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FINAL REPORT

Agency: National Institute of Justice

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Project Title: *Research to Develop Validated Methods for THC Quantification in Complex Matrices by High-resolution DART-MS-Focus on Edibles and Plant Materials*

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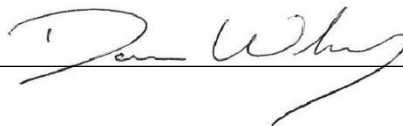
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SUMMARY OF THE PROJECT

Purpose of the Project

The hypothesis underlying this project was that the unique capabilities of direct analysis in real time – high-resolution mass spectrometry (DART-HRMS) could be used for the rapid and streamlined detection of Δ^9 -tetrahydrocannabinol, or THC (i.e., the major psychoactive component of *Cannabis sativa*), in complex plant and edible matrices. It was further proposed that the approach could be used for rapid quantification of THC in complex matrices, in a manner that enables it to be distinguished from cannabinoid isomers (i.e., molecules with the same molecular formula, such as THC and cannabidiol, or CBD), and other phytocannabinoids (i.e., natural cannabinoids) without the need for extensive sample processing steps. The goal of the project was to develop validated protocols that can be used by crime labs to rapidly identify and quantify THC in complex matrices that are difficult to analyze by conventional methods.

Major Goals and Objectives: The hypothesis was investigated through pursuit of the following four Objectives/Specific Aims:

Specific Aim I: Demonstration of the utility of DART-HRMS as a presumptive test that can be used to rapidly detect THC in complex matrices, with minimal to no sample pretreatment steps.

Specific Aim II: Demonstration of the ability to distinguish THC from CBD and other phytocannabinoids in *Cannabis* using DART-HRMS.

Specific Aim III: Development of DART-HRMS validated protocols for the quantification of THC and CBD.

Specific Aim IV: Development of optimized procedures for the recovery of THC from complex matrix edibles, beverages, and plant material for subsequent quantitative analysis of THC.

Research Questions

The questions investigated throughout the project aligned with the specific aims as outlined below:

Questions Under Specific Aim I: Can THC, CBD, and other cannabinoids standards be detected by DART-HRMS analysis? What parameters are optimal for the detection of cannabinoids? Can cannabinoids and other *Cannabis*-related molecules (i.e., terpenes) be detected in complex matrix materials including baked goods, candies, beverages, and plant materials, among others, permitting DART-HRMS to be used as a triage approach test? What are the instrument detection limits (IDLs) for THC in the various sample types?

Questions Under Specific Aim II: Can THC, CBD, and other phytocannabinoids be distinguished from one another using DART-HRMS? If so, under what conditions for each of the proposed analytical techniques are THC and CBD distinguishable?

Questions Under Specific Aim III: Can cannabinoids be quantified by DART-HRMS? Can the methods be validated? Can THC be quantified in the presence of other cannabinoids? Can THC be quantified in complex matrices, such as edibles and recreational products?

Questions Under Specific Aim IV: Can THC and CBD be extracted from complex matrix materials? What are the extraction efficiencies of different protocols? What are the effects that different complex matrices will have on the quantification of THC?

Research Design, Methods, Analytical and Data Analysis Techniques

Specific Aim I: Towards accomplishing Specific Aim I, 32 cannabinoid standards, (Cayman Chemical, Ann Arbor, MI, USA; Cerilliant Corporation, Round Rock, TX, USA), 5 terpene standards (Cayman Chemical, Ann Arbor, MI, USA), 5 cannabinoid-infused edible and 4 blank certified reference materials (CRMs) (Emerald Scientific, San Luis Obispo, CA, USA), 10 commercial personal-care products (Rad Soap Co., Albany, NY, USA; Beak & Skiff, Lafayette, NY, USA), 41 commercial hemp flower products (CBD Hemp Direct, Las Vegas, NV, USA;

