NEAFS Newsletter

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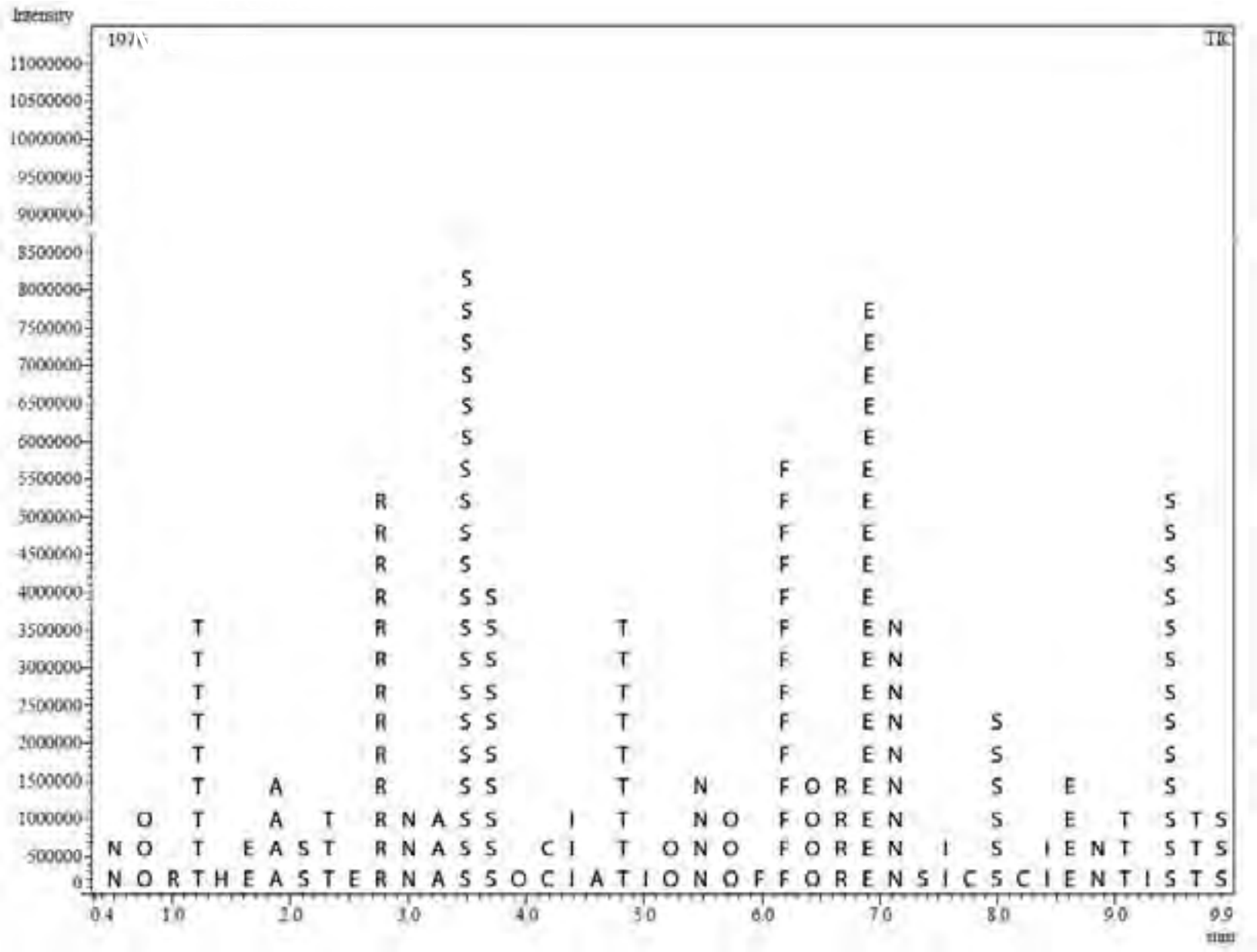

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Westchester County, NY Forensic Laboratory from Dec. 2005 to May 2011
BS in Chemistry-Fordham University
MS in Criminal Justice-LIU Post

Maria Tsocanos - President-Elect
Westchester County Forensic Laboratory, NY
Forensic Scientist in the Forensic Biology section
BS in Forensic Science - John Jay College of Criminal Justice

Adam Hall Ph.D., D-ABC - Treasurer
Director, Mass Spectrometry Facility-Barnett Institute of Chemical and Biological Analysis-
Northeastern University, Boston, MA 2014- Present
Instructor of Forensic Chemistry, Boston University School of Medicine, 2007-2014
Forensic Chemist II, MA State Police Crime Laboratory, 2002-2007
BA in Chemistry - Stonehill College
MS in Chemistry - Northeastern University
PhD in Analytical Chemistry - Northeastern University

Angela Violotti – Secretary
Connecticut Forensic Lab, Connecticut Department of Emergency Services and Public Protection, Division of Scientific Services
Forensic Science Examiner 1 for approximately 4.5 years
BS in Biochemistry – Cedar Crest College
MS in Forensic Science – Cedar Crest College

Stephanie Minero– Director
Nassau County Office of the Medical Examiner, Division of Forensic Services
Forensic Scientist in the Controlled Substance Analysis Section since 2008
BS in Forensic Science - Long Island University/CW Post
MS in Biology - Long Island University/CW Post

Elizabeth Duval - Director
Massachusetts State Police Crime Laboratory
Forensic Scientist II, 2009-present
BS Genetics, Texas A&M University
BS in Forensic Science, University of New Haven

Alanna Laureano- Director
Westchester County Department of Labs & Research, Division of Forensic Sciences Since 2007
Forensic Science Specialist and Assistant DNA Technical Leader
BS in Molecular Biology and Biochemistry- University at Albany, SUNY
MS in Forensic Biology- University at Albany, SUNY

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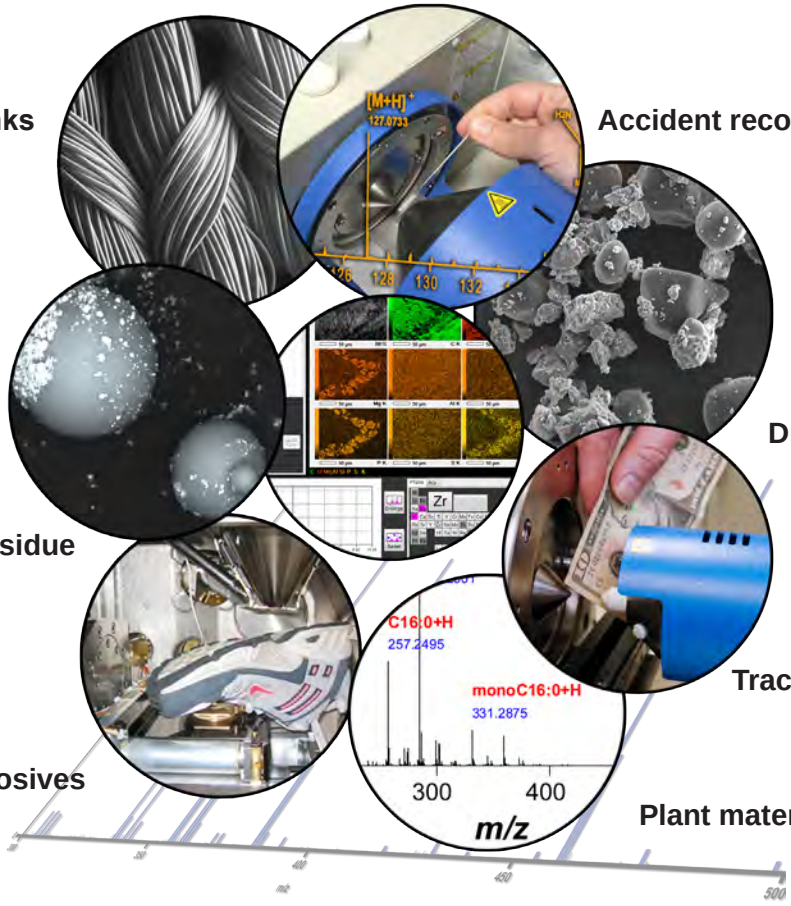

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A Message from President Tiffany A. Ribadeneyra

Happy New Year! I'm pleased to announce that we had record breaking attendance at this year's annual meeting. Congratulations to Program Chair Maria Tsocanos and her meeting staff for an extremely successful meeting. In case you were unable to attend, some highlights included honoring Dr. Lawrence Quarino with the NEAFS meritorious award as well as a fun-filled roaring 20's themed president's reception. If you had the pleasure of attending and would like a certificate of attendance, you may submit an electronic request by visiting https://www.neafs.org/neafs-annual-meetings and we would be happy to provide you with one. Don't forget to mark your calendars for the 2020 meeting from Oct. 14th-17th in Groton, CT.

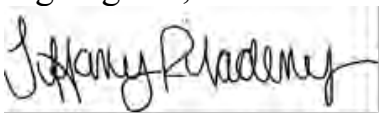
Throughout the year I have emphasized the advantages of being a NEAFS member and would be remised if I didn't summarize some of these opportunities in my final president's address. For only $50/year you can:

- Apply for a NEAFS Sponsored Training to come to your lab ($4000-$10,000)
- Attend the NEAFS annual meeting at little to no cost ($130 or less)
- Apply for a Training Scholarship Fund to attend a training ($400)
- Attend various workshops throughout the year at little to no cost
- Obtain reimbursement for professional certification such as ABC ($250) or IAI ($400)
- Access training and workshop material on the "members-only" area of our website
- Network and build lifelong professional relationships (invaluable!)

Speaking of lifelong professional relationships, I would like to closeout my tenure as President by thanking the board of directors for aiding me in a successful year. I am confident that the organization will be in capable hands under the leadership of incoming president, Maria Tsocanos. I would also like to thank the NEAFS membership for allowing me to serve as your president. I am honored and humbled by this experience. Lastly, I would like to thank my previous and current employers at Westchester and Nassau counties for supporting my involvement in NEAFS over the past 14 years. Without your support, this incredibly rewarding endeavor would not have been possible.

Our next board of directors meeting will be held remotely on February 1st. Feel free to email me at president@neafs.org if you have anything you would like the BOD to consider.

Signing Off,



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Northeastern Association of Forensic Scientists 45th Annual Meeting
The Lancaster Marriott at Penn Square, Lancaster, PA
November 12, 2019 – November 16, 2019

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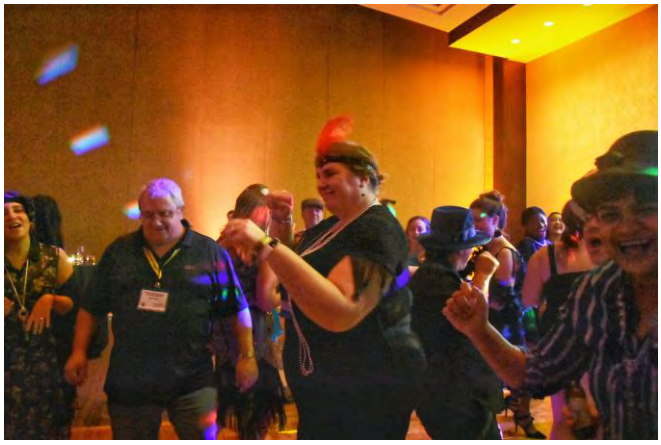

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2020 NEAFS Board of Directors and Staff

The Nominating Committee recommended the following slate of officers to the Board of Directors and an announcement was made to the Membership at the Annual Business Meeting on November 14, 2019. No additional nominations were received. The terms of office are January 1 through December 31.

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Elizabeth Duval

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Adam Hall

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Alanna Laureano

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Meritorious Service Award Recipient
Lawrence Quarino

Dear NEAFS Awards Committee,

I would like to formally recommend Dr. Lawrence Quarino as this year's recipient of the Meritorious Service Award. Larry began his career at the NYC OCME as a Forensic Biologist, and quickly rose up the ranks due to his hard work and dedication. Since then, he moved a few miles west and directs the Forensic Science Program at Cedar Crest College.

There, he serves as an educator, mentor, leader, and researcher. His program enrolls the second highest number of students in the college, and their graduates continue to make their organization proud and excel in their chosen paths. Through Larry's prolific NEAFS membership, he has been a familiar face at each meeting. He served as president in 2015 and had one of the most successful NEAFS annual meeting to date at Hershey Park, PA. During his tenure, he also instituted the Visiting Scientist Program which allocates funding for experts in our organization to travel far and wide to spread their knowledge to our member laboratories. He is a published, well respected, and certainly one of a kind member of our field and organization and should be recognized as such.

Thank you for your consideration, Stephanie Minero

Northeastern Association of Forensic Scientists

Meritorious Service Award Nomination Form

The Northeastern Association of Forensic Scientists is accepting nominations for the Meritorious Service Award.

This award is given to a NEAFS member that has a history of providing commendable service to the forensic science community by serving justice through casework, performing research advancing forensic science, training and educating forensic scientists and future forensic scientists, and overall contributions to the NEAFS organization. The nominee must have held the status of Regular Member within NEAFS for at least 10 years to be considered.

In addition to this form, a letter of recommendation outlining the nominee's contributions must be emailed to awards@neafs.org. All nominations must be received by September 1st. The winner of the NEAFS Meritorious Service Award will be announced during the annual meeting.

Nominator

First Name: Last Name:

Address:

Affiliation:

NEAFS Membership Number:

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Email Address:

Nominee

First Name: Last Name:

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Carol De Forest Research Grant Recipient
Meghan Appley (Fogerty)

Meghan is a Ph.D. candidate in the Chemistry Department at the University at Albany, has previously attended Le Moyne College in Syracuse, NY for her undergraduate degree where she majored in Chemistry and minored in Criminology, and she obtained a Master's degree in Forensic Science at the University of New Haven, CT.

Since high school Meghan has had a passion for forensic science and law enforcement. She prepared herself for a career in forensics by attending colleges that would best prepare her for her future. She immersed herself in the forensic science field through internships and research since high school. Because of these experiences she has had the desire to become a forensic chemist. Following graduating with her doctoral degree, Meghan hopes to enter the field of forensic science in order to make contributions to the work force. With an incredible interest in casework, as well as in developing, improving and validating methodologies that can improve the everyday tasks of law enforcement, Meghan strongly believes that she has the resilience, passion and determination required to pursue a career as a forensic chemist.

DEVELOPMENT OF FORENSIC METHODS FOR THE DETECTION AND IDENTIFICATION OF ILLEGALLY-TRADED ENDANGERED SPECIES OF WOOD USING MASS SPECTRAL TECHNIQUES

A. INTRODUCTION

The purpose of the research described in this application is to investigate the use of mass spectrometric analysis of headspace volatiles in combination with multivariate statistical analysis to detect and identify illegally-traded endangered species of wood. The hypothesis is that each wood species has a consistent and unique headspace chemical profile that can be used as a fingerprint signature for the detection and identification of wood species. This project is being conducted to develop a means by which to rapidly and accurately identify wood species in the field, in order to facilitate border patrol and other government agencies in combatting illegal trade in endangered species, an activity which is a major financier of illegal activities including global terrorism. It will be accomplished through pursuit of the following four specific aims:

- *Specific Aim I*: Demonstration that the headspace of illegally-traded endangered species of wood exhibit unique diagnostic chemical fingerprints.
- *Specific Aim II*: Development of a statistical analysis approach to the processing of the mass spectral data generated in Specific Aim I, to enable species identification based on chemical fingerprints.
- *Specific Aim III*: Identification of the species-specific biomarkers that enable accomplishment of Specific Aim II, for use in the rapid forensic presumptive field identification of species.
- *Specific Aim IV*: Creation of an endangered species chemical fingerprint database against which headspace profiles of woods can be screened in order to identify illegally-traded endangered species in the field.

B. BACKGROUND AND SIGNIFICANCE

Illegal logging and related trade occurs when timber is harvested, transported, processed, bought or sold in violation of national or sub-national laws. It is estimated that the global illegal timber trade nets up to $100 billion dollars annually. Illegal logging threatens some of the world's most valuable forests – from the Amazon to the Russian Far East. Illegal logging in developing countries is facilitated often by organized crime networks in conjunction with corrupt government officials, with many instances of selective logging taking place even in protected areas, due to the lack of forest law enforcement. As a result of weak forest governance, illegal timber accounts for over 70% of the timber exports of some countries, such as Peru, Bolivia and the Democratic Republic of the Congo.

The illegal felling of trees is a crime that is committed in plain sight, because when logs are harvested and sent to ports for export, it is usually impossible for border patrol agents to confirm the veracity of the claims made on the associated paperwork as to the identity of the species. In other words, most logs look alike, and illegally traded species need simply be labeled with Latin names of species that are legal to trade, in order to circumvent the law. Thus, the crime is rampant and to date, few effective strategies have been developed to address wood species identification. Current approaches include DNA profiling, morphological feature characterization and stable isotope analysis. All are costly, time consuming, and require extensive training, making them sub-optimal for field identification of logs in shipping containers. Therefore, a technique is required that will allow for the rapid forensic detection and identification of illegally-traded woods.

It is proposed that direct analysis in real time-high resolution mass spectrometry (DART-HRMS), coupled with thermal desorption gas chromatography-mass spectrometry (TD GC-MS) of the air in the vicinity of the wood (i.e. the headspace), can be used to swiftly reveal chemical signatures which when processed with multivariate statistical analysis processing tools, would enable species-level identification.

The identification of molecules in headspace volatiles through solid-phase microextraction (SPME)-facilitated analysis using a multitude of instrumental techniques has been reported for a wide range of

materials including drugs, plant material, and explosives.[2-4] In addition to SPME fibers for the collection and concentration of headspace volatiles, thermal desorption under vacuum has been utilized. In this approach, thermal desorption tubes packed with a sorbent or even filled with the material to be analyzed, can be interrogated by either DART-HRMS or GC-MS. This technique would be useful in the analysis of headspace volatiles of wood samples and could provide a means by which to alert agents to the presence of illegally-traded endangered wood species. The project presented here aims to develop and optimize a system by which headspace volatiles can be used to detect and identify a range of wood species remotely.

In order for such an approach to have practical utility in a real world context, bottlenecks often associated with analysis of headspace profiles must be addressed. The main issue with conventional approaches is the significant time required to complete the analysis by GC-MS and/or LC-MS, or other hyphenated techniques. Any analysis method that requires more than 1 -2 min of analysis is likely destined to remain underutilized because of the extent to which it delays border crossings and investigations. An analysis approach that is enjoying increased popularity in crime labs is the use of ambient ionization mass spectrometric techniques that require little to no sample preparation, and which, accordingly, cut down on sample analysis time. In this regard, the technique that has gained the most traction in crime labs in recent years is direct analysis in real time – mass spectrometry, either with unit mass or high resolution.

DART-MS utilizes an ambient ionization source, which means that samples can be analyzed directly under atmospheric conditions without pre-treatment steps (e.g. extraction or derivatization).[5] Using this approach allows for direct analysis of samples in the open-air sampling space between the ion source and the mass analyzer inlet in any phase (e.g. solid, liquid, gas or SPME fibers). This technique is beneficial for forensic science-related analyses because it requires minimal sample preparation and consumes very little time. Figure 1 illustrates the DART ion source. Samples are analyzed by suspending them for a few seconds in the "open air sample gap" between the ion source and the mass spectrometer inlet. High purity helium is exposed to a glow discharge to produce metastable helium atoms that react with water molecules in the atmosphere to create ionized water clusters. These then transfer a proton to the analyte to form $[M + H]^+$ (in positive ion mode), thus ionizing the sample. The protonated molecules then enter the mass spectrometer inlet and are detected.[1]

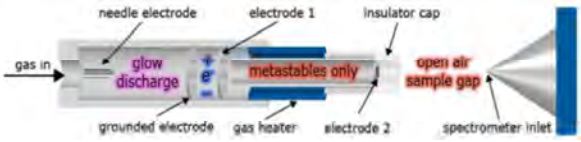


Figure 1. Illustration of DART ion source.[1]

The DART ion source is often interfaced with a high-resolution (HR) mass analyzer. By combining the ion source with a HR time-of-flight (TOF) instrument, accurate mass data can be collected. When using an instrument such as a JEOL AccuTOF DART-HRMS, the ions produced by the DART ion source can either be subjected to a low voltage (soft ionization) resulting in chemical profiles comprised of protonated precursor molecules, or the ions can be subjected to higher voltages resulting in collision induced dissociation, which produces a characteristic fragmentation signature. Both techniques are valuable and can be utilized to identify materials.

Because sample analysis requires less than 10 s, the method is well-suited to the generation of the hundreds or thousands of spectra and spectral replicates required to develop a robust statistical analysis work flow that will enable correlation of a given headspace chemical profile to a particular wood species, which would then facilitate accurate identification of the wood species that are in a particular space, such as a cargo container. Since this technique can generate large number of spectra, it is appropriate to consider how the application of multivariate statistical analysis of the data can be used to extract information that enables it to be correctly classified.

In 2009, a report was released by the National Academy of Sciences (NAS) which outlined a range of improvements that are needed in forensic science.[6] The report indicated that the strongest area in forensics is DNA analysis, not only because of the standardized procedures used, but mainly because of the use of statistical analysis processing of the results. Multivariate statistical analysis allows for

simplification and classification of complex results in an efficient and unbiased manner that is important in forensics. The data generated by DART-HRMS analysis are complex and large, not unlike the results obtained in DNA analysis. While statistical analysis processing is currently not readily applied to forensic science areas such as questioned documents, hair analysis, and shoe print analysis, it can be easily applied to MS data. Such treatment would provide 'weight' and a level of certainty to wood species identification reporting that is currently absent.[7] By applying chemometrics to headspace-derived DART-HRMS data, a classification system can be created to address issues outlined in the NAS report and enable more accurate identification of samples.

