14}	56	44	C_{14}
	C_{16}	31	69	---
C_{13}	C_{10}	29	57	---
	C_{11}	25	50	C_{11}
	C_{12}	50	75	C_{12}
	C_{14}	33	67	C_{14}
	C_{16}	20	40	---
C_{14}	C_{10}	40	57	---
	C_{11}	44	50	C_{11}, C_{14}
	C_{12}	33	75	C_{12}, C_{14}
	C_{13}	67	67	C_{13}, C_{14}
	C_{16}	40	40	C_{14}
C_{16}	C_{10}	48	52	C_{16}
	C_{11}	50	63	C_{11}, C_{16}
	C_{12}	40	67	C_{12}, C_{16}
	C_{13}	54	46	C_{13}, C_{16}
	C_{14}	50	60	C_{14}, C_{16}

Table 3. Ions responsible for discrimination of alkane mass spectra, calculated at the 99.9% confidence level. * ≤ 5% of the base peak.

TECHNOLOGY BENEFITS

The development of a confidence level associated with the identification of a controlled substance meets the requirements of a Daubert standard. While the method development uses mass spectra of controlled substances, the procedure can readily be applied to mass spectral data for other types of forensic evidence.

COLLABORATION

This project is a collaborative effort between Michigan State University (MSU) in East Lansing,

MI; the Alaska Scientific Crime Detection Laboratory (SCDL) in Anchorage, AK; and the Northeastern Illinois Regional Crime Laboratory (NIRCL) in 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). Manuscripts have been submitted for publication in *Analytical and Bioanalytical Chemistry* and *Rapid Communications in Mass Spectrometry*. A presentation may be made to the scientific working group for the analysis of seized drugs. A technical report on the project and its findings will be posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

- Bodnar-Willard, M., McGuffin, V., and Waddell-Smith, R. "Statistical Comparison of Mass Spectral Data in the Identification of Amphetamine-Type Stimulants". Oral presentation at the 64th Annual Meeting of the American Academy of Forensic Sciences. February 2013, Washington DC.
- Bodnar-Willard, M., McGuffin, V., and Waddell-Smith, R. "Informed Chemical Probability Method to Assign Statistical Confidence for Comparison of Mass Spectra". Manuscript submitted to *Rapid Communications in Mass Spectrometry*.
- Bodnar-Willard, M., Hurd, J., McGuffin, V., and Waddell-Smith, R. "Statistical Comparison of Salvinorin A Mass Spectra for Forensic Identification". Manuscript submitted to *Analytical and Bioanalytical Chemistry*.
- Bodnar-Willard, M., McGuffin, V., Waddell-Smith, R. "Statistical Comparison of Mass Spectra for Forensic Identification of Amphetamine-Type Stimulants". Manuscript submitted to the *Journal of Forensic Sciences*.

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 have the opportunity to evaluate the current version of the method and using feedback from the forensic community adjustments will be made to finalize the method developed.

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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.

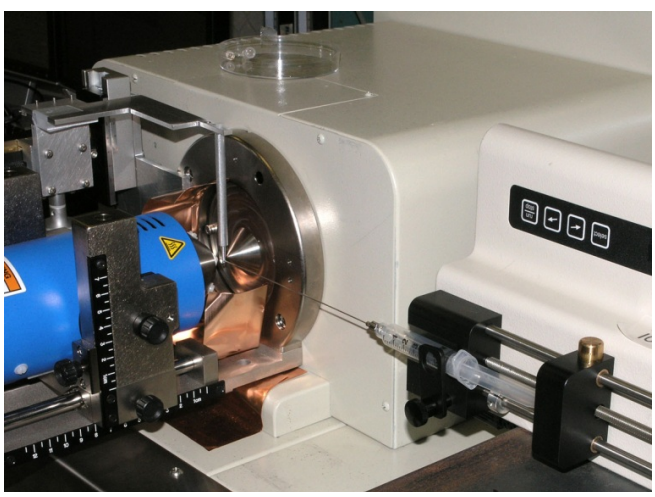

Figure 1. Headspace vapors injected into the DART-MS for analysis; blue cylinder on the left is the DART ion source. The sample syringe and syringe pump are on the right.

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 is demonstrated in the research laboratory.

To achieve these objectives:

- DART-MS spectra of vapors were collected from the major classes of ignitable liquids to build a small, proof-of-principle library.
- DART-MS spectra of common fire-debris matrices were collected after being burned with and without accelerants. Spectra were added to the library.
- The library of data generated was used 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 ml 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.

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 identical compositions producing very similar spectra.