Berkshire CBD, Brattleboro, VT, USA; Plain Jane, Berkeley, CA, USA, and Medford, OR, USA), 12 marijuana samples from Drug Enforcement Administration (DEA)-registered suppliers (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA; National Institute on Drug Abuse (NIDA) Drug Supply Program, RTI, NC, USA), and various recreational *Cannabis* products (edibles [chocolates (2); fruit chews (5); beverages (3); capsules (2)]; concentrates (6), tinctures (1); vaporizers (1); topicals (1); pre-rolls (4); marijuana flower (21)) (Garden Remedies Marijuana Dispensary, Melrose, MA, USA) were acquired. In addition, a broad range of edible samples (e.g., fruit chews/gummies, chocolates, marshmallows, beverages, baked goods) infused with cannabinoid standards were prepared in-house. All samples were analyzed by DART-HRMS over a range of m/z 60-1000 (at a rate of 1 spectrum per s) using a DART-SVP ion source (IonSense, Saugus, MA, USA) coupled to a JEOL AccuTOF high-resolution mass spectrometer (JEOL, Peabody, MA, USA). Data collected in positive-ion mode were acquired at a DART ion source grid voltage of either 250 or 350 V and the following mass spectrometer settings: orifice 1 voltage, 20 V; ring lens and orifice 2 voltages, 5 V; peak voltage, 600 V; and detector voltage, 2000 or 2100 V. Data collected in negative-ion mode were obtained at a DART ion source grid voltage of either -250 or -350 V and the following mass spectrometer settings: orifice 1 voltage, -20 or -90 V; ring lens and orifice 2 voltages, -5 V; peak voltage, 600 V; and detector voltage, 2000 or 2100 V. All DART-HRMS acquisitions were collected at a gas temperature of 350 °C and flow rate (ultra-high purity helium; AirGas, Albany, NY, USA) of 2 L/min. Samples were analyzed in replicates of 3 or 5 by inserting the closed end of a glass melting-point capillary tube (Corning, Radnor, PA, USA) into the samples and presenting it to the DART gas stream in the open-air gap between the ion source and mass spectrometer inlet for approximately 5 s. Polyethylene glycol (PEG 600) and Fomblin Y (Sigma Aldrich, St. Louis, MO,

USA) were used as the mass calibrants in all DART-HRMS acquisitions conducted in positive- and negative-ion mode, respectively. TSSPro 3.0 (Shrader Software Solutions, Grosse Pointe, MI, USA) was used for calibration, background subtraction, and peak centroid purposes. Mass Mountaineer (RBC Software, Portsmouth, NH, USA) was used for data processing of mass spectral data.

Specific Aim II: Towards accomplishing Specific Aim II, two approaches were used to investigate the differentiation of phytocannabinoids: (1) DART-HRMS analysis of cannabinoid standards in negative-ion mode under collision-induced dissociation (CID) conditions (orifice 1 voltage, -90 V) and subjecting the data to statistical analysis methods (i.e., Kernel Discriminant Analysis, or KDA); and (2) applying a derivatizing agent (*N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide, or MSTFA (Sigma Aldrich, St. Louis, MO, USA)) to cannabinoid standards and infused samples prior to interrogation by DART-HRMS. In the first approach, *m/z* values of the fragment peaks in the CID spectra enabled differentiation of 32 different cannabinoids. In the second approach, a shift in the high-resolution protonated masses of cannabinoids was used to reveal the presence of cannabinoid isomers (i.e., THC, CBD).

Specific Aim III: Calibration curves for THC and CBD were developed over a range of 10 to 150 mg/L and validated according to the U.S. Food and Drug Administration (FDA) Bioanalytical Method Validation: Guidelines for Industry. These guidelines were used because: (1) unlike many of the other guidelines that are optimized primarily for highly purified or semi-purified drug samples, the FDA guidelines better accommodate matrices that are highly complex and heterogenous, such as the food and drug samples that were the focus of this work; and (2) the FDA method validation requirements are generally much more stringent. This was deemed to be important because the successful demonstration of method validation under FDA guidelines