C. EXPERIMENTAL PROCEDURES

Specific Aim I:

The primary goal of Specific Aim I is to demonstrate that the headspaces of illegally-traded endangered species of wood exhibit unique diagnostic chemical fingerprints. To accomplish Specific Aim I, various species of wood will be acquired from the US Fish and Wildlife Forensic Research Lab as well as from local wood shops. The chemicals present in the headspace of these samples will be concentrated onto divinylbenzene/carboxen/polydimethylsiloxane coated 24-gauge SPME fibers, following shredding and transfer of the samples into vials. Additional sampling will be conducted using other sorbent materials to determine the optimal method to concentrate volatiles. Fibers will then be analyzed by direct analysis real time – high-resolution mass spectrometry (DART-HRMS). Our instrument features a (DART)-SVP ion source from IonSense Inc. interfaced with a JEOL AccuTOF high-resolution mass spectrometer. The SPME fibers will be analyzed in positive-ion mode with a gas stream temperature of 250 °C for a total of one minute over a mass range *m/z* 40-800. The DART ion source helium flow rate will be 2.0 L/min. The mass spectrometer settings will be set to: orifice 1 voltage, 20 V; orifice 2 voltage, 5 V; and the ion guide voltage, 400 V. By having the orifice 1 voltage set to 20 V there will be little to no fragmentation. Thus, the resulting spectrum will be comprised of peaks representing the protonated forms of all detected molecules (i.e. it will reveal the chemical fingerprint of the headspace). Setting the ion guide voltage to 400 V allows the detection of ions over *m/z* 40. Polyethylene glycol (PEG 600) will be used as a calibrant. TSSPro3 software will be used to calibrate, background subtract, and perform peak centroiding, and Mass Mountaineer will be used to perform mass spectral analysis.

Specific Aim II:

The primary goal of Specific Aim II is to demonstrate that statistical analysis processing of the data generated in Specific Aim I will enable species identification. If Specific Aim I is successful in showing that the headspace of wood species have diagnostic chemical signatures, then statistical analysis processing will be applied to enable identification of the material. This will be done by using algorithms coded in MATLAB. Different statistical analysis processes including data exploration approaches (e.g. principal component analysis) and classification systems (e.g. neural networks) will be used. Once the process is optimized, it will be used to create a user-friendly interface that will allow individuals to upload their sorbent-derived headspace DART-HRMS results into the platform for rapid classification and identification.

Specific Aim III:

The primary goal of Specific Aim III is to identify the molecules associated with the ability of a statistical analysis processing algorithm (developed in Specific Aim II) to enable classification to identify species. This exercise will reveal the identities of diagnostic biomarkers for specific species that are important in the detection and identification of illegally-traded endangered wood species. To do this, thermal desorption coupled with gas chromatography mass spectrometry (TD-GC-MS) will be used. A small amount (5 – 10 mg) of the shredded wood material will be placed in specialized thermal desorption tubes. The material will then be thermally desorbed using our GERSTEL Thermal Desorption Unit 3.5+. The headspace volatiles will then be concentrated using the cooling inlet system and injected onto our Agilent GC/MS. The mass spectra will be analyzed using MassHunter and compared to the NIST database in order to identify the

diagnostic molecules. It should be noted that the chemometric processing of the DART-HRMS data will have revealed which *m/z* values are important for species identification and discrimination, but the TD-GC-MS will reveal their identifies.

Specific Aim IV:

The primary goal of Specific Aim IV is to create a database against which headspace profiles can be screened in order to identify illegally-traded endangered species of wood. Following successful identification through statistical analysis, a database will be created against which wood headspace profiles can be screened to allow for remote species identification. The approach will be tested with sample unknowns.

D. PRELIMINARY RESULTS

The following data are presented to show proof-of-concept for the aims of the proposed work. The results fall under the umbrella of Specific Aims I, II and III, and illustrate that illegally-traded endangered species of wood do indeed exhibit unique chemical fingerprints which can be subjected to statistical analysis processing to obtain compound identification information.

Specific Aim I:

The focus of this Specific Aim is to show that by using SPME-facilitated DART-HRMS, the headspace volatiles of synthetic drugs can be used for identification and differentiation. For the preliminary data study, two sources of *Dalbergia nigra*, two sources of *Dalbergia retusa*, and one source of *Diospyros sp.* were selected based on their CITES status indicating the limitations on trade of these species. The samples were shredded and then transferred to scintillation vials. The mouths of the vials were sealed with aluminum foil. DVB/CAR/PDMS SPME fibers were conditioned by exposing them to a stream of dry helium gas at 250 °C for 30 min. The fibers were then exposed to the headspace for 30 min through a puncture in the aluminum foil. The SPME fibers were analyzed by DART-HRMS in positive-ion mode at 250 °C over a mass range *m/z* 40-800. Each source of wood was analyzed in replicates of ten. Data processing was accomplished using TSSPro3 and Mass Mountaineer software. The spectra of unexposed conditioned fibers were used as blanks.

Results for the headspace of each of the sources of wood species are represented as heat maps in Figure 2. Each row corresponds to an individual replicate. Similarities between spectra representing the same species were visually apparent and each species exhibited a unique signature. Furthermore, the signatures for each species were reproducible, even for those sourced from different locations.

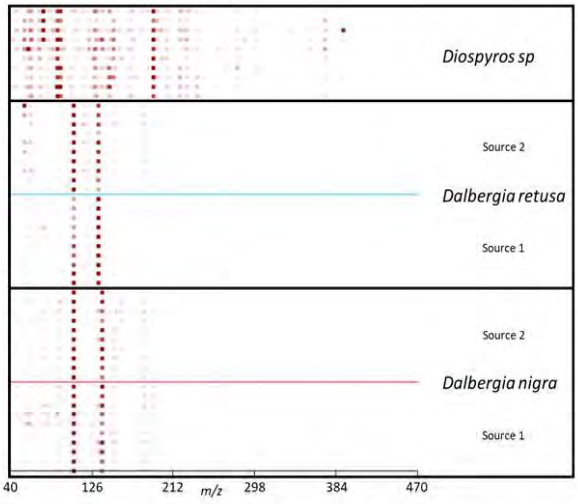


Figure 2. Heat maps representing the headspaces mass spectra of endangered species of wood. Each row represents a single run and the peaks are represented by colored bands at indicated *m/z* values whose color intensity is indicative of relative peak abundance.

Specific Aim II:

The results from the Specific Aim I experiments showed that unique headspace chemical profiles could be obtained for endangered species of wood. The data were subjected to multivariate statistical analysis processing to determine if the samples could be identified with a statistical level of certainty.

Feature masses were selected (shown in Table 1) to perform kernel discriminant analysis (KDA) which was used to determine if there were statistically significant differences between the different endangered wood species samples. Figure 3 shows the KDA plot.

George W Neighbor, Jr. Memorial Scholarship
Undergraduate Winner – Danielle Guckin

After graduating from Duquesne University with her master's degree in Forensic Science and Law, Danielle aspired to work for a crime laboratory as a Forensic Biologist. She chose this career path because DNA fascinates her. Danielle finds it enthralling how much information can be gained from a minuscule volume and with the continuous advancement of technology, the results are getting more sensitive and robust. She is interested in either remaining in the Forensic Biology division and moving up to a Lab Director position or earning her Ph.D. to work towards a position to teach Forensic Science courses at a university. The research she has been conducting as part of her master's degree has also inspired her to perform research after graduation to better Forensic Science.

Throughout Danielle's career at Duquesne University, she has become heavily involved with volunteerism, community service, and leadership. She joined the Duquesne University chapter of Habitat for Humanity my freshman year and has moved from a general member to the Advocacy Coordinator to the President of the organization chapter and finally to an executive member of the Leadership Team that was implemented by herself. She has also volunteered at UPMC Mercy Hospital and West Penn Hospital, where she cared for elderly patients to help prevent the onset of dementia. Danielle is also the Layout Editor of the D.U. Quark, a student-run scientific journal, where she learned how to manage a website, upload and peer review submitted scientific articles, and organize and compile journal issues each semester.
Danielle has also been working at Moe's Southwest Grill since 2016, where she started as a general Team Member and was promoted to a Shift Manager in 2018 due to her delegation and customer service skills, as well as her ability to handle high responsibility and pressure in an efficient manner. For the summer of 2018, she accepted an internship offer as an Autopsy and Death Investigations intern at the Allegheny County Office of the Medical Examiner. This was an amazing experience where she was able to perform autopsies with the autopsy technicians and assist the forensic investigators at crime scenes with the removal of the deceased.

Danielle believes she should be considered for the George W. Neighbor Jr. Memorial Scholarship due to her academic success, passion for the advancement of Forensic Science, and involvement in the community. She was a Girl Scout for six years which ignited her involvement in community service, leadership, and educating younger children. Through this ignition, Danielle has continued to advance these qualities at Duquesne University, in addition to performing research to advance Forensic Biology. The qualities she has gained and achievements she made caused her to be interested in a career in Forensic Biology and the education of students by teaching Forensic Science courses at a university.

George W Neighbor, Jr. Memorial Scholarship
Graduate Winner – Miranda Shaine
Boston University

After completing her first year of a Masters of Science in the Biomedical Forensic Sciences program at Boston University, Miranda is more passionate than ever about aspiring to become a forensic toxicologist. Her goals in the forensic field began during her undergraduate education. She graduated from the Honors College, magna cum laude, at The College at Brockport – State University of New York with a degree in chemistry and minors in both forensic science and criminal justice. Additionally, Miranda gained experience performing research on the effects of ionic liquids on nucleic acid secondary structures in biochemistry. She earned the 2017 Analytical Chemistry Award and the 2017-2018 Undergraduate Award in Physical Chemistry, both sponsored by the American Chemical Society for achievement at the highest level and demonstrating excellence and dedication in research and coursework, respectively. While at Brockport, what solidified her desire to become a forensic toxicologist was meeting another alumna who currently works at the North Carolina State Crime Lab, who has been a mentor of hers.

Miranda is currently working on my thesis using Surface-Enhanced Raman Spectroscopy (SERS) as a highly sensitive, non-destructive, and portable method to detect predominantly blood, as well as other various body fluids that are commonly found at a crime scene. This method utilizes the surface plasmonic resonance of SERS substrates coated with gold nanoparticles to illuminate the SERS spectral signal intensity and highlight the sensitivity of this method. Once this method is optimized and validated, it will be implemented at the Boston Police Department, and she will train the detectives on how to use this detection method for in-field sample analysis.

Miranda attended presented her research at the Northeastern Association of Forensic Scientists (NEAFS) conference and plans on presenting at the American Academy of Forensic Sciences (AAFS) conference in the upcoming year. In addition to her extensive research, she will be the teaching assistant for the instrumental analysis laboratory next semester which will enable her to have a hands-on approach and a stronger foundation in forensic toxicological practices. Concurrently, she has recently accepted the position of treasurer for the Boston University Forensic Science Society to become more involved in forensic science and be able to contribute to community building amongst future and fellow forensic scientists.

George W Chin Memorial Scholarship
Dino Robinson
John Jay College of Criminal Justice

Dino is a Graduate student at John Jay College of Criminal Justice as well as a full-time clinical technologist.

Striving to become a forensic scientist is the best objective he has set forth for himself, and he has never settled on having a backup option, because to Dino, becoming a forensic scientist was the only option. He made it his goal to maneuver in all ways necessary to make this aspiration possible.

He wishes to take his expertise in DNA extractions, sample preparation, sequencing and more, into a forensic setting where he can continue to contribute to society. He has hopes of eventually leading a team of individuals and developing advanced forensic skills and techniques that will ultimately grant him a position at the FBI headquarters.

Dino's current research efforts include studying identical twins and validating a method to differentiate between them from an epigenetic standpoint. As some research has surfaced on this topic it is wonderful to be diving into some applied forensic work associated with molecular biology. With a novel method utilizing targeted regions within the DNA that are prone to methylation, it is used to specifically identify twin A from twin B's epigenetic fingerprint by implementing a quantitative polymerase chain reaction method, normally not possible with routine short tandem repeat profiling. He is glad to be taking part of this study as it is a necessary contribution to forensic science that can help advance the field.

Dino is an achiever. He knows that the desire and dream of becoming a forensic scientist is no longer farfetched, and his knowledge will guide him to become successful. The George W Chin Memorial Scholarship will commemorate the passion and drive he has elicited in this program and will guarantee his continued success.

Dr. Peter De Forest Student Research Presentation Winner

PRESENTATIONS

Undergraduate: Ryan Zdenek, University of New Haven

"Vibrational Spectroscopic Analysis of 3D Printed polymers Pre- and Post- Manufacturing"

Graduate: Davis Watkins, Syracuse University

"Single Cell Analysis Using the DEPArray™ NxT System: Implications for Forensic DNA Mixture"

POSTERS

Undergraduate: Malorie Nitz, University of New Haven

"Investigating DNA Methylation Analysis for the Individualization of Monozygotic Twins

Graduate: Morgan Barrett, University of New Haven

"Comparison of STR Allelic Recovery Post-UV Damage Utilizing the PreCR® Repair Method: Singleplex versus Multiplex PCR Amplification"

ATTENTION STUDENTS:

Are you a current full-time undergraduate student in your junior or senior year, or are you either a part-time or full-time graduate student completing his or her degree in a forensic program at a regionally accredited institution located in the Northeastern U.S. (Connecticut, Rhode Island, Massachusetts, New Hampshire, Vermont, Maine, New Jersey, New York, and Pennsylvania)?

Then you are eligible to apply for:

George W. Neighbor Jr. Memorial Scholarship (undergraduate) - Award is $1750
George W. Neighbor Jr. Memorial Scholarship (graduate) - Award is $1750
George W. Chin Memorial Scholarship – Award is $2000

Carol De Forest Forensic Science Research Grants - Award is $2500
***Note** – eligibility is for both full-time undergraduate and graduate students

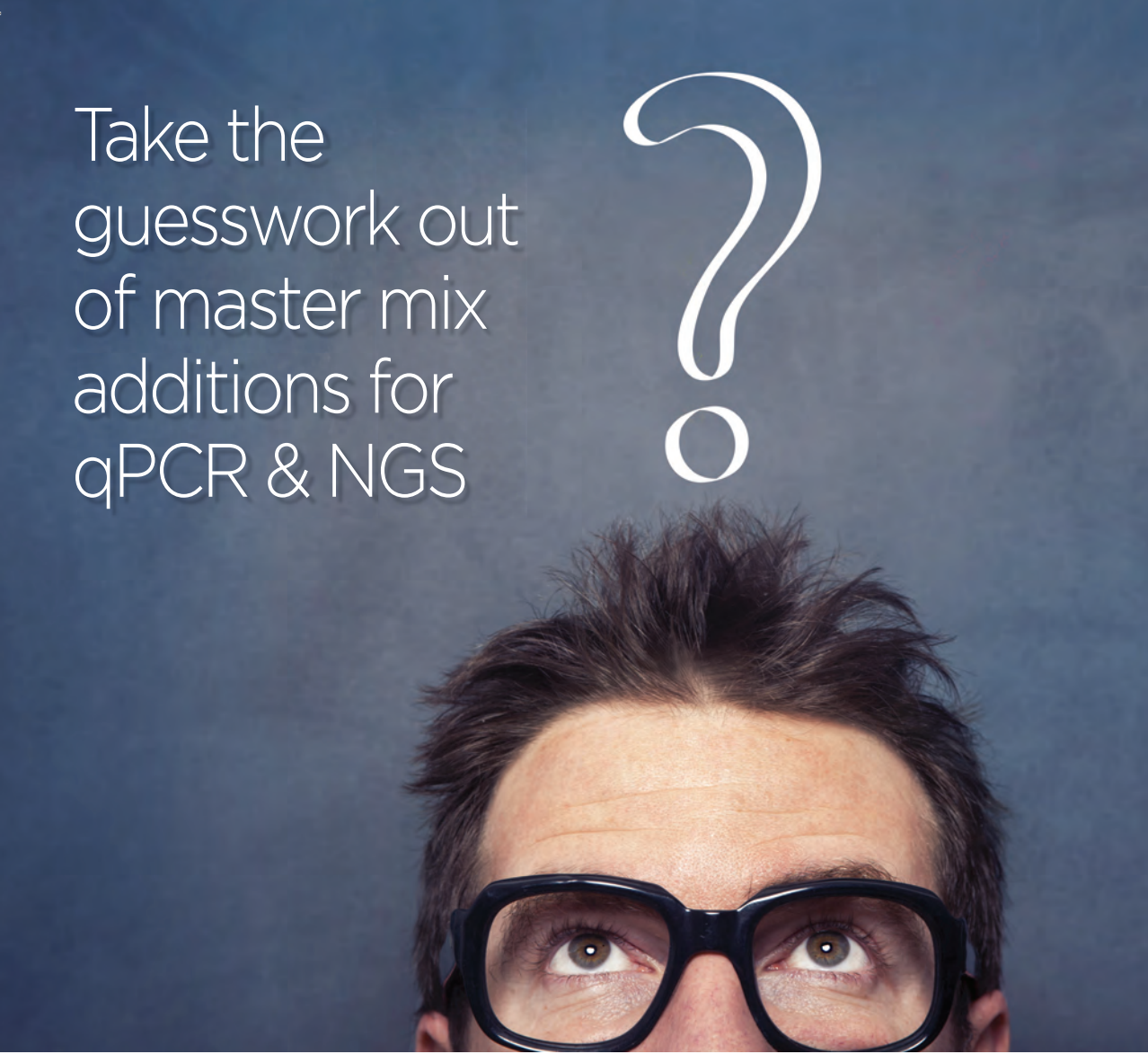
** **Note –** Two Research Grants will be Awarded.

All submission materials for either the scholarships or the research grants must be completed, and electronically submitted by April 30th. The 2020 Awards recipients will be notified no later than September1st.

For more information and Scholarship/Research Grant forms please go to http://www.neafs.org/

Questions or comments? Please email Awards@NEAFS.org.

Take the guesswork out of master mix additions for qPCR & NGS



Automate qPCR and NGS assays faster

Learn how to accurately transfer small volumes of master mix, and other critical reagents. The Artel MVS liquid handling and assay optimization platform improves assay results.

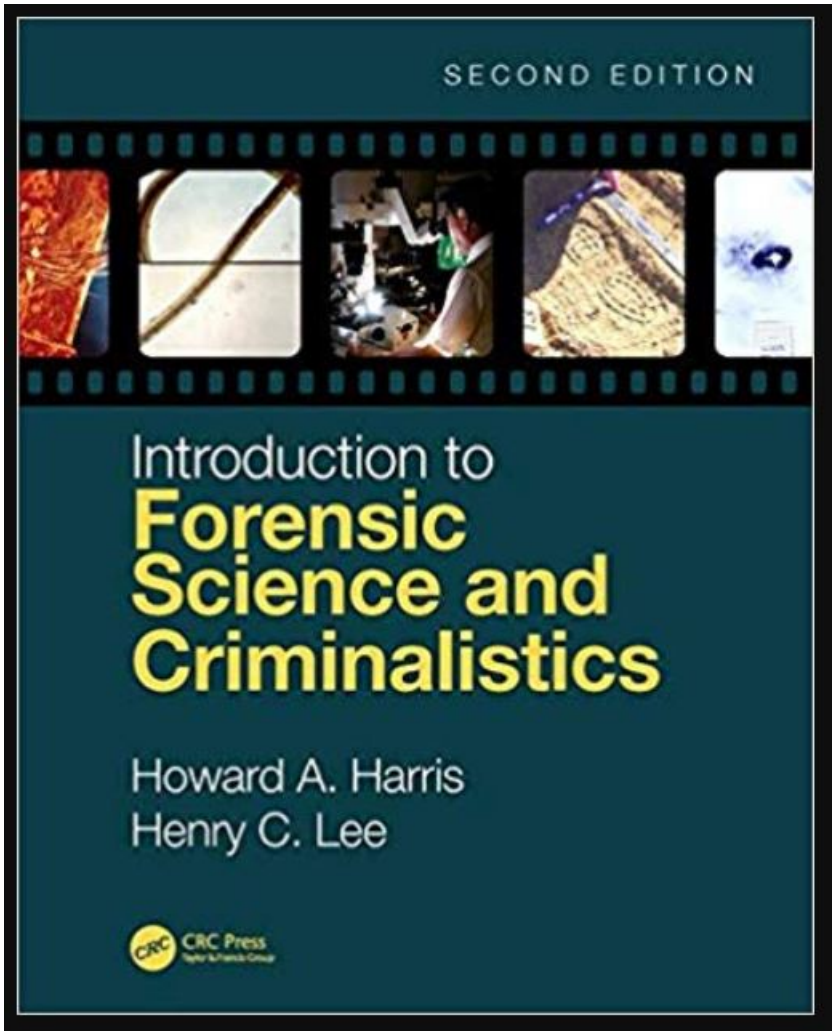
artel-usa.com/mastermix

ARTEL
Trust Your Results

Member News

Howard A. Harris and Henry C. Lee

This Second Edition of the best-selling ***Introduction to Forensic Science and Criminalistics*** presents the practice of forensic science from a broad viewpoint. The Second Edition is fully updated to cover the latest scientific methods of evidence collection, evidence analytic techniques, and the application of the analysis results to an investigation and use in court. This includes coverage of physical evidence, evidence collection, crime scene processing, pattern evidence, fingerprint evidence, questioned documents, DNA and biological evidence, drug evidence, toolmarks and fireams, arson and explosives, chemical testing, and a new chapter of computer and digital forensic evidence. Chapters address crime scene evidence, laboratory procedures, emergency technologies, as well as an adjudication of both criminal and civil cases utilizing the evidence. All coverage has been fully updated in all areas that have advanced since the publication.