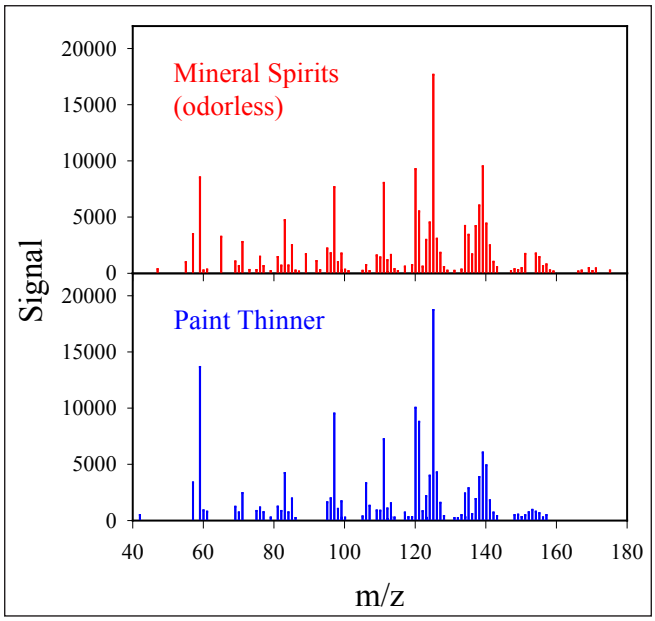

Figure 2. Vapor spectra for similar accelerants sold under different names.

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 resembled those of the accelerant vapors, the search of a burned sample spectrum against the library of accelerant vapor spectra generally matched. In only one case did the library search of the headspace vapors from a burned sample produce an incorrect result.

The aging of fire debris was examined to investigate the use of collection media and to determine the loss of chemical components by bulk evaporation. A sample of carpet and padding was ignited using regular gasoline as an accelerant. It was extinguished after burning for 3 minutes with the debris kept in a glass jar for a total of nine days. The jar was kept open to expose the sample to room air, except for a 2 to 7 hour period prior to each daily analysis when the jar was sealed to allow headspace vapors to accumulate. For comparison, samples of gasoline were left in open bottles and headspace vapors were sampled at various times.

Experiments showed that open-air aging drastically changed the debris spectra. After just one day, the fire debris spectrum showed just two peaks: one at m/z 121.100 and one at m/z 135.118. The spectrum closely resembled the spectrum of open bottle gasoline aged until only 6% of the initial volume remained. This was virtually the endpoint of the open bottle evaporation process as no change in volume was observed for several days prior to acquiring the spectrum. Yet, the vapors from the fire debris continued to change, although at a much slower pace. This suggests that aging is much faster for accelerant dispersed over the surface of fire debris than it is for liquid accelerant in an open container.

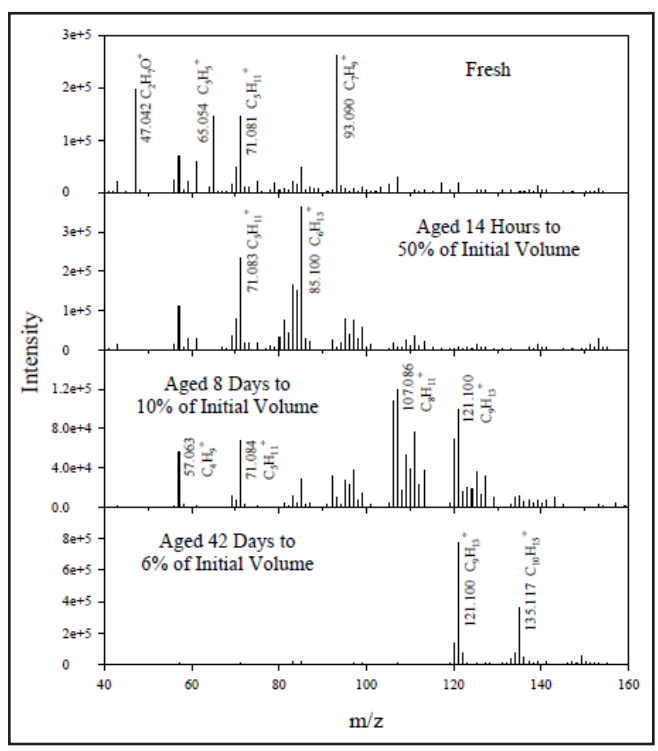

Figure 3. Spectra of vapors from regular gasoline when fresh and when evaporated down to 50%, 10%, and 6% of its initial volume.

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. With DART-MS, dropping the GC step while maintaining specificity and sensitivity, greatly speeds up the analytical process and substantially reduces data-analysis complexity.