supports the premise that the development of validated methods under the less stringent requirements of other organizations is likely possible. Thus, a next step under this initiative is to develop validated protocols using other guidelines, (e.g., the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)) in order to facilitate the opt incorporate these methods into crime laboratory workflows. Calibration curves were developed using either a 24-Pin Liquid Sampler (IonSense, Saugus, MA USA) with 384-well plates (Eppendorf, Enfield, CT, USA) or DIP-it tips and a holder (IonSense, Saugus, MA USA). All quantification experiments were performed using a DART-HRMS linear rail system, which enables semi-automated acquisitions at a user-defined speed. Deuterated counterparts of THC (THC- d_3) and CBD (CBD- d_9) were used as the internal standards, which enabled the calculation of peak area ratios between the analyte of interest (i.e., cannabinoids) and the internal standards. The developed validated protocols were used to quantify CBD in edibles prepared in-house and THC in recreational products. Research towards incorporating a derivatization step (Specific Aim II) prior to the development of calibration curves was also conducted.

Specific Aim IV: Towards accomplishing Specific Aim IV, several extraction protocols were investigated to maximize the extraction of cannabinoids from complex matrices. DisQue CEN salts (1 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate, 1 g NaCl, and 4 g MgSO₄) (Waters Corporation, Milford, MA, USA), when combined with water and acetonitrile solvents, were found suitable and therefore used to extract cannabinoids from complex edible matrices prior to launching DART-HRMS quantification experiments.

Expected Applicability of the Research

The expected deliverables of the projected included: (1) the development of a DART-HRMS presumptive test to indicate the potential presence of THC in extremely complex matrices (i.e.,

food, beverages, plant materials) that are problematic to analyze by traditional approaches; (2) methods to differentiate between cannabinoids without the requirement to separate the components by chromatography; (3) validated DART-HRMS quantification protocols that can be applied to cannabinoid-infused complex matrices; and (4) the investigation of extraction protocols to enable efficient extraction of cannabinoids from various complex matrix materials, prior to launching quantification endeavors. The relevance of DART-HRMS in the field of forensic science is not limited solely to the analysis of *C. sativa* and products derived from this plant. Rather, this approach has a wide range of uses and can aid forensic practitioners with case samples comprised of a broad range of evidence types (such as those associated with drug testing, field testing), and the development of comprehensive mass spectral databases, etc. Furthermore, the methods developed in this project have potentially far-reaching implications in other disciplines, including food science, agriculture, phytochemistry, natural products chemistry, cosmetics/personal-care products, and the *Cannabis* industry.

PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

The Vermont Forensic Laboratory (VFL) (Waterbury, VT, USA) supported the project in an advisory capacity and provided relevant information regarding the types of samples submitted to the VFL. The information provided by collaborators at the VFL assisted with decisions made throughout the duration of the project regarding the types of samples to investigate. In addition, DART-HRMS analyses involving recreational marijuana flower and *Cannabis* products were performed at IonSense (Saugus, MA, USA) by staff scientists, and the raw data files were calibrated, processed, and evaluated at the University at Albany, SUNY.

CHANGES IN APPROACH FROM ORIGINAL DESIGN AND REASON FOR CHANGE, IF APPLICABLE

Over the duration of the project, there were no changes to the agency-approved plan.

OUTCOMES

Results and Findings

Over the course of the project, cannabinoid standards, terpene standards, cannabinoid-infused edible CRMS, blank CRMs, commercial personal-care products, commercial hemp flower products, marijuana samples from DEA-registered suppliers, recreational *Cannabis* products (edibles [chocolates; fruit chews; beverages; capsules]; concentrates, tinctures; vaporizers; topicals; pre-rolls; marijuana flower), and a range of edible samples infused with cannabinoid standards prepared in-house were analyzed by DART-HRMS. Analysis in positive-ion mode confirmed that the cannabinoid and terpene standards could be detected by DART-HRMS. However, negative-ion mode was found to be insufficient for the analysis of terpenes. In most cases, DART-HRMS analyses readily revealed the presence of cannabinoids (or confirmed the absence of cannabinoids in experimental controls), in either positive- or negative-ion mode, with no sample processing steps. The DART-HRMS IDL for the developed triage approach was determined to be 1.09 µg/mL for THC and 1.29 µg/mL for CBD. However, there were a few instances where cannabinoids at very low levels in the recreational products were not detected.