Written by authors with close to one hundred years of forensic experience combined, this introductory text features comprehensive coverage of the types of forensic work done by crime laboratories for criminal cases and by private examiners for civil cases. The book's unifying vision of the role of forensic science in the justice system and of the role of the professional forensic scientist is clearly introduced in the first two chapters and reinforced throughout the text. Each chapter discusses a key case in the field and references other "real world" applications of the techniques described. The text's premise is that being a scientist is not required for understanding and using forensic science, but that a greater understanding of science lends itself to better use of the techniques of forensic science.

AMERICAN BOARD OF CRIMINALISTICS

FIELD TESTING OF THE NEW BIOLOGICAL EVIDENCE SCREENING EXAM FORENSIC DNA EXAM

Starting at the 2020 AAFS Meeting in Anaheim, CA, the ABC will begin the process of field testing two new Certification Exams: the Biological Evidence Screening Exam and the Forensic DNA Exam.

Biological Evidence Screening Exam

Qualifications:

The Applicant must possess a minimum of an earned baccalaureate degree, or equivalent, in a natural science (e.g. chemical, physical, or biological science) or forensic science from an accredited institution. Accredited institutions are those approved by regional accrediting commissions recognized by the U.S. Office of Education or foreign equivalent, and other institutions approved by the American Board of Criminalistics Board of Directors.

Experience:

Applicant must be currently working for a FSSP* (Forensic Science Service Provider) performing biological evidence screening.

Applicant must have been authorized to perform forensic casework in the field of biological evidence screening. Authorization must be obtained from a FSSP. Applicant must have a minimum of one year, full-time or equivalent, experience performing independent casework for a FSSP in biological evidence screening. Experience must be obtained after being authorized to perform forensic casework in the field of biological evidence screening.

*A FSSP, as defined by the National Commission on Forensic Sciences, is a forensic science service provider having at least one full-time analyst (however named) who examines physical evidence in criminal and/or investigative matters and provides reports or opinion testimony with respect to such evidence in United States courts of law. Forensic science service provider is used interchangeably with forensic laboratory.

AMERICAN BOARD OF CRIMINALISTICS

Forensic DNA Exam

Qualifications:

The Applicant must possess a minimum of an earned baccalaureate degree, or equivalent, in a natural science (e.g. chemical, physical, or biological science) or forensic science from an accredited institution. Accredited institutions are those approved by regional accrediting commissions recognized by the U.S. Office of Education or foreign equivalent, and other institutions approved by the American Board of Criminalistics Board of Directors.

Experience:

Applicant must be currently working for a FSSP (Forensic Science Service Provider) performing DNA analysis and interpretation.

Applicant must have been authorized to perform forensic casework in the field of DNA analysis, including interpretation. Authorization must be obtained from a FSSP.
Applicant must have a minimum of one year, full-time or equivalent, experience performing independent casework for a FSSP in DNA analysis and interpretation. Experience must be obtained after being authorized to perform forensic casework in the field of DNA analysis and interpretation.

*A FSSP, as defined by the National Commission on Forensic Sciences, is a forensic science service provider having at least one full-time analyst (however named) who examines physical evidence in criminal and/or investigative matters and provides reports or opinion testimony with respect to such evidence in United States courts of law. Forensic science service provider is used interchangeably with forensic laboratory.

The Process

This will be an extensive process, as the ABC is following ISO/IEC 17024 for validating these certification exams. The ISO/IEC 17024 process requires a minimum number of test takers that is equal to the number of questions on each exam. Once that minimum has been achieved, the certification exams will be analyzed using psychometrics (the science of measuring mental capacities and processes - dictionary.com), and cut scores determined.

AMERICAN BOARD OF CRIMINALISTICS

The ABC plans to continue to field test these two new certification exams at regional meetings and forensic biology meetings over the upcoming year until the required number of exam test takers is achieved, at which time the evaluation described above will start. Therefore, it will be some time before you are notified about your results of the exams.

At this point, the ABC plans to continue field testing:

- California Association of Criminalists meeting in late April/early May 2020.
- Mid-Atlantic Association of Forensic Scientists meeting in May 2020
- STAY TUNED FOR OTHER OPPORTUNITIES

Application

If you are interested in sitting for one or both of these field test certification exams at the AAFS meeting, please complete and submit an application by US Mail to the ABC Registrar. Applications for the AAFS sitting (February 19, 2020 in Anaheim, California) must be received by January 15, 2020. **The $250 sitting fee is waived for participation in the field tests.** Once other testing sites and dates are identified, this website will be updated with new application deadlines. Generally, the application deadline will be approximately 30 days prior to the date of the sitting.

Outcome

If you are notified you passed one or both of the field test certification exams, you will have to pay the $50 application fee to become certified. You will then be certified if you successfully complete the credentialing process (certification eligibility).

If you are notified that you did not successfully completed a field test certification exam, no personal identifying information will be retained to indicate that you sat for that ABC field test.

AMERICAN BOARD OF CRIMINALISTICS

Certification Scheme

The final certification schemes for each exam are in the processing of being finalized. Check the ABC website regularly for updates.

Study Guides

Links to the study guides for each of the field test certification exam are being finalized and will be posted on the ABC website soon. The study guides include the knowledge/skill areas for each exam associated with the tasks that use those knowledge skills and the references that should be studied for successful preparation.

If you have any additional questions, please contact the ABC Registrar.

NEAFS Sponsored Training

Have an idea for a training event?

Need to bring training to your lab?

Would you like NEAFS to subsidize and sponsor your training event?

Let's hear from you!

Interested laboratories shall complete the attached application and email it to the NEAFS Education Chairperson at education@neafs.org. Applications should be received at least 4 months in advance of the anticipated date(s) of the event. The contact person can expect to be informed by the Education Chairperson of the approval or denial of the request within 30 days of receiving the application.

Things to know:

- Due to finite amount of funds available annually, NEAFS will subsidize according to the following guidelines:
  - Small Training – 0 - 10 participants = up to $4000
  - Medium Training – 11 - 20 participants = up to $7000
  - Large Training – 21+ participants = $10,000
- If requesting more than $10,000 from NEAFS, funds may be available upon request pending approval by the Board of Directors and/or NEAFS membership.
- 20% of total participants must be NEAFS members or active NEAFS applicants.
- Training may be opened to additional labs to acquire additional funding beyond training size limitations.
- NEAFS will advertise the training on its website if registration is opened up to external (non-host) labs.
- If training funds requested exceed the guidelines and the requesting lab does not wish to open up training to outside labs then the lab is responsible for the difference in the cost of training. Registration fees for non-NEAFS members may be used.
- The number of contact hours for those claiming continuing education credit(s) as a result of the training will be determined by the instructor(s).

NEAFS Sponsored Training Request Form

Laboratory: _____

Laboratory Director/Manager: _____

Address: _____

_____

Contact Person: _____

Phone: _____

Email: _____

Requested Training:

Requested Scientist(s)/Trainer(s) (if known): _____

Approximate desired date(s) of training: _____

Number of individuals attending training: _____

Number of NEAFS members attending training _____

Request Details (to include travel/hotel costs, registration fees and training materials):

Room and Board for Presenter(s):	Yes ☐	No ☐	Approximate Cost: _____
Presenter(s) Travel:	Yes ☐	No ☐	Approximate Cost: _____
Presenter(s) Stipend:	Yes ☐	No ☐	Approximate Cost: _____
Meals for Attendees:	Yes ☐	No ☐	Approximate Cost: _____
			(Subtotal): $ 0.00
Registration Fee(s):	Yes ☐	No ☐	Approximate Cost: _____(less)

Grand Total: $ 0.00

Additional Requests:

Visit neafs.org under the merchandise tab!

GET YOUR NEAFS GEAR!

2019 Training Scholarship Fund

The Northeastern Association of Forensic Scientists (NEAFS) is proud to offer its members a 2019 Training Scholarship Fund. Regular members, in good standing, are eligible to receive up to $400 towards training, workshop or non-NEAFS meeting registration expenses. Detailed instructions and application forms are available on the NEAFS website. Simply click the “Training” link at the top of the screen and scroll down to the “NEAFS Training Scholarship Forms”. The current application period is January 1st, 2019 to December 31st, 2019. Reimbursements will be issued on a first come, first serve basis and funding is limited. If you plan to attend a non-NEAFS meeting workshop, training or course during this application period and will not be funded by your agency or any other non-NEAFS related entity, we highly encourage your swift application for the 2019 Training Scholarship Fund. Please visit the NEAFS training website to take advantage of this great NEAFS opportunity and to view upcoming training opportunities!

Dr. Jillian Conte used the scholarship fund to aid her attendance to the International Society of Forensic Genetics Workshop in September 2019.

Bayesian Reasoning in Prague at ISFG
By: Dr. Jillian Conte

I was excited to see Dr. John Butler offering a workshop in Scientific Publication during the International Society for Forensic Genetics – who better to learn from than the best? Fast forward two months when I began my registration only to find out the workshop was filled! I registered for my second choice, "Bayesian Reasoning in the framework for Bayesian Networks" with Tomas Furst, a mathematician from Prague, Czech Republic.

For those of you in casework using likelihood ratios and/or probabilistic genotyping, you know to pay close attention to how you describe your statistics to not fall into the prosecutor's fallacy. I am not longer in casework, so I needed to make sure I understood this when educating my students. Dr. Furst went through the fallacy with an easy to understand example: you see a person fishing in a lake, what is the probability that the man you see is a fisher, or what is the probability that the fisher you see is a man. These are two different probabilities and one must be careful to calculate properly.

Our workshop continued onward with framing our inferences in Bayesian Network software. Furst used a relatable example, the probability of a baby crying during the night, to help us understand the relationships between events and how they effect the probabilities. The baby awakes and cried during the night because he is wet or because he is hungry. If the baby is wet there is a high probability he is also stinky. If we become more knowledgeable about the situation, let's say we know the baby is hungry; this changes the probability of the baby crying. We used UnBBayes, a probabilistic network framework, to study this relationship. It was easy to use and quickly calculated probabilities. Furst explained how using probabilities can help large processes in business and manufacturing to identify and resolve issues. These examples really helped understand probabilistic genotyping.

The workshop was only half a day, we could have easily gone through inferences for two days, but the conference was beginning. Displayed were over 600 posters, and 60 oral presentations. I learned of forensic applications of phenotyping of human pigmentation and greying of hair, ethical and legal issues surrounding forensic genealogy and familial searching, and how non-human DNA is being used increasingly for forensic applications, such as provenancing soil samples. I shared two pieces of my research involving DNA recovery from immunochromatographic test strips and comparisons of genotyping software. Some vendors were giving out cans of beer instead of pens, as the Czech Republic is known for its beer. This entire experience was jam packed with opportunities to learn and grow as a forensic biologist. The ISFG Congress is held every two years, the next one will be held in Washington D.C. in August 2021. I encourage NEAFS members to attend, it's a wonderful opportunity to learn about forensic genetics all over the world.

Upcoming Training

February 2020

Advanced Bloodstain Pattern Analysis Workshop
Miami-Dade Public Safety Training Institute, 9601 NW 58th St., Doral, FL 33178
February 10-14, 2020

This advanced level course is designed for practitioners who have successfully completed basic instruction in Bloodstain Pattern Analysis and desire to build on that fundamental knowledge while working toward expertise in the discipline. This workshop will begin with a brief review of the basic concepts and will continue with the student applying those concepts in the analysis of bloodstain pattern crime scenes with report generation and verbal defense of findings.

Mock crime scenes with the associated clothing and physical evidence, will also be completely analyzed from documentation and stain selection through report writing. In doing so, the entire BPA methodology will be practiced and employed, including the consideration of autopsy findings and forensic biology reports.

Case specific limitations, quality assurance and context bias will also be addressed throughout the workshop.

Workshop Description: http://noslowforensic.com/new-page/

Instructor: Toby L. Wolson, M.S., F-ABC, Noslow Forensic Consultation, LLC, E-mail: Toby.Wolson@gmail.com

Further information about this workshop can be obtained at the following website: http://noslowforensic.com/new-page/ or by contacting Toby Wolson (Toby.Wolson@gmail.com).

March 2020

Bloodstain Pattern Analysis on Fabrics with an Introduction to Digital Casework Workshop
Miami-Dade Public Safety Training Institute, 9601 NW 58th St., Doral, FL 33178
March 16-20, 2020

This is an advanced bloodstain pattern analysis workshop examining the interaction of liquid blood with fabrics, textiles and clothing. Coupled with an introduction to the analysis of bloodstain casework that has been provided in a digital format. Participants must have had a minimum of an introductory level BPA workshop prior to attending this workshop.

Workshop participants will participate in practical exercises to enhance their ability to interpret complex bloodstain patterns deposited on fabrics, textiles and clothing. This will be accomplished by the participant's dripping, splashing and transferring liquid blood onto fabrics, textiles, and clothing and observing the macroscopic and microscopic interaction of the blood with the substrate. Workshop participants will also learn to evaluate bloodstain evidence from digital files by working on cases provided in a digital format. In addition, the participants will receive training in the writing of bloodstain pattern analysis reports.

Workshop Goals:

1. Enhance the participants skills for the examination of bloodstained clothing.
2. Enhance through lectures, experimentation, and laboratory practicals the participants understanding of fabric composition, construction, and finishes that can affect the appearance of the bloodstain patterns.
3. Enhance through lectures, experimentation, and laboratory practicals the participants understanding of how liquid blood interacts with fabrics.
4. Enhance the participants skills for writing bloodstain pattern analysis reports concerning the examination of clothing.
5. Enhance the participants skills for presentation of bloodstain pattern evidence on clothing as an expert witness.

Workshop Description: http://noslowforensic.com/new-page/

Instructor: Toby L. Wolson, M.S., F-ABC, Noslow Forensic Consultation, LLC, E-mail: Toby.Wolson@gmail.com

Further information about this workshop can be obtained at the following website: http://noslowforensic.com/new-page/ or by contacting Toby Wolson (Toby.Wolson@gmail.com).

NORTHEASTERN ASSOCIATION OF FORENSIC SCIENTISTS

2020 TRAINING SCHOLARSHIP FUND

OPEN APPLICATION PERIOD: JANUARY 1st, 2020- DECEMBER 31st, 2020

APPLICATION REQUIREMENTS

The Northeastern Association of Forensic Scientists (NEAFS) is proud to offer its members a Training Scholarship Fund (TSF). Members in good standing are eligible to receive up to $400 towards training, workshop or non-NEAFS meeting registration and travel expenses. Individuals will only be allowed reimbursement once per application period. Any NEAFS Annual Meeting expenses are ineligible to receive funding. Reimbursement will occur upon receipt of a certificate showing successful attendance and completion of the course along with an article summarizing the course for the NEAFS newsletter.

APPLICATION INSTRUCTIONS

Applicants must submit a *Pre-Approval Application* prior to attending the training for which they wish to obtain funding. All applications must be complete with a brief course description, statement as to how the applicant will benefit from attending the training and justification for receiving funding (i.e. insufficient employer funding or continuing education requirements).

Notification will be given to each applicant upon receipt of the *Pre-Approval Application.* This notification lets the applicant know that their submission has been received by the Awards Chair at NEAFS and is being reviewed. Applicants can expect to be informed of the acceptance or rejection of their application within 60 days of receiving this *Pre-Approval Application* notification.

Upon successful attendance and completion of the training, all pre-approved applicants must submit a *Reimbursement Application* along with supporting documentation. Whenever possible, a certificate should be provided as proof of attendance and completion. If a certificate is not issued, or is unavailable, a letter from the organizer/ instructor verifying the applicant's successful attendance and completion shall suffice. Each Training Scholarship Fund recipient is required to contribute to NEAFS and its members by publishing a written article in the Newsletter. *Reimbursement Applications* will only be considered complete when accompanied by a 1000-word (minimum) course summary.

All application materials can be found on the NEAFS website. Please submit all inquiries, applications and supporting documentation to: awards@neafs.org.

NORTHEASTERN ASSOCIATION OF FORENSIC SCIENTISTS

TRAINING SCHOLARSHIP FUND

PRE-APPROVAL APPLICATION

Instructions: To be completed prior to attending the workshop/meeting eligible for reimbursement.

Applicant Information

First Name: _____ Last Name: _____

Organization/Agency: _____

Street: _____

City: _____ State: _____ ZIP Code: _____

Phone: _____ Fax: _____ E-Mail: _____

NEAFS Member Number: _____

ABC Status: ☐ Diplomate ☐ Fellow ☐ Board Member ☐ Affiliate ☐ Exam Committee

Training Information

Course Title: _____

Sponsor/Host Organization: _____

Meeting Dates & Times: _____

Location: _____ Travel Dates & Times: _____

Course Description: _____

_____

_____

Attendance Benefit to Applicant: _____

_____

_____

_____

_____

Expenses

Registration Cost: _____ Travel Cost: _____

Justification for Reimbursement:

_____

_____

_____

_____

_____

Will you be reimbursed by your agency or any other non-NEAFS related entity for any expenses incurred as a result of attending the above training? ☐ Yes ☐ No

If "Yes", provide funding agency and amount: _____

Have you been funded to attend all or part of a workshop or training course this year? ☐ Yes ☐ No

If "Yes", provide cost of workshop(s)/training(s) attended and amount compensated: _____

_____

_____

FOR OFFICIAL USE ONLY: Reference #: _____ Date Received: _____ Initials: _____

NORTHEASTERN ASSOCIATION OF FORENSIC SCIENTISTS

TRAINING SCHOLARSHIP FUND

REIMBURSEMENT APPLICATION

Instructions: To be completed upon successful completion of the workshop/meeting approved for reimbursement.

Applicant Information

First Name: _____ Last Name: _____
Organization/Agency: _____
Street: _____
City: _____ State: _____ ZIP Code: _____
Phone: _____ Fax: _____ E-Mail: _____

Training Information

Course Title: _____
Sponsor/Host Organization: _____
Meeting Dates & Times: _____
Location: _____ Travel Dates & Times: _____
Instructor/Organizer: _____
Phone: _____ Fax: _____ E-Mail: _____

*If applicable, provide proof of attendance and successful completion by attaching a certificate to this form.

NOTE: Each applicant must write a 1000-word (minimum) course summary to accompany this form. Applicants awarded full or partial workshop reimbursement will have their course summaries published in the NEAFS Newsletter. The preferred format is a Microsoft Word document. Reimbursement applications submitted without a course summary will be considered incomplete and ineligible for reimbursement.

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Expense(s) Incurred: _____

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Will you be reimbursed by your agency or any other non-NEAFS related entity for any expenses incurred as a result of attending the above training? ☐ Yes ☐ No

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Feasibility of the Analysis of Fentanyl Analogs in Postmortem Blood Using Biocompatible Solid-Phase Microextraction (BIOSPME®) followed by Direct Analysis in Real Time Mass Spectrometry (DART-MS) and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Introduction

From 2015 to 2017, there was a 65% increase in drug-related overdose deaths in Pennsylvania (1). In 52% of these cases, fentanyl and fentanyl-related substances were identified in decedents, with heroin being the second most frequently identified substance (1). Due to the frequency at which these illicitly manufactured fentanyl analogs are emerging, it is increasingly difficult for laboratories to keep up with the development of methodology that can detect these compounds in post-mortem samples. The fentanyl epidemic has also caused an increase in post-mortem toxicology casework due the surging number of drug-related overdose deaths. Therefore, the development of more efficient techniques for screening post-mortem samples for fentanyl and its analogs would greatly benefit toxicology laboratories.

A major advancement in sample preparation and analyte extraction came in 1989 with the introduction of solid-phase microextraction (SPME) (2). Some benefits of this methodology include the elimination of extraction solvents as well as fast and simple extractions. SPME is also compatible with separation/detection techniques, which are already available in some laboratories.

More recently, the application of in-vivo SPME in forensic toxicology research as received some attention since it allows for the direct analysis of biological fluids and organs without having to collect the actual samples (3). A concern regarding in-vivo SPME is that other components of biological fluids, such as macromolecules, will bind to the SPME fiber in place of the analyte of interest. To avoid this, biocompatible SPME (BioSPME®) was developed (3). BioSPME® fibers contain a small metal core which contains a coating of either C-18 or mixed-mode chemistry all secured by a pipette tip (Fig. 1). Inside the metal core are functionalized silica particles, which are embedded in an inert binder (4). The binder will exclude large biomolecules commonly found in biological matrices and allow smaller molecules, such as drug compounds, to penetrate and absorb onto the silica particles in the fiber (4). Due to this novel design, the need for protein precipitation steps is eliminated making BioSPME® useful for direct sampling of biological matrices (4). Additionally, these fibers allow for sample concentration and cleanup to occur simultaneously which reduces sample processing, ultimately speeding up the extraction process (4). An additional benefit is that these fibers are compatible with common solvents used in liquid chromatography (4).