COLLABORATION

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

DISSEMINATION

Research findings and results were presented at the 2011 annual meeting of the MFRC and at the 2011 Fall Meeting of the Midwestern Association of Forensic Scientists (MAFS). A technical report on the project and its findings was posted on the MFRC website.

PUBLICATIONS AND PRESENTATIONS

- Jones, R., McClelland, J., Reinot, T. "Rapid Arson Sample Analysis Using DART Mass Spectrometry". Oral 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". Oral presentation at the 40th Annual Meeting of the Midwestern Association of Forensic Scientists, Chicago, IL. September 2011.

IMPLEMENTATION

The project established proof-of-concept for rapid arson sample analysis using DART mass spectrometry. The PI has applied for federal funding to continue the work. The investigator plans to host a workshop on DART-MS application in forensic arson analysis.

CONTACTS

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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 to assist in the identification of individuals. For highly degraded DNA, the number of methods is limited because of differing levels of discriminatory power and differing tolerance levels. A common strategy used for processing such cases is to systematically test DNA recovered from a sample until usable results are obtained. This is both resource intensive and time consuming. A method is needed that provides the DNA analyst with significant information on both the quality and the quantity of DNA recovered from a sample.

TECHNOLOGY DESCRIPTION

This project targets the development of an assay that provides information on the quality and quantity of both nuclear (nDNA) and mitochondrial DNA (mtDNA). Previous work on this subject resulted in a multiplex polymerase chain reaction (PCR) assay Quantitative Template Amplification Technology (QTAT) for the quantification and characterization of human nuclear DNA. This project builds upon that work by enhancing the Q-TAT assay to including quantification of mitochondrial DNA, and by evaluating the ability of the Q-TAT assay to detect degradation in chromosomal DNA.

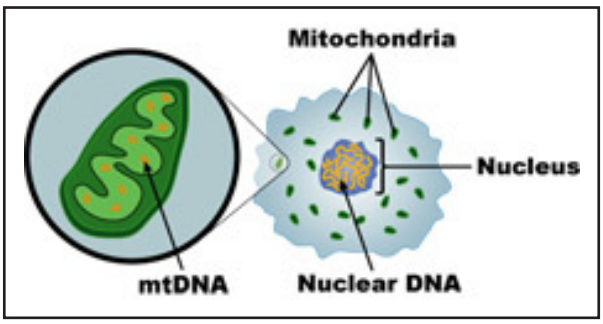

Figure 1. Nuclear and Mitochondrial DNA (Source: NIJ)

METHODOLOGY

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

ACCOMPLISHMENTS AND ONGOING WORK

Two primer sets labeled with the fluorescent dye NED (i.e., mt97 reverse and mt287 reverse) were developed to promote the amplification of targets in mtDNA in the D-loop vicinity close to the origin of replication for the mitochondrial genome. It was found that the mtDNA primers promoting the amplication of the mt287 product were suitable for quantifying mtDNA in a sample thus showing that the Q-TAT assay is capable of simultaneously quantifying both nuclear and mitochondrial DNA.

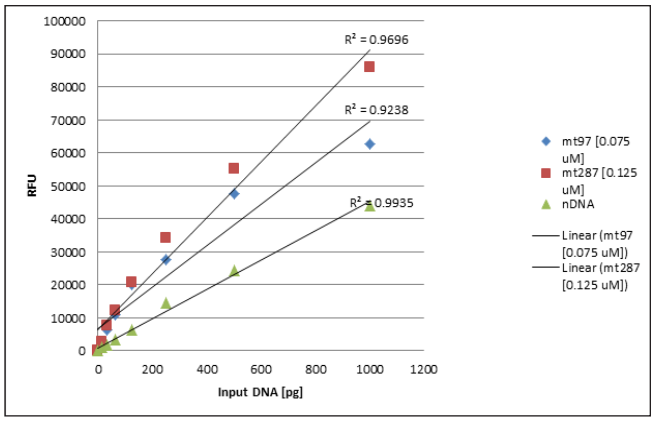

Figure 2. Standard curves produced from known amounts of input male DNA. The RFU values shown represent total area under the different amplicon peaks in the electropherograms.