Two investigations for differentiating cannabinoids demonstrated proof-of-concept that can be further refined for optimal inclusion in forensic workflows. The first investigation produced an internal validation accuracy of 91.88% when performed on a KDA model containing 32 cannabinoids. These results demonstrate that cannabinoids can be differentiated when statistical analysis techniques are applied to DART-HRMS data collected in negative-ion mode at an orifice

1 voltage of -90 V. The second investigation showed that by incorporating a pretreatment step (i.e., the derivatizing agent MSTFA) prior to DART-HRMS analysis, THC and CBD could be differentiated when both are present in complex matrices (i.e., edibles, oils, balms).

THC and CBD calibration curves were prepared as described above and created using DART-HRMS semi-automated capabilities. Quality control (QC) standards and the guidelines recommended by the FDA enabled the development of validated protocols. These protocols were relied upon for the quantification of CBD in chocolates and fruit chews prepared in-house using known amounts of CBD, for which percent (%) recoveries could be calculated. These % recoveries were determined to be 76.2-84.3% for the CBD-infused chocolates and 96.7-99.0% for the CBD-infused fruit chews. The DART-HRMS protocols were also used to quantify the THC content in recreational chocolates and fruit chews. Because these samples were *not* prepared in-house and there was no prior direct knowledge of the amounts of THC used in their preparation, the quantification results were directly compared to the %THC listed on the product labels (since % recoveries could not be calculated). The analysis yielded 0.155 and 0.143 %THC by mass in the chocolates and 0.109 and 0.118 %THC by mass in the fruit chews. These results aligned with the %THC levels reported on the labels (i.e., 0.1 and 0.11 %THC in the chocolates; 0.15 and 0.17 %THC in the fruit chews). The cannabinoid content of the edibles analyzed in this study was extract using the DisQue CEN salts, water, and acetonitrile. This combination of these salts and solvents was found efficient for subsequent DART-HRMS analysis and cannabinoid quantification, and the matrices analyzed did not appear to interfere with the accurate quantification of cannabinoids. Furthermore, the process of applying the derivatizing agent to extracts obtained from cannabinoid-infused edibles was also found to be successful.

From the perspective of the Specific Aims, the major findings of the project were: (1) the development of a triage approach to rapidly identify the possible presence of *Cannabis*-related molecules (i.e., cannabinoids, terpenes) in a variety of complex cannabinoid-infused matrices, including edibles prepared in-house, CRMs, commercial personal-care products, *C. sativa* plant material (both hemp and marijuana varieties), and recreational THC products; (2) development of proof-of-concept for two methods (i.e., statistical analysis of data obtained by DART-HRMS in negative-ion mode under CID conditions; and the introduction of a derivatization step) for the differentiation of cannabinoids; (3) development of validated DART-HRMS semi-automated protocols for rapid quantification, and application of the methods to quantify CBD and THC in chocolate and fruit chew/gummy matrices; and (4) application of extraction protocols to various matrices that are troublesome to analyze by conventional chromatography methods. Furthermore, it is important to highlight that the results obtained by DART-HRMS for either triage or quantification purposes were achieved in a fraction of the time it would have taken to analyze the samples by traditional chromatography approaches. It is concluded that by combining the results of Specific Aims II, III, and IV, validated DART-HRMS protocols for the differentiation and quantification of cannabinoids in all complex matrix types, and will circumvent many of the challenges associated with performing these analyses by conventional methods.

Activities/Accomplishments

The accomplishments associated with these findings appear in the form of 11 articles (8 journal articles published, 2 articles submitted to journals that are under review at the time of the submission of this report, and 1 manuscript in preparation); 10 conference presentations; 5 accepted/invited conference abstracts; 2 STEM outreach presentations; 1 ACS local section meeting presentation; a method for the rapid triage of complex matrices for the presence of

cannabinoids; validated methods for the quantification of cannabinoids in edibles post-extraction; and the training of 2 postdoctoral associates and 3 graduate students.