The purpose of this study is to investigate the in-vivo biocompatible solid-phase microextraction technique, BioSPME®, for the extraction of fentanyl and its analogs from postmortem blood samples. BioSPME® was developed as an extraction method which could quickly extract drugs from biological fluids without the binding of macromolecules, which was a concern for previous SPME techniques. Figure 1 shows an example of one of the BioSPME® tips used in this study.

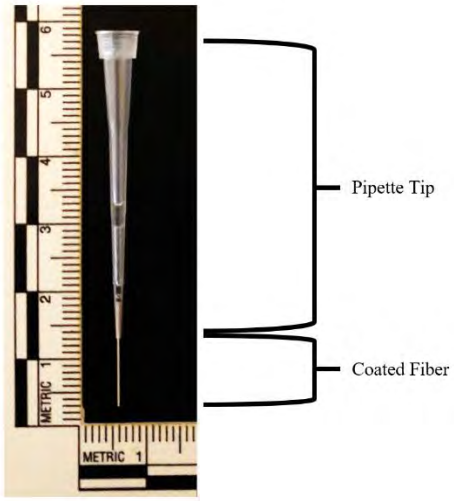


FIG 1 – *BioSPME® pipette tippet manufactured by Sigma-Aldrich.*

Grant, et al. (5) previously reported an initial investigation into the analysis of fentanyl in postmortem blood samples by extracting them with BioSPME® fibers followed by screening with gas chromatography-mass spectrometry (GC-MS) and confirming with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Using this method, BioSPME® extraction was compared to toxicology results and was found to be consistent with fentanyl blood concentrations over 21.4 ng/mL (5). In this study, the feasibility for the analysis of fentanyl and six fentanyl analogs in post-mortem blood was investigated utilizing BioSPME® methodology. The method being developed uses both direct analysis in real time mass spectrometry (DART-MS) as a screening method followed by LC-MS/MS for confirmation.

Reagents and Standards

Fentanyl, norfentanyl, fentanyl analog drug standards and internal drug standards were purchased from Cerilliant (Round Rock, TX) and Cayman Chemical (Ann Arbor, MI). Drug standards purchased from Cerilliant include fentanyl, fentanyl-D_5, norfentanyl oxalate, norfentanyl-D_5 oxalate, acetyl fentanyl, acetyl fentanyl-$^{13}C_6$, 4-ANPP, 4-ANPP-D_5, furanyl fentanyl HCl, (±)-cis-3-methylfentanyl HCl, and 4-fluoro-isobutyryl fentanyl. Drug standards purchased from Cayman Chemical include cyclopropyl fentanyl HCl and cyclopropyl fentanyl-D_5 HCl. HPLC grade methanol, LC-MS grade acetonitrile, and HPLC grade water was purchased from EMD Millipore Corporation (Darmstadt, Germany). LC-MS grade formic acid was purchased from Thermo Scientific (Rockford, Illinois).

BioSPME Fibers

BioSPME® fibers (Supelco, Bellefonte, PA) containing a small metal core with coatings of C18 or C8-SCX Mixed Mode chemistry were utilized in this study. BioSPME® fibers can be inserted into hypodermic needles or pipette tips. In this study, BioSPME® LC tips were used. The BioSPME® tips were donated by Supelco.

Instrumentation

Analysis was performed by LC-MS/MS utilizing a Shimadzu UFLC System coupled with an AB SCIEX 3200 QTRAP triple quadrupole tandem mass spectrometer. The LC system consisted of a CBM-20A HPLC controller, two LC-20AD pumps (S/N: L20104451592 US L and S/N: L20104451591 US L), a DGU-20A3 vacuum degasser, a SIL-20AC autosampler (S/N: L20174559177 US A), and a CTO-20AC column oven. All results were analyzed using Analyst version 1.5 software. An Acentis® Express biphenyl column (50 mm x 2.1 mm, 2.7 μL) column (Supelco, Bellefonte, PA) was utilized as well.

BioSPME Extraction Procedure

Initially, the BioSPME® fibers were conditioned for 20 minutes in 50:50 (v/v) HPLC grade methanol (VWR Analytical, Lot #16I194014) / HPLC grade water solution (Honeywell, Lot #DS285-F-US) while agitating at 700 rpm. Following this, the fibers were washed in HPLC grade water for 10 seconds and then placed into 500 μL of a spiked blood sample (Lampire Biological Laboratories, Lot #18K20121) while agitating at 700 rpm for 40 minutes. Next, the fibers were removed from the blood sample and washed in 500 μL of 10 mM sodium phosphate buffer for 10 seconds followed by another wash in HPLC grade water (Honeywell, Lot #DS285-F-US) for 10 seconds at 700 rpms. The fibers were then placed into 1-mL glass inserts in the well plate containing 100 μL of HPLC grade methanol while agitating at 700 rpms. The glass inserts were dried at 55°C for 30 minutes under a gentle stream of Nitrogen using a TurboVap instrument. The samples were then reconstituted into 40 μL of 0.1% (v/v) formic acid (Thermo Scientific, Lot #RF2184301) in HPLC grade water (Honeywell, Lot #DS285-F-US). Figure 2 depicts the described process for analysis via LC-MS/MS.

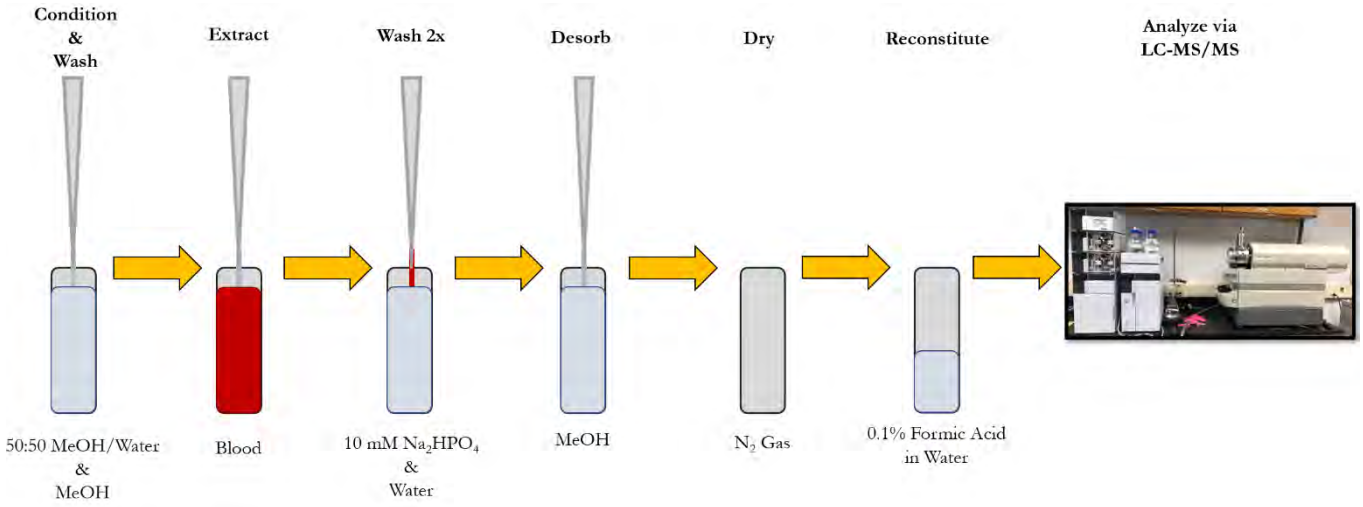


FIG 2 – *BioSPME® pipette tippet extraction procedure.*

DART-MS

For analysis using DART-MS, 100 ng/mL fentanyl and fentanyl analog mixture samples prepared in both methanol and bovine blood were extracted using mixed mode BioSPME® tips. The fentanyl mixture included fentanyl, cis-3-methylfentanyl, acetyl fentanyl, cyclopropyl fentanyl, 4-fluoroisobutyryl fentanyl (4-FIBF), 4-ANPP, furanyl fentanyl, and norfentanyl. In order to prepare these tips, the extraction process was stopped before the desorption step. This is because the DART allows for the analytes to be analyzed on the tips themselves rather than going through a desorption step into solvent. Overall, the DART-MS method is much quicker and involves much less sample preparation than conventional methods. A photograph showing the setup for analysis can be seen in Figure 3. The parameters used for the DART-MS are listed below in Figure 4.

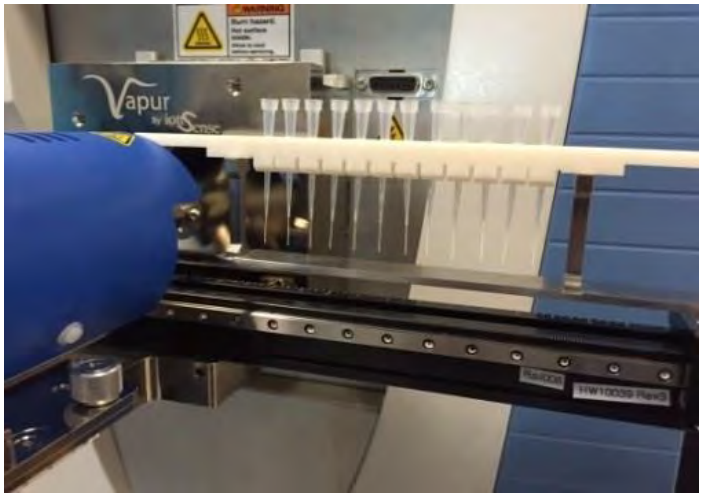


FIG 3 – *Analysis setup for BioSPME® tips using DART-MS. Photograph provided by IonSense.*

DART Methods

- Temperature: 300°C
- Rail Speed: 0.3 mm/sec
- Polarity: Positive
- Ionization Gas: Helium

JEOL AccuTOF Settings

- Detector voltage: 2200 V
- Spectrum Monitoring Parameters
  - Acquisition Range: 100-500 m/z
  - Polarity: Positive
  - Data Sampling Interval: 0.5s
  - Recording Interval: 1.0s
- Inlet Parameters
  - Needle Voltage: 0V
  - Orifice 1 Voltage: 2V
  - Orifice 2 Voltage: 5V
  - Ring Lens Voltage: 3V
  - Orifice 1 Temp: 120°C
  - Desolvation Chamber Temp, Desolvating Gas, Nebulizing Gas, Sweep Orifice 1 Voltage: OFF
- Analyzer Parameters
  - Ion Guide Peak Voltage: 800V
  - Ion Guide Bias Voltage: 27V
  - Focus Voltage: -150V
  - Condenser Lens Voltage: 10.0V
  - Quadrupole Lense Voltage: 10.0V
  - Right/Left Lens Voltage: 1.5V
  - Top/Bottom Lens Voltage: 4.4V
  - Pusher Bias Voltage: -0.44V
  - Reflection Votlage: 980V
  - Advanced Analyzer
    - Pusher Volate: 778.0V
    - Pulling Voltage: -778.0V
    - Suppress Voltage: 0.20V
    - Flight Tube Voltage: -7000V

Thermo QExactive Settings

- Positive/Negative Ion Mode
- Resolution: 70,000 @ 2Hz
- Fragmentation: none, HCD Gas Off
- Scan Settings: 1 μ-scan by 100 ms max inject time
- AGC Target: Ultimate Mass Accuracy ($5e^5$)
- Capillary Temperature: 200°C

FIG 4 – *DART-MS parameters.*

Overall, the results showed that the 100-ng/mL fentanyl in methanol standard shows lower recovery than the blood samples. This is because extracting in more than 10% organic solvent with SPME fibers detracts from the overall extraction efficiency of the fibers. Additionally, the 100-ng/mL fentanyl in spiked-blood extracts showed strong fentanyl signals with no visible signs of matrix interference. These results can be seen in Figures 5 through 8.

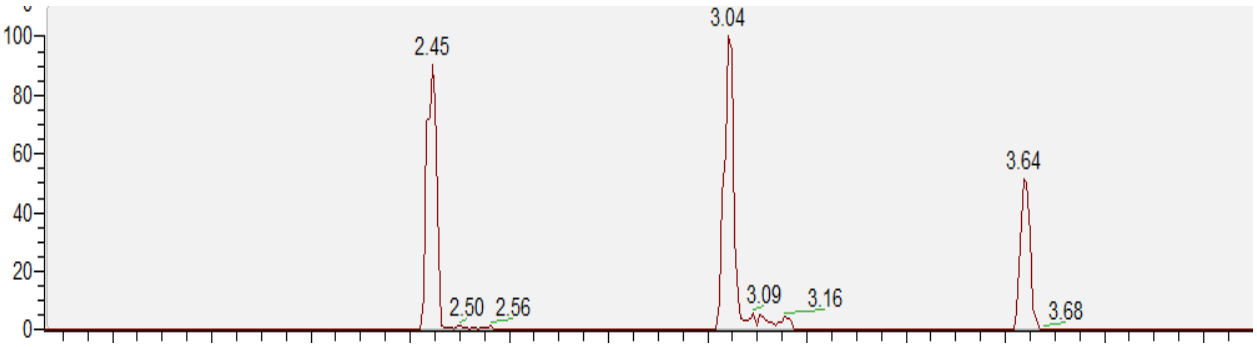


FIG 5 – *EIC of fentanyl from 100 ng/mL fentanyl in methanol extraction*

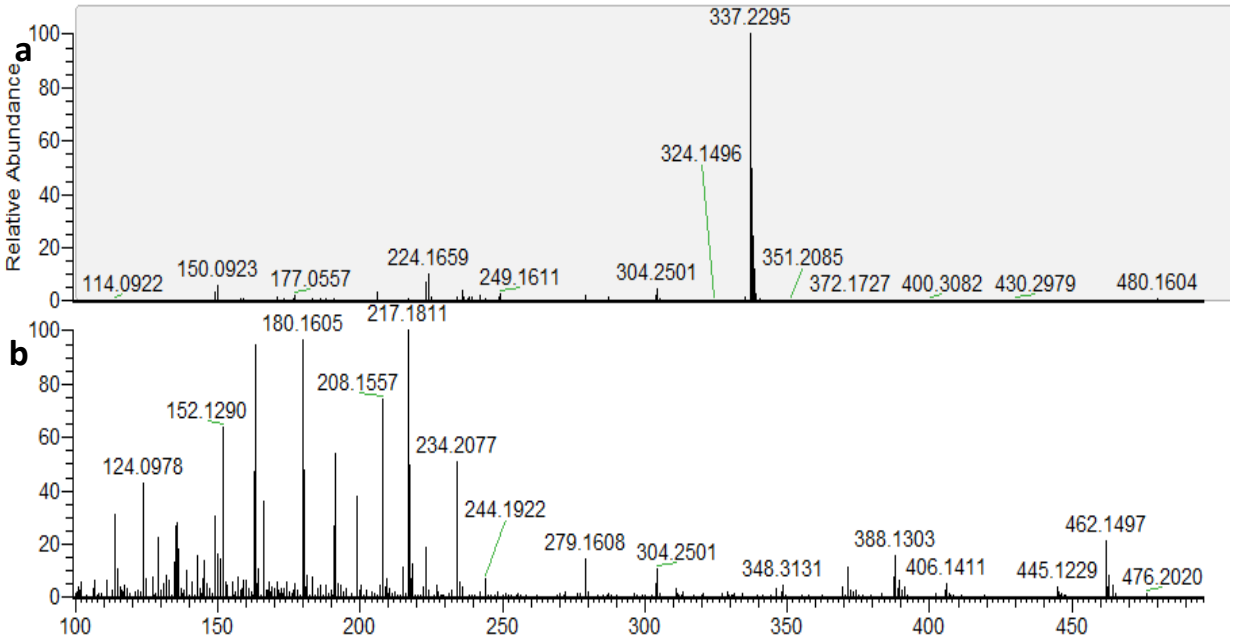


FIG 6 – *a. 100 ng/mL fentanyl in methanol extract.*
b. Blank methanol extract.

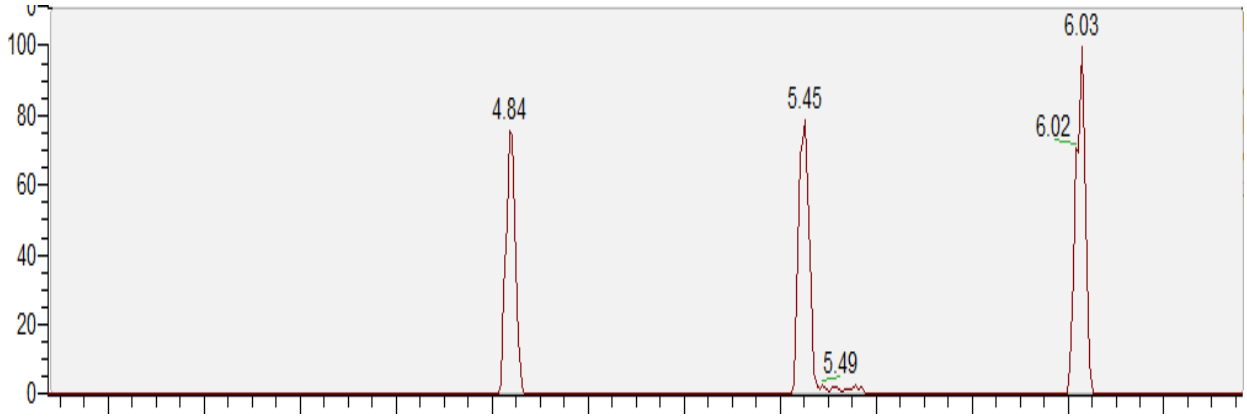


FIG 7 – *EIC of fentanyl from 100 ng/mL fentanyl in blood extraction.*

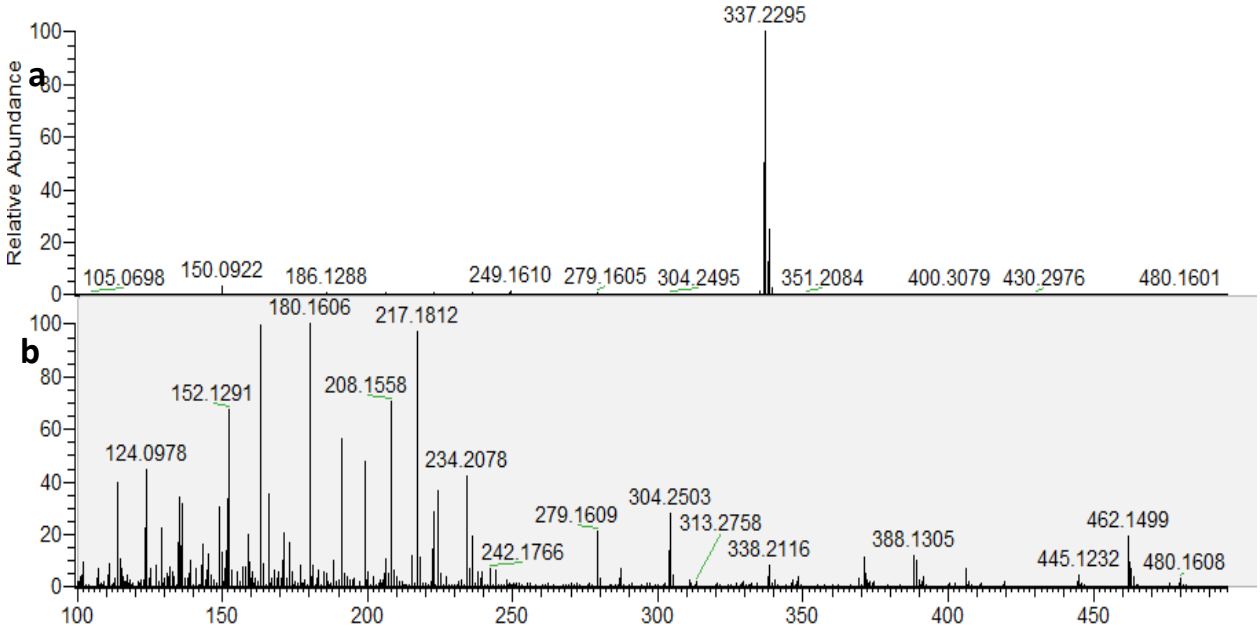
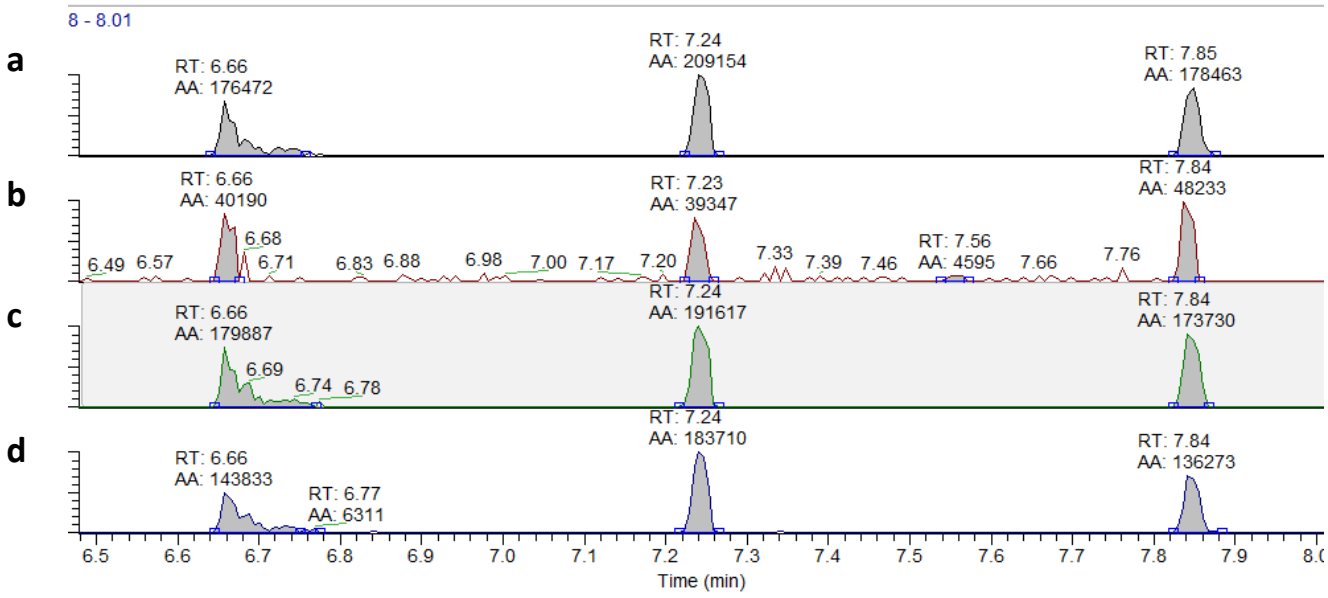


FIG 8 – *a. 100 ng/mL fentanyl in blood extract.*
b. Blank blood extract.