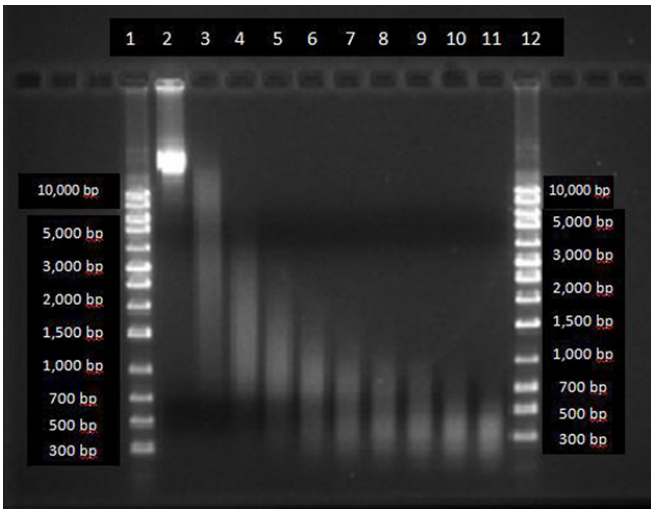

Figure 3. Detection of template degradation (each line represents 10 minutes of increased exposure (left to right) of DNA to 95° C. Lanes 1 and 12 are size standards, lane 2 is intact genomic DNA).

To determine the ability of the Q-TAT assay to detect DNA degradation, five micrograms of genomic DNA, extracted from a freshly procured blood sample, were suspended in 25 µl of ultrapure water and subjected to degradation catalyzed by DNase 1 and also by heating at 95° C in a thermal cycler. Samples were removed from the cycler at 10 minute intervals (up 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 were chilled, 1 µl of each was diluted further five-fold with TE-4 and 1 µl amplified in the Q-TAT assay.

It was found that the enzymatic treatment was incapable of degrading the genomic DNA as extensively as heating and that heat exposure to 95° C lead 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 disappear from STR profiles faster than low molecular weight loci.

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

Degradation ratios, obtained from the quantity estimate of DNA in the sample produced using the SRY amplicon versus that obtained using Amel-X+Y at each heating time point showed that the first degradation ratio of greater than 1.0 occurs at the t = 40 minute time point. Also, that the largest degradation ratio obtained was 1.78 and occurred at the t = 90 minute time point. The gradual increase in degradation ratios through the time course appeared to follow a linear function.

Using a one-way ANOVA, with Tukey's multiple comparison test, the degradation ratio became significantly different from t = 0 at the t = 60 minute time point ($p<0.01$). The time point corresponds to a ratio of approximately 1.5, and samples that have experienced degradation according to the research model would be expected to display a significant difference in relative fluorescence units (RFU) in large and small STR loci if a degradation ratio equal to or greater than 1.5 were obtained in the Q-TAT assay.

Ninety-one male reference samples available in the Tulsa Police Laboratory were evaluated with

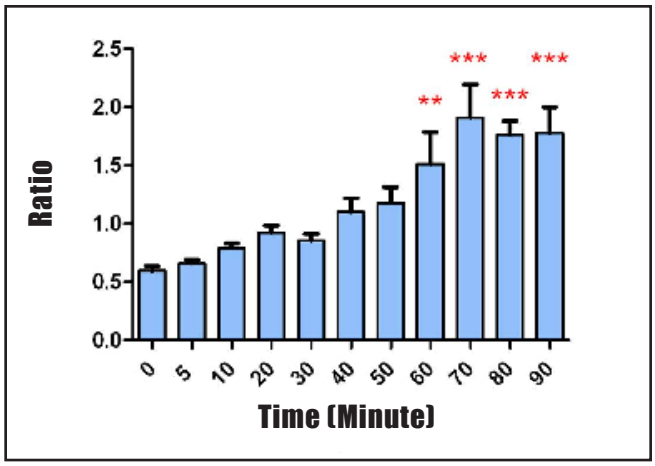

Figure 4. Degradation ratios obtained from each timepoint of the high-molecular weight sample throughout the degradation experiment. At the t=60 time point, the ratios become very significant ($p<0.01$).

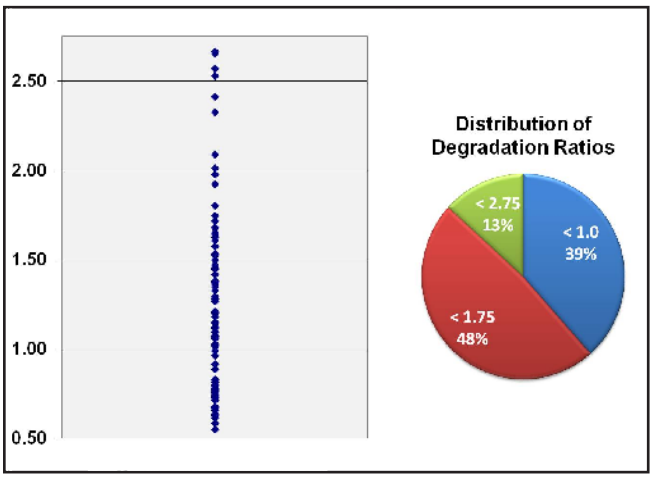

Figure 5. Fluorescence in small Q-TAT amplicons produced from 91 reference male samples.

the Q-TAT assay to assess the quality of DNA in reference samples. It was found that about 87% of all samples had ratios of less than 1.75 which could serve as the threshold level for concluding that a DNA sample exhibits less than optimal integrity.