Limitations

Although the advancements of the project were slowed down because of the impact of state-mandated COVID-19 restrictions on movement, the progress on the project was significant.

ARTIFACTS

Publications, conference papers, and presentations

**Graduate student; **Postdoctoral associate*

Publications

1. Arathala, P.;** Chambers, M.I.;* Musah, R.A. *Investigating Protonated Cannabinoid Dimers: A Combined Mass Spectral and Computational Approach. In preparation.*
2. Chambers, M.I.;* Garosi, B.;* Musah, R.A. *DART-MS Facilitated Quantification of Cannabinoids in Complex Edible Matrices – Focus on Chocolate and Gelatin “Gummy” Candies. Submitted.*
3. Chambers, M.I.;* Beyramysoltan, S.;** Garosi, B.;* Musah, R.A. *Combined Ambient Ionization Mass Spectrometric and Chemometric Approach for the Differentiation of Hemp and Marijuana Varieties of Cannabis sativa. J. Cann. Res. (2023). doi: 10.1186/s42238-023-00173-0.*
4. Chambers, M.I.;* Musah, R.A. *DART-HRMS Triage Approach Part 2 – Application to the Detection of Cannabinoids and Terpenes in Recreational Cannabis Products. Forensic Chem. (2023), 33, 100469. doi: 10.1016/j.forc.2023.100469.*

5. Chambers, M.I.;* Giffen-Lemieux, J.E., Musah, R.A. *Rapid Detection and Quantification of Hallucinogenic Salvinorin A in Commercial Salvia divinorum Products by DART-HRMS*. **ACS Omega**. (2023), 8, 761-770. doi: 10.1021/acsomega.2c06106.
6. Beyramysoltan, S.;** Chambers, M.I.;* Osborne, A.M.;* Ventura, M.I.;* Musah, R.A. *Introducing “DoPP”: A Graphical User-Friendly Application for the Rapid Species Identification of Psychoactive Plant Materials and Quantification of Psychoactive Small Molecules Using DART-MS Data*. **Anal. Chem.** (2022), 94, 16570-16578. doi: 10.1021/acs.analchem.2c01614.
7. Ventura, M.I.;* Beyramysoltan, S.;** Musah, R.A. *Revealing the Presence of Tryptamine New Psychoactive Substances using Fused “Neutral Loss” Spectra Derived from DART High-resolution Mass Spectra*. **Talanta**. (2022), 246, 123417. doi: 10.1016/j.talanta.2022.123417.
8. Chambers, M.I.* Musah, R.A. *DART-HRMS as a Triage Approach for the Rapid Analysis of Cannabinoid-infused Edible Matrices, Personal-care Products and Cannabis sativa Hemp Plant Material*. **Forensic Chem.** (2022), 27, 100382. doi: 10.1016/j.forc.2021.100382.
9. Appley, M.G.;* Chambers, M.I.;* Musah, R.A. *Quantification of Hordenine in a Complex Plant Matrix by Direct Analysis in Real Time - High-Resolution Mass Spectrometry: Application to the “Plant of Concern” Sceletium tortuosum*. **Drug Test. Anal.** (2022), 14, 604-612. doi: 10.1002/dta.3193.
10. Beyramysoltan, S., Abdollahi, H. and Musah, R.A. *Workflow for the Supervised Learning of Chemical Data: Efficient Data Reduction-Multivariate Curve Resolution (EDR-MCR)*. **Anal Chem.** (2021). 93, 5020-5027. doi: 10.1021/acs.analchem.0c01427.
11. Chambers, M.I.;* Appley, M.G.;* Longo, C.M.;* Musah, R.A. *Detection and Quantification Psychoactive N,N-Dimethyltryptamine Content in Ayahuasca Brews by Ambient Ionization*

High-Resolution Mass Spectrometry. **ACS Omega**. (2020), 5, 28547-28554. doi: 10.1021/acsomega.0c03196.