Each fentanyl analog, as well as fentanyl and norfentanyl, were detected from the blood extracts spiked with the fentanyl mixture; however, matrix suppression was observed as the signal of the fentanyl compounds was lower (approx. 1E5 and 5E4 signal levels). These results can be seen below in Figure 9.



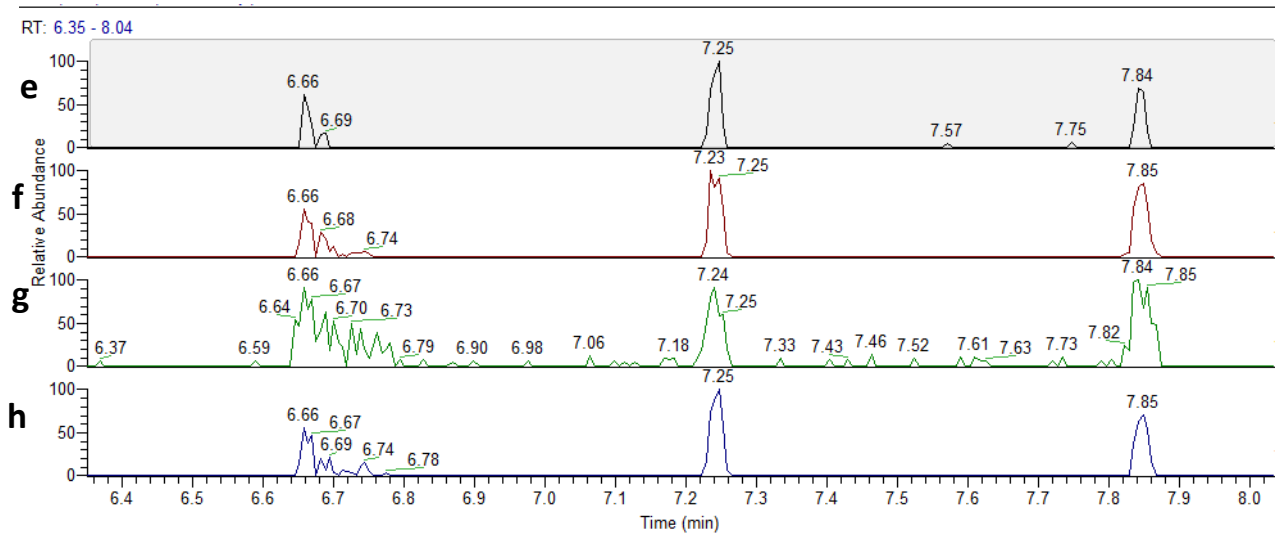


FIG 9 – *a. Fentanyl, b. Norfentanyl, c. Acetyl fentanyl, d. Cis-3-methyl fentanyl, e. Furanyl fentanyl, f. FIBF, g. 4-ANPP, h. Cyclopropyl fentanyl from 100 ng/mL fentanyl mix in blood extraction.*

LC-MS/MS

LC-MS/MS was used as a confirmation technique in this method. Analysis was performed using a Shimadzu UFLC System coupled with an AB SCIEX 3200 QTRAP triple quadrupole tandem mass spectrometer. An Acentis® Express biphenyl column (50 mm x 2.1 mm, 2.7 μL) column (Supelco, Bellefonte, PA) was utilized and all results were analyzed using Analyst version 1.5 software.

First, enhanced product ion (EPI) spectra, collision energies (CE), and declustering potentials (DP), were obtained for each of the drug standards. Table 1 shows the EPI results of the certified reference standards for the drugs obtained by MS/MS.

TABLE 1—*EPI information for all drug standards.*

Compound	Q1 (m/z)	Q3 (m/z)	Collision Energy (V)	Declustering Potential (V)
Fentanyl 1	337.5	188	31	46
Fentanyl 2	337.5	105	55	46
Fentanyl-D_5 1	342.5	188	32	47
Fentanyl-D_5 2	342.5	105	55	47
Norfentanyl 1	233	150	26	36
Norfentanyl 2	233	84	25	36
Norfentanyl-D_5 1	238	84	27	36
4-ANPP 1	281.5	188	23	35
4-ANPP 2	281.5	105	44	35
4-ANPP-D_5 1	286.5	188	23	36
4-ANPP-D_5 2	286.5	105	44	36
Acetyl Fentanyl 1	323	188	32.5	47.5
Acetyl Fentanyl 2	323	105	53	47.5

Acetyl Fentanyl-D_5 1	329	188	32	47
Acetyl Fentanyl-D_5 2	329	105	54	47
Cyclopropyl Fentanyl 1	349	188	33	47.5
Cyclopropyl Fentanyl 2	349	105	57.5	47.5
Cyclopropyl Fentanyl-D_5 1	354	188	33	49
Cyclopropyl Fentanyl-D_5 2	354	105	57.5	49
Furanyl fentanyl 1	375.5	188	56	47
Furanyl fentanyl 2	375.5	105	30	47
4-fluoroisobutyryl fentanyl 1	369.5	188	32	51
4-fluoroisobutyryl fentanyl 2	369.5	105	58	51
(±)-3-cis-methylfentanyl 1	351.5	202	32	50
(±)-3-cis-methylfentanyl 2	351.5	105	55	50

Next, the chromatography was optimized by adjusting the column oven temperature, run time, flow rate, and gradient vs isocratic column flow programming. The column and mobile phases were held constant during optimization. The weak mobile phase (A) consisted of 0.1% (v/v) formic acid in HPLC grade water and the strong phase (B) consisted of 0.1% (v/v) formic acid in acetonitrile. Each liquid chromatographic separation was investigated using a 250 ng/mL drug mixture which contained norfentanyl, fentanyl, acetyl fentanyl, 4-ANPP, cyclopropyl fentanyl, (±)-cis-3-methylfentanyl, furanyl fentanyl, and 4-fluoroisobutyryl fentanyl (FIBF).

It was determined that an isocratic method using 30% B was the optimal method for chromatographic separation of the compounds. The optimized separation of these compounds can also be seen below in Figure 10.

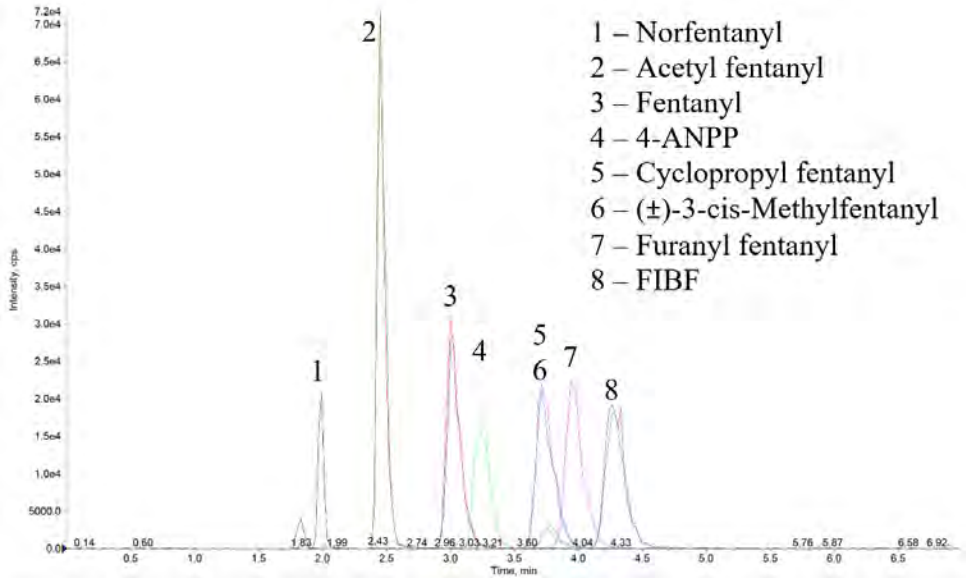


FIG 10 – *LC separation of fentanyl, norfentanyl, and six fentanyl analogs using an isocratic method (30% B).*

PDMS/DVB mixed mode fibers were consistently shown to produce results with overall higher peak areas than C-18 fibers for fentanyl and its analogs. Due to this observation, PDMS/DVB fibers were used throughout the rest of the study. A series of calibration curves were analyzed from three separate extractions performed on the same day. The concentrations utilized ranged from 0.05 ng/mL to 100 ng/mL. For all quantitative analysis, the first ion was utilized as the quantitative ion. The second ion was used as the qualifier ion. The acceptance criteria for this experiment was the presence of both ion pairs for each analyte. Figure 11 shows an example of a calibration curve produced from this portion of the study.

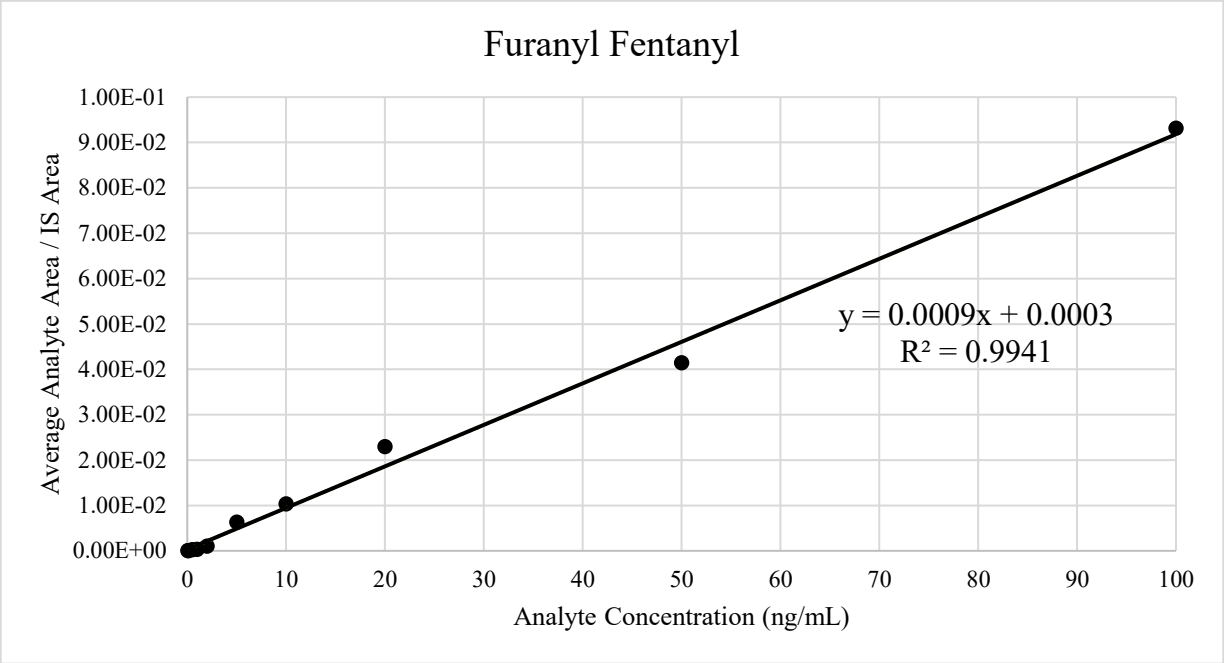


FIG 11 – *Calibration curve for furanyl fentanyl.*

Lastly, the peak areas for each concentration analyte was pooled and averaged. The LINEST function on Excel was used to calculate the limit of quantitation and limit of detection for each analyte. The results for this data analysis can be seen in Table 2.

TABLE 2—*Figures of merit from calibration curves.*

Compound	**R^2**	**LOD (ng/mL)**	**LOQ (ng/mL)**
Fentanyl	0.9845	4.8	16.0
Norfentanyl	0.9272	10.7	35.8
4-ANPP	0.9941	2.9	9.8
Acetyl Fentanyl	0.9792	5.6	18.6
Cyclopropyl Fentanyl	0.9910	3.6	12.2
Furanyl fentanyl	0.9949	2.8	9.2
4-fluoroisobutyryl fentanyl	0.9912	3.6	12.0
(±)-3-cis-methylfentanyl	0.9931	3.2	10.6

In order to gain sensitivity, all further extractions were performed and analyzed in the Toxicology Department of Health Network Laboratories (HNL) in Allentown, PA using their Sciex 5500 Qtrap Mass Spectrometer coupled with a Shimadzu HPLC system. A Restek Ultra Biphenyl (50 mm x 2.1 mm, 5 μL) column was utilized and all results were analyzed using Analyst and MultiQuant software. A method previously optimized and validated by HNL was used for the remaining experiments. This method included the following analytes: fentanyl, norfentanyl, norcarfentanil, acetyl fentanyl, furanyl fentanyl and 4-ANPP. The method utilized a weak mobile phase (A) consisting of 0.1% formic acid, 2 Mm ammonium acetate, and 2% acetonitrile in water and a strong mobile phase (B) consisting of 0.1% formic acid, 2 Mm ammonium acetate, and 10% water in acetonitrile. The method also utilized a 0.5000 mL/min flow rate, 40°C oven temperature, and a 10 μL injection volume. The separation for the analytes using this method can also be seen below in Figure 12. The EPI data utilized by this method can be seen below in Table 3.

FIG 12 – *LC separation of fentanyl, norfentanyl, norcarfentanil, and 3 fentanyl analogs using HNL's method.*

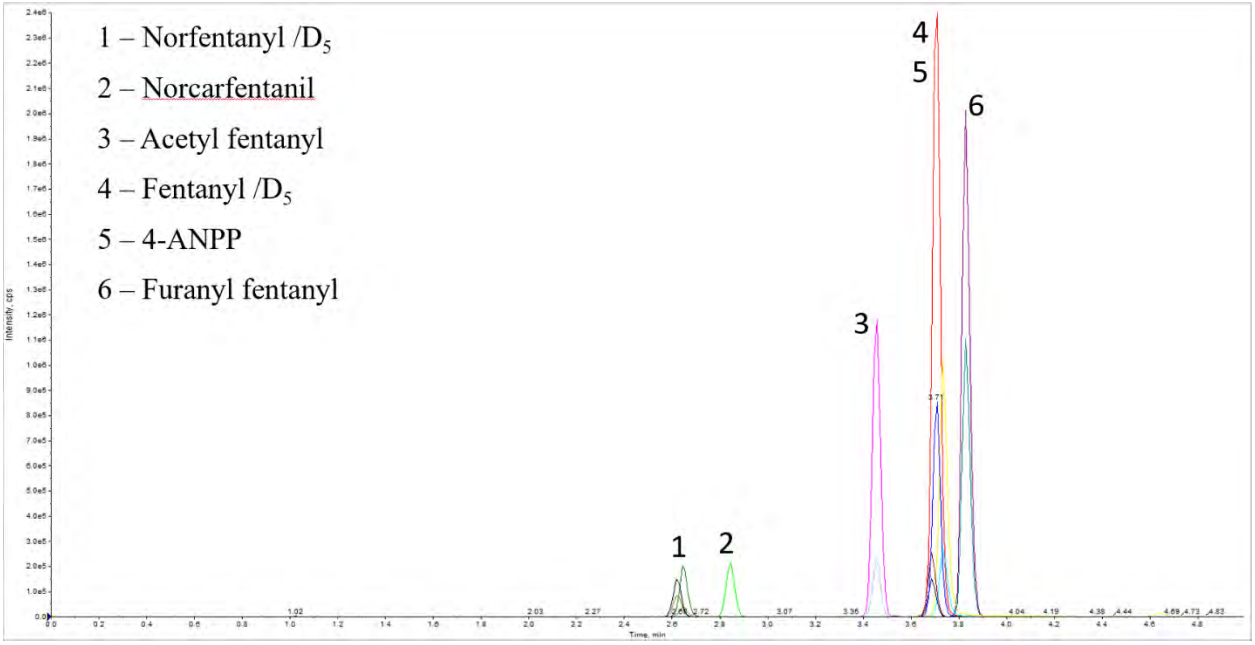


TABLE 3—*EPI information for all compounds.*

Compound	Q1 (m/z)	Q3 (m/z)	Collision Energy (V)	Declustering Potential (V)
Fentanyl 1	337.200	188.100	45	51
Fentanyl 2	337.200	105.100	49	51
Fentanyl-D_5 1	342.246	188.200	60	51
Fentanyl-D_5 2	342.246	105.000	110	51
Norfentanyl 1	233.200	84.000	35	41
Norfentanyl 2	233.200	150.1	23	41
Norfentanyl-D_5 1	238.099	84.400	50	41
Norfentanyl-D_5 2	238.099	84.100	50	41
4-ANPP 1	281.120	188.100	40	1
4-ANPP 2	281.120	104.900	60	1
Acetyl Fentanyl 1	323.130	188.100	51	66
Acetyl Fentanyl 2	323.130	105.100	73	66
Furanyl Fentanyl 1	375.071	188.100	45	81
Furanyl Fentanyl 2	375.071	105.00	70	81
Norcarfentanil 1	291.061	231.100	19	56
Norcarfentanil 2	291.061	142.100	21	56

A single calibration curve was analyzed using the following concentrations: 2.5, 5, 12.5, 20, and 25 ng/mL. Based upon these results, the limit of detection, limit of quantitation, and upper limit of linearity were determined. Those results can be

seen below in Table 4. The acceptance criteria for these figures of merit included the presence of both ions for each analyte and the transition relative abundances within ± 20% of target, relative to the calibrators. It is also important to note that blank samples were analyzed after the 20 and 25 ng/mL calibrators. The results indicated that no carryover was present after analyzing these concentrations.

TABLE 4—*Figures of merit from the calibration curve extracted using BioSPME® and analyzed on the Sciex 5500.*

Compound	R^2	LOD (ng/mL)	LOQ (ng/mL)	ULL (ng/mL)
Fentanyl	0.99073	2.5	2.5	25
Norfentanyl	0.98079	2.5	5	25
4-ANPP	0.98180	2.5	2.5	25
Acetyl Fentanyl	0.98618	2.5	2.5	25
Furanyl fentanyl	0.98763	2.5	2.5	25
Norcarfentanil	0.97904	2.5	5	25

Casework

A total of 22 case samples were analyzed in this study. The case samples were donated by the Lehigh County Coroner's Office in Allentown, PA. Samples submitted for analysis were either heart or femoral blood collected in grey top tubes containing sodium fluoride/potassium oxalate. Cases were picked based on their history of suspected illicit drug use. Additional 'negatives' were added to ensure the lack of false positive results. These 'negative' samples were collected under the assumption that the history of the decedent did not involve illicit drug use. All cases were completed by HNL using their current validated supported liquid extraction method. Additionally, these cases were extracted using the BioSPME® fibers to be analyzed by DART-MS and LC-MS/MS.

All 22 case samples analyzed using DART-MS yielded negative results. Although no fentanyl compounds were identified, two of the cases did show evidence of methamphetamine, which did coincide with the results seen by HNL.

In order to ensure that negative samples were not analyzed further, the BioSPME® samples were screened by LC-MS/MS on the Sciex 5500 at HNL prior to selecting the samples presented. Table 5 shows the ions and expected retention times for each analyte.

TABLE 5—*Ions and expected retention times for each analyte.*

Compound	Description	Associated IS	Precursor Ion	Quant Ion	Qual Ion	Retention Time
Fentanyl	Analyte	Fentanyl-D5	337.300	188.2	105.1	3.69
Norfentanyl	Analyte	Norfentanyl-D5	233.111	84.0	150.1	2.62
4-ANPP	Analyte	Fentanyl-D5	281.120	188.10	104.90	3.74
Acetyl Fentanyl	Analyte	Fentanyl-D5	323.130	188.1	105.1	3.46
Furanyl fentanyl	Analyte	Fentanyl-D5	375.01	188.10	105.00	3.83
Norcarfentanil	Analyte	Fentanyl-D5	291.061	231.100	142.100	2.84
Fentanyl-D_5	Internal Standard	N/A	342.246	188.2	105.0	3.69
Norfentanyl-D_5	Internal Standard	N/A	238.099	84.400	84.100	2.62

The acceptance criteria used for the BioSPME® experiment to determine qualitative (LOD) and quantitative (LOQ) results are as follows. The retention times of the analytes and the internal standard for case samples must fall within ± 0.200 minutes

of the retention time of the calibrators. Ion ratios between the quantitation ion and the qualifier ion are set based on the mean of ion ratios of the calibrators. The ion rations must be within ± 20% of target, relative to the calibrators. Refer for Table 4 for limit of detection and limit of quantitation. Lastly, the recovery of the internal standard needs to be between 50% and 200% of the area of the calibrator's internal standard. Below are the LOD results for the positive case samples analyzed using both HNL's existing extraction and the experimental BioSPME® extraction (Figure 13). The highlighted boxes show the inconsistent results.