Results and findings from this project demonstrate that the Q-TAT assay can be effective in allowing a DNA typing laboratory to both quantify and characterize DNA recovered from a biological sample. With the inclusion of the primers for mt97 and mt287 amplicons, the Q-TAT assay detects degradation of mitochondrial DNA in the same way as nuclear DNA.

The only complication associated with evaluating mtDNA is the need to establish the minimal number of cell equivalents of mtDNA needed to produce a complete mtDNA sequence since Q-TAT quantity estimates are in cell equivalents rather than in molecular weight.

TECHNOLOGY BENEFITS

The benefits of the Q-TAT assay to the forensic community are three-fold. First, the method provides a rather complete picture on the quantity and quality of the DNA recovered from evidence, including the integrity of both nuclear and mitochondrial DNA. This information can be very valuable in deciding among the different STR kits and DNA typing technologies available in the laboratory. Second, the assay requires the same instrumentation as required to produce an STR profile from a sample. Third, no new instrumentation will be required for the laboratory.

COLLABORATION

The project is a collaborative effort between the DNA laboratory of Oklahoma State University's (OSU) Department of Forensic Sciences in Tulsa, OK; and the Tulsa Police Laboratory (TPL) in Tulsa, OK. TPL provided 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, TPL provided validation and casework experience.

DISSEMINATION

Project findings and results were presented at annual meetings of the MFRC. A manuscript is currently being prepared for submission to the *Journal of Forensic Sciences* or *Forensic Sciences International*. A technical report on the project and its findings has been posted on the MFRC website.

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.

- Allen, R. "Application of the Q-TAT Quantification Assay Relevant to the Forensic DNA Laboratory". Presentation at the MFRC Annual Meeting, Columbus, OH. May 2012.

- Allen, R. "Quantification and Characterization of Human DNA with Q-TAT". Training session for DNA scientists from Sri Lanka. OSU, Department of Forensic Sciences, Tulsa, OK. July 2012.

IMPLEMENTATION

The Q-TAT assay is routinely used by the Tulsa Police Laboratory and has resulted in savings of $50,000-$100,000 per year on reagents and service contracts. The assay is also being used to quantify DNA in 6-8 new labs around the world. The Department of Forensic Sciences at OSU has hosted training sessions to support the needs of the labs. Maven Analytical, Inc., has purchased the licensing rights for the assay from OSU and makes kits available commercially.

CONTACTS

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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," noted the lack of a robust statistical framework for pattern evidence analysis. It also noted the lack of study into the range of possible distortion of evidence. To address these concerns, 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) and transfer of the unique features to a substrate.

TECHNOLOGY DESCRIPTION

In an earlier study, a shape analysis software was developed to study patterns of change and variation in biological structures. The software was based on a shape change measurement technique called Geometric Morphometric (GM) analysis, and involved placement of landmark points, curves or outlines on either two- or three-dimensional images. This project builds upon that software by exploring the distortion in fingerprints with regard to pressure and substrate, and by applying it to footwear evidence to test if shoe size can be determined without a scale of reference.

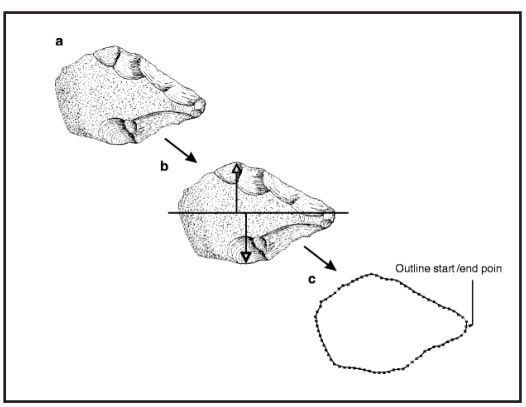

Figure 1. Bitmap data transformed from scans of biface illustrations (a) into Cartesian XY coordinates (b) using geometric morphometric assessment tools and 75 equidistant points (c).