Presentations

1. **Pittcon Conference and Expo 2023 Annual Conference**, Philadelphia, PA; “High-end Sweets - Application of Ambient Mass Spectrometry for the Forensic Analysis of Cannabinoid-infused Complex Matrices,” Garosi, B., Chambers, M.I., Musah, R.A. March 21, 2023. Accepted Poster Presentation.
2. **Pittcon Conference and Expo 2023 Annual Conference**, Philadelphia, PA; “High on the Successful Development of an Ambient Ionization Mass Spectrometric Approach for the Detection and Quantification of Cannabinoids in Complex Plant and Edible Matrices,” Musah, R.A., Chambers, M.I., Garosi, B. March 20, 2023. Invited Oral Presentation.
3. **The 2023 Emerald Conference**, San Diego, CA; “Livin’ the High Life - Detection, Differentiation, and Quantification of Cannabinoids in Edible and Non-Edible Complex Matrices by Ambient Ionization Mass Spectrometry,” Garosi, B., Chambers, M.I., Musah, R.A. March 2, 2023. Accepted Poster Presentation.
4. **The 2023 Emerald Conference**, San Diego, CA; “Weeding Out the Methods to Enable Rapid Differentiation of Fiber-type and Drug-type Varieties of *Cannabis sativa*: A Combined Ambient Ionization Mass Spectrometric and Chemometric Approach,” Chambers, M.I., Beyramysoltan, S., Garosi, B., Musah, R.A. March 2, 2023. Accepted Poster Presentation.
5. **American Academy of Forensic Sciences Annual Conference**, Orlando, FL; “Getting into the Weeds—A Combined Ambient Mass Spectrometric and Chemometric Approach for Differentiating Hemp and Marijuana Varieties of *Cannabis sativa*,” Musah, R.A., Chambers, M.I., Beyramysoltan, S., Garosi, B. February 14, 2023. Accepted Poster Presentation.

6. **Northeastern Association of Forensic Scientists 2021 Annual Meeting**, Niagara Falls, NY; “Trick or Weed - Application of Ambient Mass Spectrometry for the Detection and Quantification of Cannabinoids in Complex Matrices,” Garosi, B., Chambers, M.I., Musah, R.A. October 19, 2022. Oral Presentation.
7. **Northeastern Association of Forensic Scientists 2021 Annual Meeting**, Niagara Falls, NY; “Things WE’ED Like to Know – How to Differentiate Hemp and Marijuana Varieties of *Cannabis sativa* with a Combined Ambient Mass Spectrometric and Chemometric Approach,” Chambers, M.I., Beyramysoltan, S., Garosi, B., Musah, R.A. October 19, 2022. Oral Presentation.
8. **The Emerald Conference 2022**, San Diego, CA; “Applications of Direct Analysis in Real Time - High-resolution Mass Spectrometry to the Analysis of *Cannabis sativa* Plant Material and Derived Products,” Chambers, M.I., Musah, R.A. March 1, 2022. Poster Presentation.
9. **American Academy of Forensic Scientists 2022 Annual Conference**, Seattle, WA; “Best Buds – Ambient Ionization Coupled with Mass Spectrometry for the Forensic Analysis of Cannabinoid-Infused Complex Matrices,” Chambers, M.I., Musah, R.A. February 25, 2022. Oral Presentation.
10. **Eastern New York American Chemical Society Future Leaders Virtual Meeting**; “Starting the Year on a High Note – Application of Direct Analysis in Real Time – High-resolution Mass Spectrometry to the Analysis of Complex Cannabis Matrices,” Chambers, M.I., Musah, R.A., January 19, 2021. Oral Presentation.
11. **Northeastern Association of Forensic Scientists 2021 Annual Meeting**, Newport, RI; “Development of Novel Approaches for Efficient Cannabinoid Detection and Quantification