		Fentanyl		Norfentanyl		4-ANPP		Acetyl Fentanyl	
		HNL	BioSPME®	HNL	BioSPME®	HNL	BioSPME®	HNL	BioSPME®
Case Sample	TS-19-001	+	+	+	-	+	-	-	-
	TS-19-002	+	+	+	+	-	-	-	-
	TS-19-006	+	+	+	+	+	+	-	-
	TS-19-012	+	+	+	+	+	-	-	-
	TS-19-013	+	+	-	-	+	+	-	-
	TS-19-015	+	+	-	-	-	-	-	-
	TS-19-016	+	+	+	-	-	-	+	-
	TS-19-018	+	+	+	-	+	-	-	-

FIG 13 – *Comparison of LOD results for casework. All samples were negative for norcarfentanil and furanyl fentanyl.*

Figure 14 shows the LOQ results of the case samples extracted at HNL as well as using the BioSPME® extraction.

		Fentanyl		Norfentanyl		4-ANPP		Acetyl Fentanyl	
		HNL	BioSPME®	HNL	BioSPME®	HNL	BioSPME®	HNL	BioSPME®
Case Sample	TS-19-001	9.3	9.5	1.2	-	0.9	-	-	-
	TS-19-002	11.2	5.4	1.8	10.5	-	-	-	-
	TS-19-006	38.7	85.1	1.0	3.9	2.1	7.8	-	-
	TS-19-012	10.9	8.4	4.0	2.9	0.9	-	-	-
	TS-19-013	4.6	33.7	-	-	0.7	7.6	-	-
	TS-19-015	1.0	3.6	-	-	-	-	-	-
	TS-19-016	8.0	10.1	5.6	-	-	-	1.0	-
	TS-19-018	13.8	13.2	1.4	-	2.5	-	-	-

FIG 14 – *Comparison of LOQ results for casework based on a single BioSPME® curve. All samples were negative for norcarfentanil and furanyl fentanyl. All concentrations shown are in ng/mL.*

Limitations

The results above have a few limitations which must be considered. The limit of detection and quantitation for the compounds extracted using BioSPME® fibers and analyzed on the DART-MS have not been determined. A 100-ng/mL spiked solution containing all of the analytes was analyzed by DART-MS, and each compound was detected, proving it is possible to detect fentanyl compounds using BioSPME® in conjunction with DART-MS. It is possible that the case samples screened negative due to the concentrations being below the limit of detection on the DART-MS.

The LC-MS/MS results show that the qualitative detection of these analytes utilizing the BioSPME® fiber is possible. In order to do quantitative work using the BioSPME® fiber, the assay would need to be validated according to the SWGTOX guidelines. The lowest calibrator used in this study was 2.5 ng/mL, so it is possible that the inconsistent results between HNL and the BioSPME® fibers are due to this 2.5 ng/mL cutoff.

Conclusions and Future Work

Overall, the feasibility of using BioSPME® fibers for the extraction of fentanyl, norfentanyl, and its analogs from blood samples has been shown to produce promising results. The results so far indicate that the assay can be successfully used qualitatively; however, quantitative work needs further experimentation in order to draw any conclusions. To do this, lower calibrators will be analyzed in order to determine the true limit of detection and quantitation of the assay. The results from the DART-MS show that the detection of these compounds directly from the fiber is also possible; however, not at low concentrations. Further studies should be completed to optimize the DART-MS conditions and determine the limit of detection and quantitation for these compounds. The combination of BioSPME® fibers and LC-MS/MS thus far has shown

that a quick and simple detection of these compounds is possible, which shows potential for the method's use in forensic toxicology.

Resources

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The Optimization of the Gas Chromatographic-Mass Spectrometric Analysis of Thirty Fentanyl Analogues

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ABSTRACT: Forensic laboratories are overwhelmed with emerging fentanyl related compounds. At the beginning of 2019, the National Forensic Laboratory Information System reported that fentanyl reports have increased 10 times from 2014 to 2017. Fentanyl and fentanyl analogues are frequently sold as heroin and are widely available increasing the risk of overdose. The standard analysis technique that forensic laboratories most often employ to analyze seized drugs, including fentanyls, is gas chromatography-mass spectrometry. With the vast number of different fentanyl analogues emerging there are issues with unresolved compounds using only one gas chromatographic column. To address the overwhelming amount of samples and challenges fentanyl analogues pose to crime labs this project was performed to develop the optimum gas chromatographic-mass spectrometric conditions to improve upon the separation and resolution of fentanyl analogues. Different variables in gas chromatographic methodology were investigated including: split ratio, injector temperature, injection volume, and the oven temperature program. In total, seven columns with different stationary phases of varying degrees of polarity were evaluated to discern the best stationary phase type for analyzing fentanyl compounds. An optimized gas chromatographic-mass spectrometric method is reported.

KEYWORDS: forensic science, drug chemistry, fentanyl, fentalogs, gas chromatography-mass spectrometry

Introduction

Opioid abuse has become an epidemic in the United States. Among drug related deaths in 2016, 66% were due to opioid overdose (1). According to the National Forensic Laboratory Information System (NFLIS) the number of fentanyl reports increased 10 times from 2014 (5,531 reports) to 2017 (56,530 reports). The 2019 NFLIS report showed nationwide increasing trends in the abuse of the following fentanyl analogues: 3-methylfentanyl, 4-fluoroisobutyryl fentanyl, acetyl fentanyl, acryl fentanyl, carfentanil, cyclopropyl fentanyl, fentanyl, furanyl fentanyl, and U-47700 (2). Fentanyl and its analogues are an increasing source of the opioid overdoses, which make up the major part of drug-related deaths; the main mechanism of death being respiratory depression (3). Commonly, when fentanyl is distributed illegally, it is often mixed with heroin and cocaine (1, 4). Fentanyl analogues and new psychoactive substances (NPS) are appearing regularly and are usually packaged without reporting the actual concentration (3, 5). In February of 2018 the DEA temporarily scheduled fentanyl-related substances that have similar structures. This order is in effect till February 2020 (6). New substances are constantly being added to the controlled substances list since the chemical structure is easily manipulated in clandestine labs. Because crime labs are faced with a vast amount of new and different analogues that are structurally similar it makes analysis difficult for traditional analytical techniques (7).

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) (8) is a highly reliable resource used by forensic laboratories in the United States for their guidelines on how to develop and validate seized-drug testing methods (4). Gas chromatography-mass spectrometry (GC-MS) is listed within these guidelines and is considered the gold standard technique for seized-drug analysis. With forensic laboratories facing an overwhelming workload of casework validating a method that comprises multiple standards can be very time-consuming, not to mention very costly to perform. The information reported in this study presents comparisons of multiple gas chromatographic-mass spectrometric parameters regarding analysis of fentanyl analogues with the aim to develop the best optimized method for resolving fentanyl analogues.

Experimental

Chemicals

All of the drug standards used were purchased as certified standards from commercial sources. The name of the standard and lot # are listed in Table 1 and 2. All fentanyl and fentanyl analogue standards were purchased at an initial concentration

of 1 mg/mL in methanol, except for one carfentanil standard at 100 μg/mL in methanol, and the standards in the shaded blue boxes in Table 2 which were purchased in 1-mg size as powder.

Table 1. Standards purchased from Cerilliant, Inc.(Round Rock, TX, USA).

Drug	**Lot #**	**Drug**	**Lot #**
acetyl fentanyl	FC08011601	remifentanil	FF10131501
fentanyl	FE04231502	alfentanil	FE12291404
sufentanil	FE11191502	carfentanil *(100 μg/mL)*	FE04241710

Table 2. Standards purchased from Cayman Chemical Co., (Ann Arbor, MI, USA).

Drug	**Batch #**	**Drug**	**Batch #**
4-anilino-*N*-phenethylpiperdine (4-ANPP)	0510160	acryl fentanyl	0513702
4-anilino-*N*-phenethylpiperdine (4-ANPP)	0477298-42	acryl fentanyl	0484313-16
isobutyryl fentanyl	0484505	alfentanil	0505032-9
isobutyryl fentanyl	0499402-16	carfentanil	0508010
cyclopropyl fentanyl	0517150	furanyl fentanyl	0484909
cyclopropyl fentanyl	0508967-18	furanyl fentanyl	0537068-6
β-methyl fentanyl	0494309-19	W-15	0482784
β-methyl fentanyl	0494309-23	*ortho*-fluorofentanyl	0539799-4
para-methoxy butyryl fentanyl	0484889	*ortho*-fluorofentanyl	0490394-26
β-hydroxythiofentanyl	0486122	*meta*-fluorofentanyl	0481790-28
α-methyl fentanyl	0499458-8	*meta*-fluorofentanyl	0481790-26
α-methyl fentanyl	0499485-12	*para*-fluorofentanyl	0487106
(±)-*cis*-3-methyl fentanyl	0504267	*para*-fluorofentanyl	0482912-32
(±)-*cis*-3-methyl fentanyl	0517885-3	crotonyl fentanyl	0523691-5
(±)-*trans*-3-methyl fentanyl	0490381	crotonyl fentanyl	0545072-4
(±)-*trans*-3-methyl fentanyl	0538769-1	remifentanil	0507288
4-fluoroisobutyryl fentanyl (FIBF)	0490753-34	remifentanil	0544055-3
4-fluoroisobutyryl fentanyl (FIBF)	0490753	ocfentanil	0488443
ortho-fluorobutyryl fentanyl	0546047-2	ocfentanil	0488443-22
ortho-fluorobutyryl fentanyl	513603	sufentanil	0535925-3
meta-fluorobutyryl fentanyl	0487107	thiofentanyl	0494637
meta-fluorobutyryl fentanyl	0541951-1	thiofentanyl	0551784-2
para-fluorobutyryl fentanyl	0487736	valeryl fentanyl	0513184
para-fluorobutyryl fentanyl	0523974	valeryl fentanyl	0495866-11
para-methoxy butyryl fentanyl	0495945-20	butyryl fentanyl	0482640-25

Note: Standards in the shaded blue boxes in Table 2 were purchased as 1-mg powder.

n-Alkane calibration standards were analyzed on the Thermo Scientific Trace 1310 gas chromatograph and the Agilent Technologies 6890N Series GC system once a day and any day before fentanyl standards were analyzed. These standards were purchased from Restek (catalog #: 31633, lot #: A098207, exp.: 10/2020), (catalog #: 31633, lot #: A0135311, exp.: 03/2025).

Laboratory chemicals utilized to dilute standards were purchased from Fisher Chemicals (Hampton, NH, USA). These included LC-MS grade methanol (lot #: 170824TF) and certified ACS cyclohexane (lot #: 982113).

Instrumentation

Four different instruments were used for this study.

The gas chromatograph used for the initial feasibility study was an Agilent Technologies 7890A Series GC system (serial number: US10829030) coupled with an Agilent Technologies 5975C Network Mass Selective Detector (serial number: US82515930). The autosampler used for this gas chromatographic instrument was an Agilent Technologies 7683B Series Injector (serial number: US72110714).

The gas chromatograph used to collect data for analysis on the HP-1 column was an Agilent Technologies 6890N Series GC system (serial number: US10521014) coupled with an Agilent Technologies 5973 mass selective detector (serial number: US44621461). The autosampler used for this gas chromatographic instrument was an Agilent Technologies 7683B Series Injector (serial number: US72110720).

The gas chromatograph which housed the Rxi-624 and Rxi-17 columns was an Agilent Technologies 7890A Series GC system (serial number: US10729005) coupled with a dual flame ionization detector. The autosampler used for this GC-FID was an Agilent Technologies 7683B series injector (serial number: US80810921).

The gas chromatograph, otherwise utilized, was a Thermo Fisher Scientfic Trace 1310 GC (model: Trace 1300 series, serial number: 715102031) coupled with a dual flame ionization detector and a Thermo Fisher Scientific ISQ LT single quadrupole mass spectrometer. The autosampler used for this gas chromatographic instrument was a Thermo Fisher Scientific TRI PLUS RSH (serial number: 352146). Xcalibur software from Thermo Fisher Scientific was used to analyze data.

Columns

Technical information about the seven gas chromatographic columns used in this study are listed in Table 3. All of the columns used were purchased from Restek Corp. (Bellefonte, PA) with the exception of column number 5 (Table 3), which was purchased from Agilent Technologies (Santa Clara, CA). The first one, an Rxi-5sil ms column with a stationary phase comprised of 5% 1, 4-bis (dimethylsiloxy) phenylene and 95% dimethyl polysiloxane was used in the feasibility study, in the 7890A gas chromatograph. The second column was the primary column used in the 1310 gas chromatograph. This was an Rxi-5ms column with a 5% diphenyl: 95% dimethyl polysiloxane. The third column was used in the Agilent Technologies 6890N Series GC, with a non-polar 100% dimethyl polysiloxane phase, and is used as a general-purpose column (part number: 19091S-933, serial number: US8550456H). Other columns were investigated as options for possible use for complimentary sample information. These include a Rtx-5 amine column, a Rtx-200ms column, a Rxi-624Sil ms column, and a Rxi-17Sil ms column.

Table 3. Gas chromatographic column information.

#	Column	Stationary Phase	Column Dimensions	Serial #
1	Rxi-5Sil MS	5% 1, 4-*bis* (dimethylsiloxy) phenylene: 95% PDMS	30 m x 0.25 mm ID, 0.25 μm	1406587
2	Rxi-5ms	5% diphenyl: 95% PDMS	30 m x 0.25 mm ID, 0.25 μm	1487174
3	HP-1	100% PDMS	30 m x 0.25 mm ID, 0.25 μm	US8550456H
4	Rtx-5 amine	5% diphenyl: 95% PDMS	30 m x 0.25 mm ID, 0.5 μm	1480901
5	Rtx-200ms	100% trifluoropropylmethyl polysiloxane	30 m x 0.25 mm ID, 0.25 μm	1487938
6	Rxi-624Sil MS	6% cyanopropyl:94% PDMS	30 m x 0.25 mm ID, 1.4 μm	1624584
7	Rxi-17Sil MS	50% diphenyl: 50% PDMS	30 m x 0.25 mm ID, 0.25 μm	1630853

Note: PDMS = polydimethylsiloxane

Results and Discussion

Feasibility Study

A feasibility study was performed to assess retention behavior of six fentanyl analogues. The Agilent Technologies 7890A Network GC system coupled with a 5975C Network Mass Selective Detector and fitted with the Rxi-5Sil ms column (column #1, Table 3). The initial GC conditions were as follows: injection mode, split ratio 5:1; injector temperature, 250°C; injection volume, 1.0 μL; carrier gas, Helium; oven temperature program, initial temperature at 60°C (2 min hold) followed by ramp at varying rates (°C/min) up to 300°C (10 min hold). The varying rates were as follows: 9°C/min, 12°C/min, 15°C/min, 18°C/min, 20°C/min, 25°C/min, and 30°C/min.

Retention behavior was assessed in the feasibility study using the GC-MS conditions described above. Table 4 shows the data from the six fentanyl standards. At a rate of 9°C/min the retention times were near 30 minutes and at a rate of 30°C/min the retention times were observed before 12 minutes, a 50% decrease. The data for fentanyl and three fentanyl analogues can be seen in Figure 1. This data demonstrates the utility of a steep ramp rate of 30°C/min, which decreased the total run time in half versus the ramp rate of 9°C/min.

Table 4. Retention times (minutes) for six fentanyl standards analyzed at seven separate ramp rates (n=2)

Rate	Alfentanil	Butyryl Fentanyl	Fentanyl	Furanyl Fentanyl	Isobutyrl Fentanyl	Valeryl Fentanyl
9°C/min	27.50 ± 0.01	26.34 ± 0.08	25.77 ± 0.04	28.25 ± 0.01	25.71 ± 0.13	27.12 ± 0.12
12°C/min	21.91 ± 0.01	21.05 ± 0.08	20.64 ± 0.08	22.50 ± 0.01	20.54 ± 0.08	21.63 ± 0.11
15°C/min	18.48 ± 0.01	17.76 ± 0.08	17.49 ± 0.14	19.04 ± 0.01	17.36 ± 0.06	18.28 ± 0.16
18°C/min	16.19 ± 0.00	15.53 ± 0.08	15.25 ± 0.07	16.74 ± 0.00	15.18 ± 0.07	16.00 ± 0.15
20°C/min	15.04 ± 0.00	14.38 ± 0.04	14.75 ± 0.92	15.59 ± 0.00	14.11 ± 0.11	14.82 ± 0.11
25°C/min	13.00 ± 0.00	12.33 ± 0.00	12.09 ± 0.01	13.53 ± 0.01	12.04 ± 0.01	12.71 ± 0.01
30°C/min	11.64 ± 0.01	11.00 ± 0.01	10.77 ± 0.00	12.16 ± 0.01	10.71 ± 0.00	11.36 ± 0.01

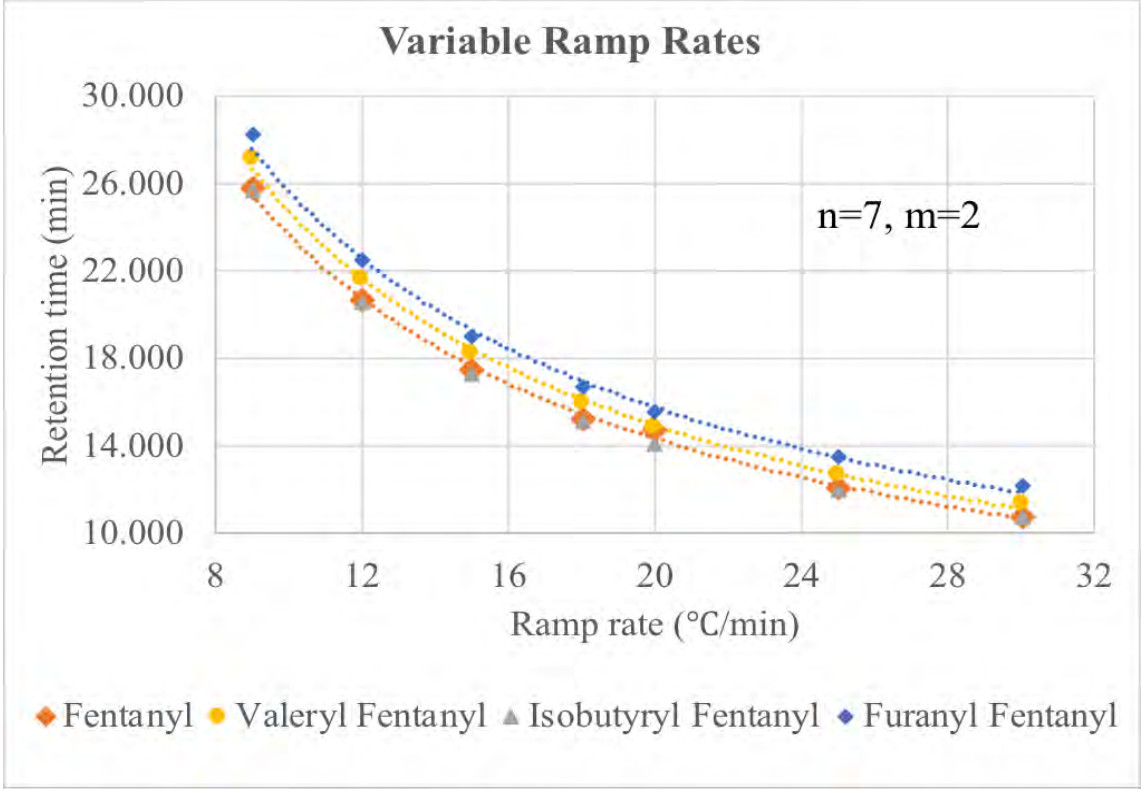

Fig. 1 Retention behavior in response to an increasing ramp rate for five fentanyl analogues.

GC-MS Optimization

Split Ratio

An optimized GC-MS method was developed using a Thermo Fisher Scientfic Trace 1310 GC coupled with a dual flame ionization detector and a Thermo Fisher Scientific ISQ LT single quadrupole mass spectrometer. The primary column used for the optimization was a 30 m x 0.25 mm ID coated with Rxi-5ms (0.25 μm) (column #2, Table 3). The first parameter optimized was the split ratio. Three different split ratios were investigated: 10:1, 30:1, 50:1. Fentanyl standards were analyzed at concentrations of 100 μg/mL. An n-alkane and fentanyl certified reference standards were utilized as calibration standards for all data collected. The retention times for peaks that appeared in the chromatograms corresponded with reference samples and the mass spectra matched the appropriate fentanyl analogue through a library search.

Figure 2 shows the data for the para-methoxybutyryl fentanyl standard analyzed at split ratios of 10:1 and 30:1, respectively. A split ratio of (10:1) was determined from the ideal chromatogram peak shape, being taller and sharper, observed in Figure 2 (top) as opposed to the shorter peak in Figure 2 (bottom). As expected, there was also an increase in peak area using a split ratio of (10:1) versus a split ratio of (30:1), indicating the GC method was more sensitive using a split ratio of (10:1).