METHODOLOGY

A specific objective of the project is to determine similarity limits between impression objects and to provide an understanding of the range and nature of distortion possible when the object is transferred to a substrate. To achieve the goals:

- Impression evidence specimens were acquired from the crime laboratory partner. Landmark points, curves, and outlines were placed on digital images of the specimens using TPSdig software. Landmark coordinates were saved in data files.
- Shape information was visualized by plotting landmark positions in Procrustes superimposition, a method of optimally matching one shape to another. The approach was used to explore distortion in fingerprints with regard to pressure and substrate. It was also applied to footwear evidence to test if size could be determined without a scale in the image.
- Integrated Morphometrics Package (IMP) freeware was used to statistically analyze the information obtained. Principle Component Analysis (PCA) was applied to visualize the principal variations of shape to determine which shape aspect was responsible for the most variation. Canonical Variate Analysis (CVA) was used to analyze relationships between groups of variables to determine if shape information can distinguish between different categories of data.

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.

Landmarks were placed on 18 minutiae points of digital fingerprint images using TPSdig software. Landmarks were chosen at locations of bifurcation and terminations to maximize the area of the entire print. Orientation of minutiae was not a factor. The same landmarks were used for each print.

Figure 1. Landmark placement of fingerprint. 18 minutia points were selected.

Coordinates were saved in data files and constitute the measured set of information about a single specimen. The data were then extracted and the prints compared statistically.

It was found that fingerprint transferred in a very reliable manner regardless of press and substrate. For example, the variance was very low with a Root Mean Square of scatter of 0.029. This suggests that distoration of a print under different experimental conditions (pressure and substrate) is quite small: around 3% of the overall pattern.

PCA scores were determined for digitization of fingerprint images (10 measures each). PCA analysis determined that 56% of the variance in the prints was movement of the ridge structure in the fingertip portion of the print while the bottom portion remained almost stationary.

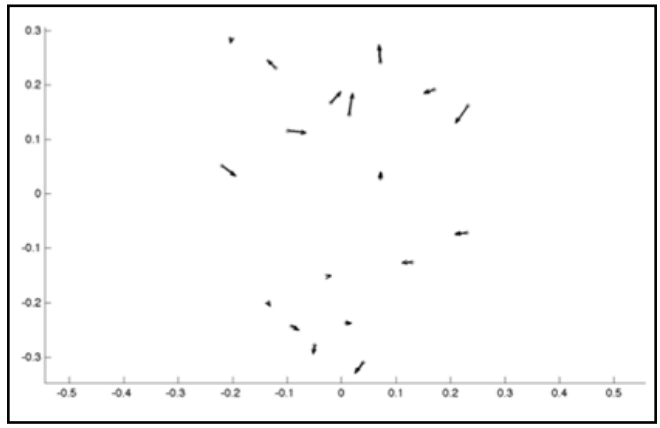

Figure 2. Deformation pattern implied by PCA axes. The units are differences in landmark positions when the shape is in a Procrustes superimposition, which is in Procrustes distance units. This plot displays a very large amplitude change in shape, shown with exaggerated changes at each landmark so that the overall pattern of relative changes can be clearly seen. The arrows show the magnitude of the movement of the landmark positions on the fingerprints as you move across the axis from left to right. The principal distortion is at the tip of the finger where the arrows grow. These methods detect very small changes, so it is common in the field to plot them several times actual size, so that the pattern is easily seen. The image shows the relative change in landmark position, not magnitudes.

CVA score plots separated specimens into groups. In cross validations, there was a high correct assignment to pressure-based substrates in all groups except 10 print cards where the results were not pronounced. This indicates that 10 print cards may be relatively immune to pressure affects. Substrate differences were always detectable, an effect that increased with increasing pressure.

For the analysis of footwear impressions, three brands of shoes were used: Nike Lunar, Converse

• Substrate	Correct assignment to Pressure
Computer Paper	86.7%
Re-tabs	93.3%
Soft gloss	91.67%
Ten-print	40%
Random expectation	33%

Figure 3. CVA of data grouped by substrate 10 prints cards which were fairly immune to pressure differences.

All Stars, and Sperry Topsiders. The brands were chosen due to differences in sole patterns.

Soles were scanned on a flatbed scanner. Landmarks were placed on the digital images of the soles at ending patterns at defined positions of the shoe. This method of identifying a class characteristic (shoe size) based on shape only with no scale needed, worked for some types of shoes.

For the Sperry Topsider, for example, no clear pattern emerged and shape differences did not correlate well with shoe size. In the Converse, there was a nice linear trend shape change with size. Nikes did not have a clean grouping.

Yet, only one shoe (size 11) did not line up according to size. Attempts to match right to left shoes were successful with the error being one half shoe size. Error could be related to the manufacturing process.

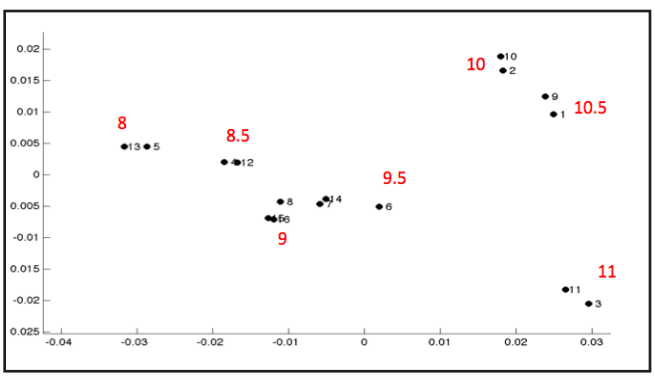

Figure 4. PCA plot for all 16 Converse brand sneakers. The values along the two axes are the specimen scores along the PCA axes, in units of Procrustes distance. This plot shows the differences between shoes of different size are large compared to the difference betwen shoes of the same size.

Wear rate of accidental characteristics was difficult to quantify and will form the basis of future work. Wear trends could easily been seen on the heel and toe portion while the rest of the shoe was basically unaltered. There was indication that accidental characteristics in areas other than the heel and toe may remain unaltered for a great length of time. In this project they remained the same over 7 weeks.

Figure 5 and 6. Baseline and week 7 of footwear show as sole wear trends.

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

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

DISSEMINATION

Project findings aired on TV and were also presented at annual meetings, seminars, and symposia. A final report was posted on the MFRC website while a manuscript was prepared for submission to the *Journal of Forensic Sciences*.

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". Oral 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". Oral Presentation at the 64th Annual Meeting of the American Academy of Forensic Sciences, Atlanta, GA. February, 2012.

- Bush, M., Langenburg, G., Gross, S., Bush, P., Sheets, D. "A Statistical Investigation of Distortion in Fingerprints: A Geometric Morphometric Approach". Oral Presentation at the NIJ Pattern and Impression Evidence Symposium, Clear Water, FL. August, 2012.

- Donnelly, L. "Fingerprints: Distortion with Regard to Diameter of Surface Touched". Thesis, Forensic Chemistry program, Buffalo State College, New York. Mary Bush mentor.

- Bush, M., Torres, A., Langenburg, G., Gross, S., Bush, P., Sheets, D. "Distortion in Fingerpints: A Statistical Investigation Using Shape Measurement Tools". Manuscript submitted to the *Journal of Forensic Science*.

- Channel 4 local news in Buffalo, New York, did a segment on the footwear impression portion. It aired on August 2, 2011, and was called "Footprints may help defeat criminals".

- Information on the research was presented in the form of a webinar. It was entitled "Shape Measurement and Statistical Tools: Application to Fingerprints and Footware Evidence". It was made available through the Idaho State Police Forensic Service. August 2012.

IMPLEMENTATION

The project and its findings were highlighted in the Forensic Science Foundation Award workshop "Applications of Geometric Morphometrics in 2D and 3D for Forensic Comparisions" at the Annual Meeting of the American Academy of Forensic Sciences, Atlanta, GA.

The scientific working group on shoeprint and tire tread evidence (SWGTREAD) expressed interest in the footwear portion of the project. Commentary was received for the work that involved placing the accidental characteristics. Future work will involve collaboration with SWGTREAD.

The statistical software used in the project is available as freeware and can be found at :
http://www3.canisius.edu/~sheets/

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

FORENSIC TECHNOLOGY NEED

The study of gunshot-related blood spatter is a common and often critical task for investigators. Relatively few studies have examined the dynamics of firearms-related blood spatter. In part this is due to the lack of realistic models to simulate cranial gunshot wounding and the formation of blood spatter. This lack of suitable models makes it difficult to answer case related questions.

TECHNOLOGY DESCRIPTION

In this project, a physical model of the human head is constructed that can be used to test theories relevant to cranial gunshot wounds and to demonstrate gunshot-related blood spatter. The model is based on anatomically accurate dimensions and best available simulant materials.

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

METHODOLOGY

The objective of the project is to design and develop a realistic model that provides forensic scientists with a tool to simulate the formation of blood spatter and to understand the mechanism of spatter projection. This is accomplished by:

- Identifying and obtaining materials that adequately simulate relevant anatomical features of the human head.
- Constructing the physical model that permits the visualization of intra-cranial dynamics and external spatter formation.
- Demonstrating the use of the model for case specific reconstruction experiments.
- Compiling a set of gunshot-related video clips to be added to the widely used blood pattern analysis (BPA) video library.
- Demonstrating the use of the model for studying critical cranial mechanistic components such as tail splashing and intra-cranial cavitation effects.