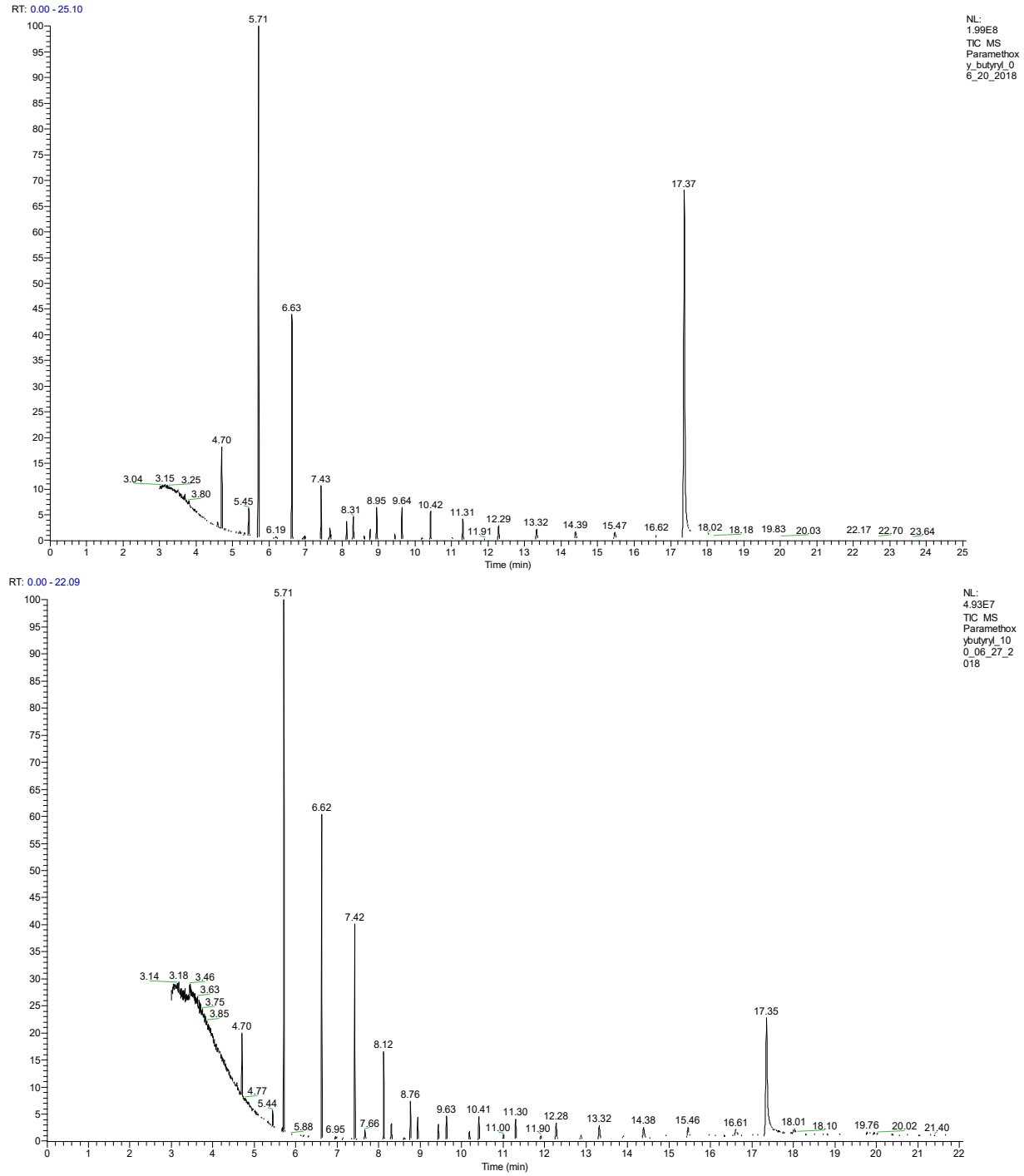

Fig. 2 Gas chromatography-mass spectrometry analysis of *p*-methoxybutyryl fentanyl (100 μg/mL). Total ion chromatograms (TIC) at a split ratio of 10:1 (top) and at a split ratio of 30:1 (bottom).

Injection Volume

The next parameter investigated was the injection volume. Three different injection volumes were evaluated, 0.7 μL, 1.0 μL, 2.0 μL. An injection volume of 2.0 μL was considered too large for the injection insert and would contaminate the injection port. Figure 3 shows acetyl fentanyl analyzed via GC-MS at injection volumes of 0.7 and 1.0 µL, respectively. The acetyl fentanyl eluted at a retention of 14.1 min. The other peaks in the TIC are from the n-alkane calibration standard. An injection volume of 1.0 µL was considered to be the optimum volume because it proved to be the most sensitive without producing carryover contamination.

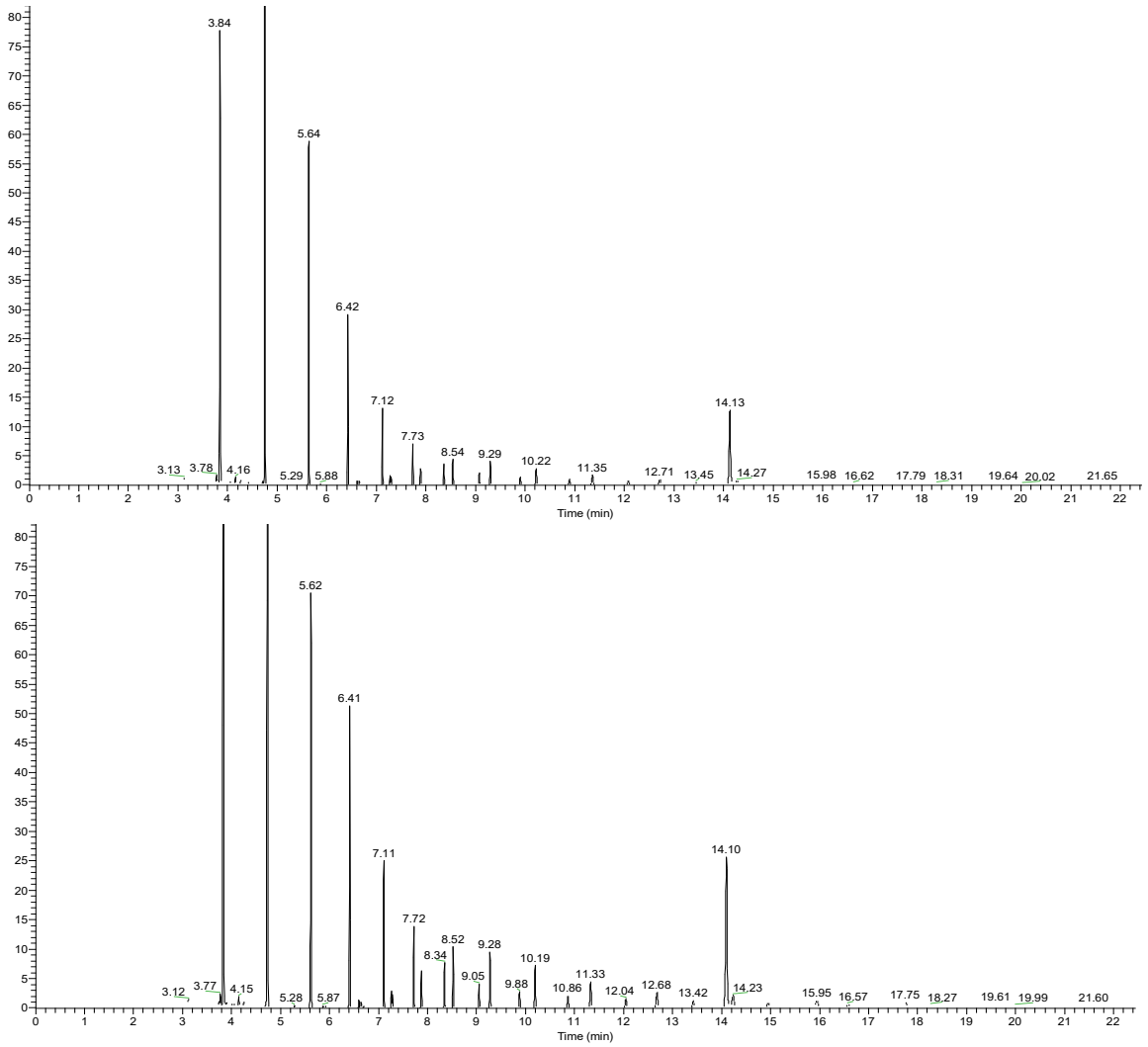

Fig. 3 TIC of acetyl fentanyl (100 μg/mL*)* at two injection volumes: 0.7 μL (top), 1.0 μL (bottom).

Injection Port Temperature

Three different injection port temperatures were evaluated to assess which temperature would yield the best chromatographic peak. The injector temperatures investigated were 260°C, 280°C, and 300°C. Figure 4 shows a plot of detector response (peak area counts) versus injection port temperature data for fentanyl standards analyzed by GC-MS. Only nine fentanyl standards are shown for clarity. Standards which demonstrated a general trend, having no contributable significance, were omitted. From this data it was determined that the GC method was most sensitive using an injector temperature of 280°C versus 260°C. The detector response seemed to decrease at 300°C (Figure 4) which may indicate decomposition at this temperature.

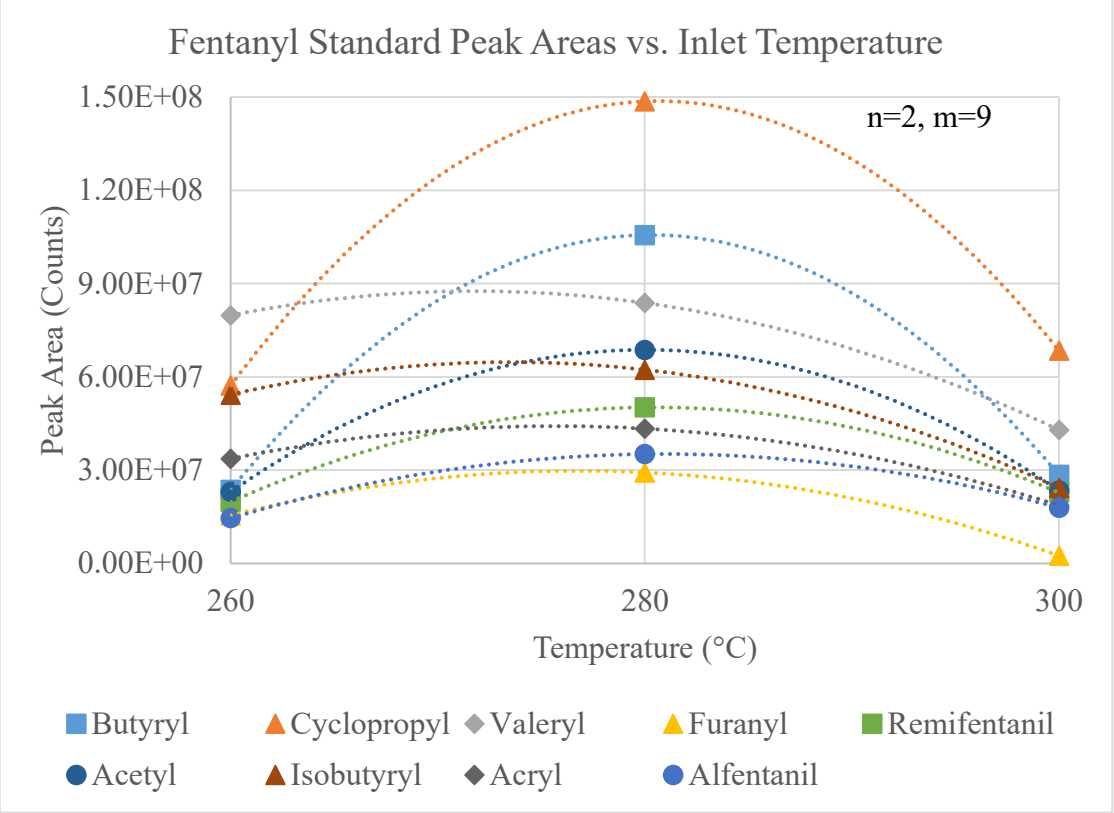

Fig. 4 Plot of fentanyl standards analyzed via GC-MS using three different injector temperatures (n=2).

Gas Chromatographic Oven Temperature Program

Several different gas chromatographic oven temperature programs were evaluated in order to obtain the most optimum oven temperature program to resolve the fentanyl compounds. The optimized split ratio (10:1), injection volume (1 μL) and injection port temperature (280°C) were used with these different oven temperature programs. The different oven temperature program parameters are shown in Table 5.

Eight oven program optimizations in all were utilized in the evaluation. Resolution and total run time were the major factors considered to optimize the method. Tables 6 and 7 show the calculated resolution for the fentanyl standards used to optimize the GC oven program.

The resolution equation used was as follows:

$$\text{Resolution} = 1.18 \times [(t_{R2} - t_{R1}) / (W_{0.5h1} + W_{0.5h2})]$$

The t_R and $W_{0.5h}$ variables represent the retention times and width at half peak height, respectively, for the two compared compounds.

Table 5. Oven temperature program optimizations.

Instrument Method	Oven Program	Ramp Rate(s)	Run Time (minutes)
Default	60°C (2.00 min hold) 15°C/min to 300°C (10.00 min hold) Post Run: 60°C (2.00 min hold)	1	28.00
Opt. 1	60°C (2.00 min hold) 30°C/min to 240°C 8°C/min to 300°C (9.50 min hold)	2	25.00

Opt. 2	60°C (2.00 min hold) 30°C/min to 270°C 8°C/min to 300°C (6.50 min hold)	2	19.25
Opt. 3	60°C (2.00 min hold) 30°C/min to 240°C 4°C/min to 300°C (6.50 min hold)	2	29.50
Opt. 4	60°C (2.00 min hold) 30°C/min to 250 °C 4°C/min to 300°C (2.50 min hold)	2	22.33
Opt. 5	60°C (2.00 min hold) 30°C/min to 270°C (4.00 min hold) 4°C/min to 300°C (2.50 min hold)	2	22.00
Opt. 6	60°C (1.00 min hold) 30°C/min to 260°C (4.00 min hold) 4°C/min to 300°C (2.50 min hold)	2	24.17
Opt. 7	60°C (1.00 min hold) 30°C/min to 280°C (4.00 min hold) 4°C/min to 300°C (2.50 min hold) *Carrier gas flow was increased from 1.0 mL/min to 1.4 mL/min	2	22.00
Opt. 8 (Final Method)	60°C (1.00 min hold) 30°C/min to 270°C (8.00 min hold) 8°C/min to 300°C (2.50 min hold)	2	22.25

The retention times observed from implementing optimization 1 (Table 5) GC oven temperature program demonstrated that the fentanyl standards were eluting in the temperature area of 270°C. Table 8 shows the changes made to the GC oven temperature program in order to optimize the GC method. In optimization 1, retention times were observed to elute in the temperature area of 270°C, however from optimization 1 to 2 there was a general decrease in resolution. Optimization 3 had the best resolution to that point, but the total run time was near half an hour. Resolution was generally retained from optimization 3 to 4 and the total run time was decreased to near 20 minutes, using the W-15 standard as the end marker. Introducing a plateau in optimization 5 demonstrated similar resolution results to optimization 4. There was a general decrease in resolution for both modifications analyzed in optimizations 6 and 7. Optimization 8 demonstrated an increase in resolution from optimization 5.

Table 6. Resolution determined between adjacent eluted fentanyl standards analyzed using optimizations 1-4.

Compound	Opt. 1	Opt. 2	Opt. 3	Opt. 4
4-ANPP	19.18	15.17	21.39	20.55
Remifentanil	22.32	20.46	22.92	24.47
Acetyl	6.51	5.56	6.78	7.05
Isobutyryl	2.72	2.79	3.33	2.81
Fentanyl	2.86	2.84	2.87	3.07
Acryl	9.17	7.81	10.39	10.56
Butyryl	9.80	9.19	10.78	10.99
Cyclopropyl	6.39	6.05	7.13	7.61
Valeryl	8.14	8.25	8.17	8.76
β-hydroxythio	3.80	3.70	5.02	5.02
Alfentanil	11.72	10.89	14.72	14.35
Paramethoxybutyryl	5.84	6.44	6.05	6.23
Furanyl	37.71	37.39	39.38	36.35
W-15				

Table 7. Resolution determined between adjacent eluted fentanyl standards analyzed using optimizations 5-8.

Compound	Opt. 5	Opt. 6	Opt. 7	Opt. 8
Remifentanil	21.63	21.49	20.33	21.05
FIBF	11.07	10.47	9.91	10.61
Trans-3-methyl fentanyl	0.00	0.00	0.00	0.00
Fentanyl	1.51	N/A	1.31	1.53
Meta-fluorobutyryl fentanyl	0.90	N/A	1.18	0.87
Thiofentanyl	1.81	N/A	1.32	1.79
Para-fluorobutyryl fentanyl	3.17	2.85	2.92	3.13
Ortho-fluorobutyryl fentanyl	7.75	7.32	7.40	8.16
Ocfentanil	10.18	10.18	9.59	11.19
Carfentanil				

Table 8. Modifications to GC oven temperature program during optimization of GC method.

Optimization	30°C/min to	Hold	2nd Ramp Rate
1	240°C	N/A	8°C/min
2	270°C	N/A	8°C/min
3	240°C	N/A	4°C/min
4	250°C	N/A	4°C/min
5	270°C	4 min	4°C/min
6	260°C	4 min	4°C/min
7	280°C	4 min	4°C/min
8	270°C	8 min	8°C/min

*Initial temperature, 60°C, final temperature, 300°C

After several changes to the parameters it was observed that holding at a temperature of 270°C for eight minutes, after first ramping at 30°C/min, then performing a second ramp rate of 4°C/min till 300°C offered the optimum separation between analogues. By introducing a plateau of 8 minutes in optimization 8, there was enough time for the fentanyl analogues to elute in a wider range of time. The total run time was able to be reduced to 22.25 minutes, which incorporates the retention time of W-15, by utilizing an initial hold time of 1.00 minute and then a gas saver time of 1.00 minute, as well. The final optimized GC conditions were as follows: injection mode, split 10:1; injector temperature, 280°C; injection volume, 1.0 μL; carrier gas, helium; carrier gas saver time, 1.00 minute. The final GC oven was temperature programmed as follows: initial temperature, 60°C; initial hold, 1.00 min; temperature program rate, 30°C/min to 270°C (8.00 min hold), 8°C/min to 300°C; final hold, 2.50 min. Figure 5 shows the separation on the Rxi-5ms column at these conditions of three mixtures each containing 10 different fentanyl compounds each at a concentration of 100 μg/mL.

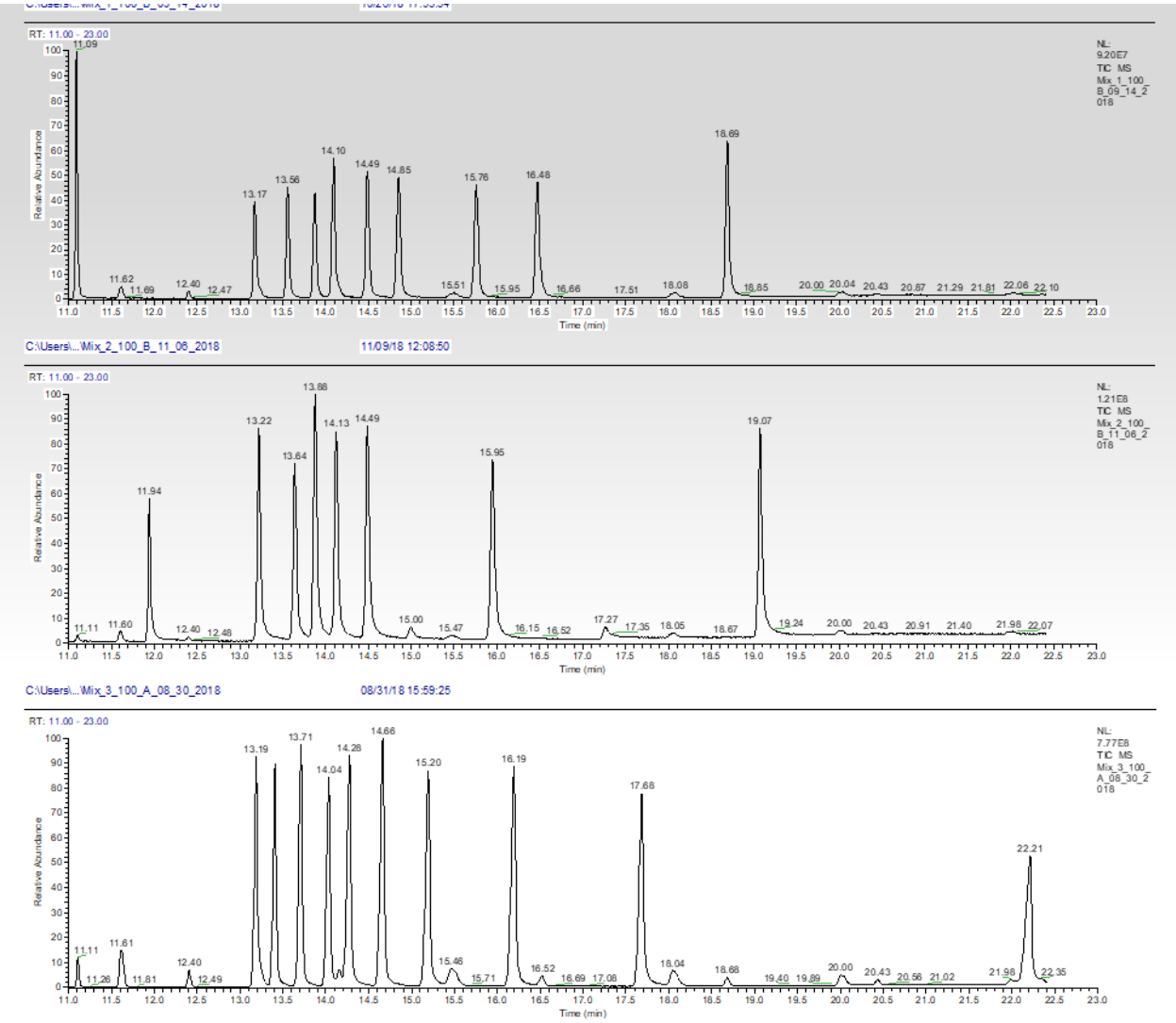

Fig. 5 Gas chromatographic-mass spectrometric analysis of three mixtures of ten fentanyl analogues (100 μg/mL) utilizing a Rxi-5ms column (30 m x 0.25 mm ID, 0.25 μm).

Linear Retention Indices

Two other GC columns were installed into this instrument, Rtx-5 amine (column #4, Table 3) and Rtx-200ms (column #5, Table 3). The final GC-MS method was also loaded onto the Agilent Technologies 6890N Series GC system, fitted with an HP-1 column (column #3, Table 3). The method was also loaded onto the Agilent Technologies 7890A Series GC-FID system, fitted with both Rxi-624Sil ms (column #6, Table 3) and Rxi-17Sil ms (column #7, Table 3) columns.

All thirty fentanyl standards were analyzed using the final GC method conditions, as described above, and linear retention indices were determined (Tables 9 and 10). The linear retention indices were calculated using the equation as follows:

$$\text{Linear Retention Index (RI)} = [100n \times (t_c - t_z / t_{z+1} - t_z)] + 100z$$

Figure 6 shows the data from ten fentanyl standards analyzed simultaneously. An injection from a mixture of standards was injected onto the front FID and then a second injection from the same mixture of standards

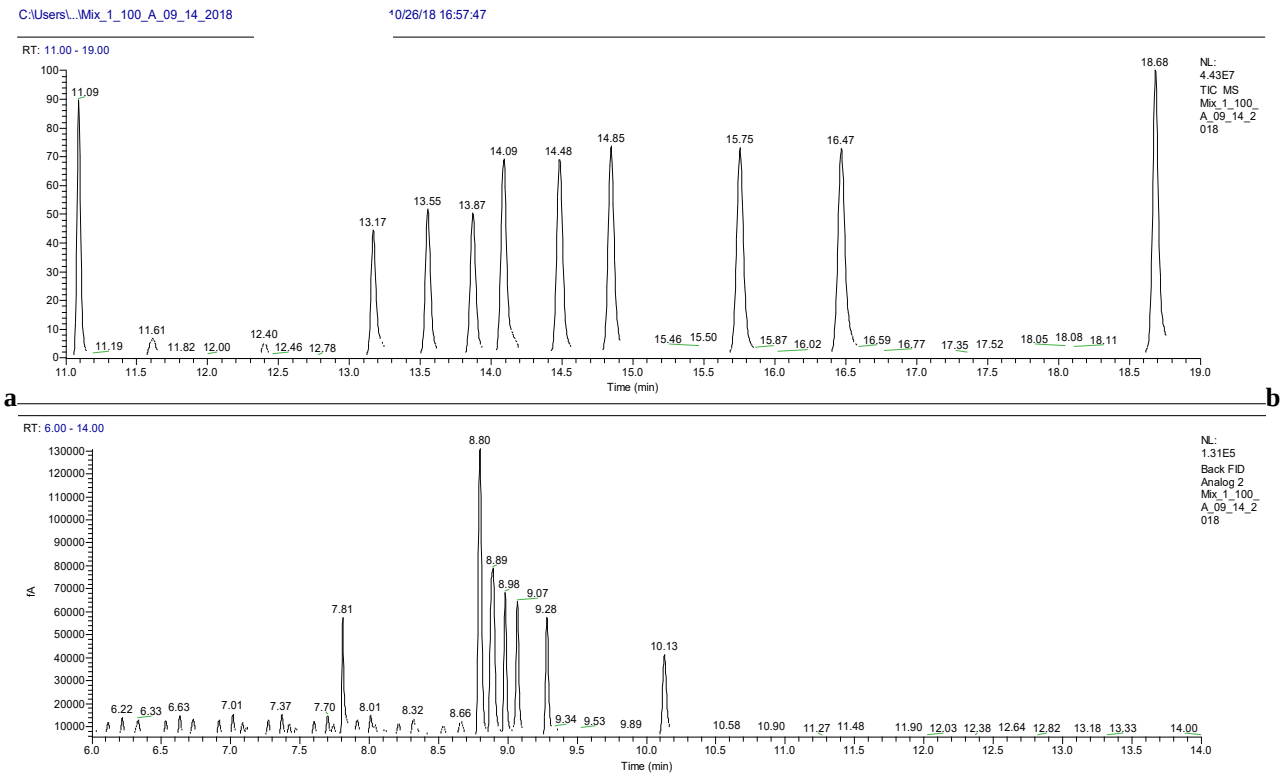

Fig. 6 Gas chromatograph-mass spectrometry analysis of ten fentanyl analogues (100 μg/mL) utilizing an (**a**) Rxi-5ms column and a (**b**) Rtx-200ms column. Total ion chromatograms (TIC) displayed using 8-minute ranges.

Table 9. Linear retention indices calculated for thirty fentanyl standards on three columns (n=3)

Compound	Rxi-5ms	Rtx-5 amine	Rtx-200ms
4-ANPP	2539.48 ± 0.82	2573.83 ± 0.65	2798.57 ± 0.00
Remifentanil	2637.79 ± 0.00	2665.14 ± 0.00	3143.16 ± 1.82
Meta-fluorofentanyl	2752.69 ± 1.06	2786.49 ± 0.84	3223.02 ± 1.37
FIBF	2753.92 ± 1.84	2784.56 ± 1.45	3208.73 ± 1.37
Acetyl fentanyl	2755.76 ± 0.00	2796.62 ± 0.84	3238.89 ± 2.38
Para-fluorofentanyl	2774.19 ± 2.44	2806.88 ± 0.59	3256.35 ± 1.37
Ortho-fluorofentanyl	2788.33 ± 1.41	2822.20 ± 0.59	3218.25 ± 1.37
β-methyl fentanyl	2794.47 ± 0.00	2829.08 ± 1.48	3190.53 ± 1.82
Isobutyryl fentanyl	2801.84 ± 2.44	2831.43 ± 0.34	3174.74 ± 1.82
Fentanyl	2811.01 ± 0.00	2849.12 ± 0.90	3226.98 ± 2.38
Trans-3-methyl fentanyl	2811.82 ± 0.93	2846.56 ± 0.34	3219.05 ± 1.37
Meta-fluorobutyryl fentanyl	2821.20 ± 1.27	2856.19 ± 0.90	3275.40 ± 2.75
Thiofentanyl	2825.48 ± 1.27	2866.99 ± 1.02	3247.62 ± 1.37
Acryl fentanyl	2825.89 ± 0.35	2869.55 ± 0.68	3242.86 ± 1.37
Para-fluorobutyryl fentanyl	2835.88 ± 1.77	2874.66 ± 1.56	3311.11 ± 3.64
Ortho-fluorobutyryl fentanyl	2848.32 ± 0.00	2894.11 ± 0.59	3271.43 ± 1.37
Cis-3-methyl fentanyl	2849.13 ± 0.93	2893.91 ± 0.34	3253.17 ± 0.00
α-methyl fentanyl	2859.73 ± 1.77	2904.91 ± 0.68	3289.68 ± 1.37
Butyryl fentanyl	2871.56 ± 1.22	2918.47 ± 1.36	3278.57 ± 2.75
Sufentanil	2879.31 ± 0.35	2927.90 ± 0.90	3208.73 ± 1.37
Ocfentanil	2892.15 ± 1.77	2940.08 ± 0.59	3422.16 ± 1.01
Cyclopropyl fentanyl	2927.22 ± 1.22	2971.32 ± 1.77	3307.14 ± 3.64
Crotonyl fentanyl	2937.61 ± 0.00	2981.34 ± 1.02	3373.02 ± 2.75
Carfentanil	2953.72 ± 1.97	2992.34 ± 1.23	3380.16 ± 3.64
Valeryl fentanyl	2971.05 ± 1.27	3005.66 ± 1.13	3374.60 ± 2.38
β-hydroxythiofentanyl	3020.53 ± 0.40	3095.94 ± 2.59	3609.56 ± 1.91
Alfentanil	3050.29 ± 2.00	3082.41 ± 0.85	3651.14 ± 2.16

Para-methoxybutyryl fentanyl	3120.18 ± 1.44	3149.57 ± 0.85	3788.34 ± 2.02
Furanyl fentanyl	3145.33 ± 0.00	3186.96 ± 0.74	3646.57 ± 1.44
W-15	3363.09 ± 2.80	N/A	N/A

were injected onto the back FID. The Rxi-5ms column was fitted onto the front FID and the Rtx-200ms and Rtx-5 amine columns were fitted onto the back FID. The fentanyl standards elute in a tighter range, with the majority of standards eluting before 11.00 minutes, using the Rtx-200ms column versus the Rxi-5ms column, as seen in Figure 5. Therefore, while the Rtx-200ms column cannot resolve all of the fentanyl analogues in the mixture it has potential to serve as a screening column for these compounds.

Resolution, according to the linear retention indices produced, was accomplished between all thirty fentanyl standards using the HP-1 column, which was not able to be achieved utilizing the Rxi-5ms column. The Rtx-5 amine column did not provide data that was significantly different than data produced from utilizing the Rxi-5ms column. This is most likely due to having the same exact stationary phase with the exception of the Rxi-5ms column being chemically treated for analyzing basic drugs. Fentanyl standards eluted in the range of 14.00-26.00 minutes using the Rxi-17sil ms column and provided some complementary information for compounds analyzed on the more non-polar columns, Rxi-5ms and HP-1. The fentanyl compounds eluted in a range from 21.00-31.00 minutes utilizing the Rxi-624sil ms column and had a more difficult time with coming off the column as noted in Table 10. The film thickness was greater for the Rxi-624sil ms column, relative to all columns in this study, which may have affected the resolution of the fentanyl compounds.

Table 10. Linear retention indices calculated for twenty-nine fentanyl standards on three columns (n=3)

Compound	HP-1	Rxi-17sil ms	Rxi-624sil ms
4-ANPP	24667.24 ± 0.99	3023.63 ± 0.09	2517.93 ±1.38
Remifentanil	2560.68 ± 0.00	3167.11 ± 0.11	2632.63 ± 1.78
Acetyl fentanyl	2671.26 ± 0.00	3325.38 ± 0.15	2758.56 ± 1.74
Meta-fluorofentanyl	2674.71 ± 0.00	N/A	2744.79 ± 1.70
FIBF	2678.54 ± 0.66	3223.12 ± 0.22	2741.54 ± 1.71
Para-fluorofentanyl	2688.51 ± 0.00	3289.06 ± 0.15	2768.85 ± 1.60
Ortho-fluorofentanyl	2701.15 ± 0.00	3320.46 ± 0.26	2776.87 ± 1.68
β-methyl fentanyl	2710.34 ± 0.00	3314.02 ± 0.15	2773.01 ± 1.98
Isobutyryl fentanyl	2715.33 ± 0.66	3307.31 ± 0.26	2777.59 ± 2.38
Fentanyl	2724.14 ± 0.00	3360.82 ± 0.18	2802.41 ± 1.46
Trans-3-methyl fentanyl	2728.74 ± 0.00	3330.85 ± 0.00	2797.42 ± 2.24
Thiofentanyl	2736.78 ± 0.00	3398.35 ± 0.15	2820.20 ± 1.73
Acryl fentanyl	2737.93 ± 0.00	3410.44 ± 0.23	2820.56 ± 1.70
Meta-fluorobutyryl fentanyl	2742.53 ± 0.00	3308.91 ± 0.26	2810.09 ± 1.48
Para-fluorobutyryl fentanyl	2758.62 ± 0.00	3338.67 ± 0.18	2829.36 ± 1.72
Ortho-fluorobutyryl fentanyl	2775.86 ± 0.00	3376.22 ± 0.15	2837.92 ± 1.30
Cis-3-methyl fentanyl	2778.16 ± 0.00	3370.27 ± 0.11	2837.15 ± 1.45
α-methyl fentanyl	2786.21 ± 0.00	3392.47 ± 0.11	2847.95 ± 1.76
Butyryl fentanyl	2800.75 ± 0.00	3412.07 ± 0.07	2858.79 ± 1.95
Ocfentanil	2809.22 ± 0.43	3479.75 ± 0.14	2901.68 ± 1.90
Sufentanil	2810.46 ± 0.00	3417.46 ± 0.04	2855.29 ± 1.51
Cyclopropyl fentanyl	2841.10 ± 0.75	3514.36 ± 0.25	2910.88 ± 1.87
Crotonyl fentanyl	2847.07 ± 0.00	3545.67 ± 0.12	2927.99 ± 2.19
Carfentanil	2861.02 ± 0.43	3537.24 ± 0.25	N/A
Valeryl fentanyl	2882.94 ± 0.00	3503.66 ± 0.15	2952.56 ± 2.35
β-hydroxythiofentanyl	2910.59 ± 0.00	*N/A	*N/A
Alfentanil	2936.24 ± 0.43	*3622.38 ± 0.15	*N/A
Para-methoxybutyryl fentanyl	3020.29 ± 0.35	*3658.67 ± 0.10	*N/A
Furanyl fentanyl	3038.73 ± 0.35	*3754.90 ± 0.14	*N/A

*Note: GC-MS method was modified so that these compounds would elute. The final temperature was increased to 310°C and held for an additional 12.00 minutes. For compounds denoted with *N/A, the modification was not enough for these compounds to elute.

Conclusions

With forensic laboratories overwhelmed by increasing amounts of casework pertaining to fentanyl compounds, and the analogue trends changing unpredictably, a gas chromatographic-mass spectrometric method was needed to address the analysis of fentanyl analogues. Identification of fentanyl analogues commonly rests on confirmative analysis by way of gas chromatography-mass spectrometry. Identification is not solely significant for fentanyl but for fentanyl analogues, which possess different schedules and their own geographic trends (1). Optimization of a qualitative gas chromatographic-mass spectrometric method, suitable for forensic casework, was principle for the capability of resolving and identifying multiple fentanyl analogues.

The solution to obtaining the best resolution between fentanyl analogues was achieved by optimizing crucial parameters of the gas chromatographic-mass spectrometric method. Parameters for separating fentanyl and 29 of its analogues investigated were stationary phase, injection volume, split ratio, injection port temperature and gas chromatographic oven temperature program. Sufficient resolution for identification of the 30 fentanyl compounds was accomplished using a 100% dimethylpolysiloxane column (HP-1, 30 m x 0.25 mm ID, 0.25 μm). Six other columns with more polar stationary phases were not able to achieve the same resolution of these compounds. However, the Rtx-200ms column could be used as a screening column since most of the fentanyl compounds elute in a short retention time window. By slowing down the ramp rate as much as possible around a temperature of 270°C, where the majority of fentanyl analogues elute on the non-polar column 100% PDMS), the best resolution was observed. The final optimized GC conditions for this column were as follows: injection mode, split 10:1; injector temperature, 280°C; injection volume, 1.0 μL; carrier gas, helium; carrier gas saver time, 1.00 minute. The final GC oven was temperature programmed as follows: initial temperature, 60°C; initial hold, 1.00 min; temperature program rate, 30°C/min to 270°C (8.00 min hold), 8°C/min to 300°C; final hold, 2.50 min. The total run time for this method is 22.25 minutes. Linear retention index data for 30 fentanyl compounds is also reported on the six different stationary phases which was previously unreported.

Acknowledgements

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Historical facts – NEAFS

- "NEAFS was founded in 1975 by a group of dedicated forensic scientists dedicated to improving the professional status and technical capabilities of individuals engaged in all phases of forensic science." "To accomplish its goals, NEAFS conducts continuing education seminars featuring workshops and special training sessions. The Annual Meeting…presents a contagious atmosphere of scientific exchange and social congeniality."Mark Lewis, President 1980
- The first Editor of the newsletter in 1976 was R.E. Gaensslen
- The first meeting of the Executive Board was on May 1, 1976 by President Angelo Fatta. Also in attendance were Vincent Crispino, R.E. Gaensslen, Thomas Kubic, Carl Moller and Alexander Stirton.
    - On this first meeting, it was stated that there were 211 members and this number included applicants. Six of those members were upgraded to Regular members.
    - The first annual meeting was being discussed. The annual meeting was to be a one day meeting on or about October 23, 1976. Tentative sites were John Jay College or C.W. Post College. The schedule was: 8am-12pm Coffee and Registration, business meeting and split sessions; Lunch; 1pm-5pm two general interest talks, split sessions, mixer and dinner. The split sessions included serology, microscopy, arson, toxicology and drug identification. The general interest talks would be short and would be concerning aspects of forensic science that would be unfamiliar or unusual to most members.
- NEAFS was incorporated by the State of Connecticut on May 12, 1976. Vincent Crispino, Thomas Kubic and Henry Lee were the Incorporators.
- The NEAFS newsletters were published by the Forensic Sciences Foundation which was located in Maryland.
- A joint meeting was held on April 15-16 with MAAFS in New Jersey as well as the Annual Meeting of NEAFS on October 29th in 1977.
- Dr. Peter De Forest chaired the Hairs and Fibers Session during the Second Annual Meeting. Alexander Stirton chaired the Serology Session and Dr. Jesse Bidanset chaired the Toxicology Session during the Second Annual Meeting.
- The newsletters included information from other regional organizations as well as NEAFS.
- In 1977, the BOD acted as an ad hoc Education Committee and set up two courses intitled: "Forensic Microscopy" and "Introduction to the Forensic Applications of Infrared Spectroscopy".
- A luncheon was held during the 3rd Annual meeting of NEAFS and consisted of salad, a choice of roast beef or filet of sole, dessert and a beverage for $6.00. Cocktails were $1.50 and beer and wine were $1.00.
- In 1978, the annual meeting was increased to a two day program instead of one day.
- George Neighbor volunteered to chair the Paint analysis program for the 1978 Annual Meeting.
- In 1978, NEAFS sponsored a training course entitled "Basic Bloodstain Analysis" and it was taught by Dr. Henry Lee, Dr. R.E. Gaensslen and Dr. Peter De Forest. This course was held at the University of New Haven.
- George W. Neighbor was the Secretary of NEAFS in 1978.
- Thomas A. Kubic was voted in as a Life Member of NEAFS while he was President in 1978.
- In 1979, Chris Chany was approved to become a Provisional member from a student member and Peter Diaczuk was approved to be a Corresponding member.
- George W. Neighbor was President-elect in 1980.
- Travel reimbursement for mileage was 17 cents/mile in 1980.
- NEAFS had 400 members in 1980.
- In May 1980 in Louisville Kentucky, NEAFS participated in the first multi-regional association meeting.
- George W. Neighbor had a BA degree in Chemistry from Rider College and a MS in Forensic Science from John Jay College. He worked as a Principal Forensic Chemist for the NJSP in the North Regional Laboratory in Little Falls, NJ where he supervises the trace evidence and bio-chemical units. Prior to working with the NJSP, He has

twenty years of industrial research experience in materials analysis. He served as Secretary for two terms (1978-79) and was a member of the AAFS and the Forensic Science Academy. George became President of NEAFS in 1981 – the 7th year in NEAFS history. George stated at the end of his President's message in the March 1981 newsletter "Now you can call me George, or you can call me G.W., or you can call me George W., or you can call me Hi Neighbor". In 1989, George presented "Trace Evidence Never Grows Old" during the Criminalistics Session.

- In 1997, the Scholarship award was renamed the George W. Neighbor Jr. Memorial Scholarship
- In 1980, the Annual Meeting budget was $2000.
- 1980 Goals of NEAFS
    - Exchange ideas and information among professionals in the field
    - Promote recognition of forensic science as an important part of the justice system
    - Sponsor and organize seminars, workshops, and special training sessions
    - Represent the membership on national issues affecting forensic science
    - Encourage research and development
    - Stimulate implementation of new methods and techniques
    - Establish professional standards
    - Provide advice on educational curricula, legislation and other matters affecting the profession
    - Arbitrate professional disputes
    - Foster friendship and collegiality among the forensic scientists of the Northeast
- For the 10th Annual Meeting, the room rate was $55 (single or double).
- The 12th annual meeting was the first meeting held in New England in Peabody, MA. A clam bake was scheduled.
- The door prizes that were given out at the 11th Annual Meeting were a Commador 64 Computer, Cannon AE1 Camera, Reflecting Telescope and an AM-FM radio.
- Our current method of visiting the exhibitor booths and obtaining confirmation of the visit goes back to at least the 9th Annual Meeting in 1983.
- The door prizes given out at the 14th Annual Meeting which was donated by Perkin-Elmer were a Video Cassette Recorder, Compact Disk Player, Scientific Programmable Calculator, Cordless Telephone and a Sony Walkman.

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