ACCOMPLISHMENTS AND ONGOING WORK

Anatomical structures of relevance to cranial gunshot wounding were defined as brain, skull bone, skin tissue, and blood. A thorough review of the literature showed that common simulant materials for soft tissue are ballistic soap and gelatin of varying concentrations; for skin different types of leather, polyurethane-coated polyamide fleece, and polyurethane sponges coated with silicone; for bone polyurethane composites; and for brain gelatin, silicone gel, and siloxane-based polymers.

An experimental set-up was constructed to test and assess the brain simulants. A series of tests on 5%, 10%, and 15% gelatin were conducted. It was found that there is a direct relationship between the water content of the gel and its efficiency to absorb the kinetic energy of the projectile. Indications are that the most suitable gelatin surrogate for sheep brain contains a water mass fraction of 5 to 10%.

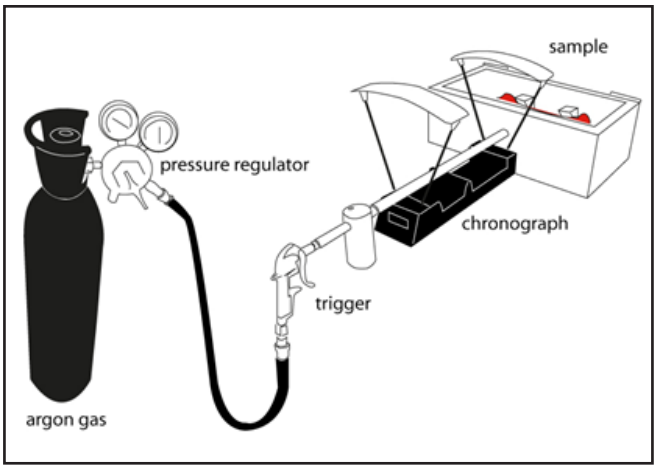

Figure 2. Low velocity depth of penetration apparatus.

A small indoor test range was then constructed and tests were conducted to determine the performance of the facility. It was found that the medium-velocity testing range performed well and that high speed video recordings of ballistic experiments involving pig shoulder bone, sheep brain, and brain simulant materials looked good.

A number of discriminatory tests were developed to show maximum separation between two rheologically different fluids. Tests were used to evaluate the performance of candidate blood simulant materials. They were also used to measure and compare the following items: the final drip stain diameter, the total number of observable spines and scallops of a drip stain, the calculated angle of impact of a drip stain, the number of spatter stains found in the upper regions of an impact pattern, and resolution of a transfer pattern.

It was found that spreading and splashing of passively dripped bloodstains is more Newtonian than previously thought. By applying the concept of effective viscosity to passive dripping models, the shear-thinning properties of blood were not found to influence the final spread diameter and number of observable spines and scallops of a resultant stain. These findings encourage creating water-based fluids as candidate SBSs. To date, water-based mixtures of 50% v/v honey and 30% glycerol with 10% w/v starch produce patterns with the most blood-like characteristics.

To assist with the validation of the model, a study was undertaken of cranial gunshot wounds from criminal cases, and head gunshot wound photographs from 53 U.S. victims were compiled. Gunshot wounds from eight caliber classes were represented.

Photographs were organized in MS Power Point by caliber class of the involved firearm as determined by crime scene findings, Medical Examiner reports and Firearm Section reports. Medical Examiner reports were also used to label photographs as entrance or exit wounds.

The research is currently focusing on design and construction of a realistic blast headform. This is accomplished through consultation and collaboration with the University of Canterbury (NZ) and the University of Nebraska-Lincoln (USA).

TECHNOLOGY BENEFITS

The new model assists forensic scientists investigating cases involving cranial gunshot deaths and instructors of forensic training courses. It is suitable for the scientific simulation of cranial gunshot wounding and associated splatter formation. In the course of testing the model, a comprehensive set of high-speed videos is generated providing a valuable new teaching resource.

COLLABORATION

This project is a collaborative effort between the Institute of Environmental Science and Research (ESR) in Christchurch, NZ; the University of Otago (UO) in Dunedin, NZ; and the Kansas City Police Crime Laboratory (KCPCL) in Missouri. The project brings together forensic scientists from New Zealand and the United States. The ESR and UO design, develop and construct the physical model. ESR also provides the high speed digital

camera and backlighting system for high speed video imaging. The KCPCL provides the test site and conducts the testing model.

DISSEMINATION

Project results and findings will be presented at national and international forensic meetings. Depending upon the outcome of the project, a number of manuscripts might 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

No publications or presentations have resulted from this project yet.

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.

The KCPCL will host a demonstration of the model and invitations to participate will be 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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