- in Edibles, Beverages, Personal-care Products and Plant Materials.” Chambers, M.I., Musah, R.A. November 3, 2021. Oral Presentation.
12. **LCGC Webcast co-sponsored by JEOL USA, Inc.;** “New Frontiers in Complex Matrix Analysis,” Musah, R.A., October 7, 2021. Virtual Oral Presentation.
 13. **Pittcon Conference and Expo 2021 Annual Conference,** New Orleans, LA, Virtual; “*Yes, We Can(nabis) Use Direct Analysis in Real Time – High-Resolution Mass Spectrometry for Detection of Cannabinoids in Plant Material and Edible Complex Matrices.*” Musah, R.A. March 11, 2021. Oral Presentation.
 14. **American Academy of Forensic Scientists 2021 Annual Conference,** Houston, TX, Virtual; “*Candies and Cannabinoids – Rapid Identification of Cannabinoids in Plant Material and Edible Complex Matrices by Ambient Mass Spectrometry.*” Chambers, M.I., Musah, R.A. February 17, 2021. Oral Presentation.
 15. **Northeastern Association of Forensic Scientists 2020 Annual Meeting,** Virtual; “*Who’s Ready for Halloweed? – Detecting Cannabinoids in Candies, Chocolates and Other Edible Products by DART-HRMS.*” Chambers, M.I., Musah, R.A. October 15, 2020. Oral Presentation.
 16. **Hofstra University Forensic Science Seminar Course,** Virtual; “*Graduate Studies and Forensic Research.*” Chambers, M.I., Musah, R.A. September 21, 2020. Oral Presentation.
 17. **New York State Police Virtual Forensic Internship;** “*Forensic Research and Graduate Studies.*” Chambers, M.I., Musah, R.A. August 7, 2020. Oral Presentation.
 18. **American Society for Mass Spectrometry,** Virtual; “*An Ambient Mass Spectral Technique for the Rapid Detection of Cannabinoids in Plant Material and Complex Edible Matrices.*” Chambers, M.I., Musah, R.A. May 31, 2020. Poster Presentation.

19. **Pittcon Conference and Expo 2020 Annual Conference**, Chicago, IL; “*Bake Sale – An Ambient Mass Spectral Technique for the Rapid Detection of Cannabinoids in Complex Edible Matrices.*” Chambers, M.I., Musah, R.A. March 5, 2020. Oral Presentation.

Websites

1. Presentations and progress on the project are featured at the PI’s website:

<https://rabi-musah.squarespace.com/>

IMPLICATIONS FOR CRIMINAL JUSTICE POLICY AND PRACTICE IN THE U.S.

This project has resulted in the development of innovative approaches for the rapid and routine analysis of complex matrices (e.g., plant material, edibles, beverages, concentrates, oils, topicals), and will, to varying extents, overcome numerous challenges encountered during analyses of THC, CBD, and other *Cannabis*-related molecules by traditional methods (i.e., chromatography-based instrumentation). These are: (1) **Rapid Triage Approach for the Detection of Cannabis-related Molecules** – The incorporation of the DART-HRMS triage step into current forensic workflows will dramatically reduce the time, major instrument, human resource, and other financial investments required to detect *Cannabis*-related molecules, and circumvent the risks associated with damaging or contaminating chromatography-based instruments; (2) **Differentiation of Cannabinoids** – The utilization of statistical analysis techniques or the introduction of a derivatization step prior to analysis by DART-HRMS enables the differentiation of cannabinoid isomers (i.e., THC and CBD) without requiring chromatographic separation of sample components; (3) **Validated Quantification Protocols** – A semi-automated DART-HRMS approach to determine the cannabinoid content in cannabinoid-infused edible matrices reduces the sample analysis time and circumvents the aforementioned risks associated with analyzing complex “sticky” samples by chromatography; and (4) **Extraction of Cannabinoids from Complex**

Edibles – The use of extraction salts designed to remove matrix ingredients from samples prior to instrumental analysis, combined with DART-HRMS quantification protocols, demonstrated the ability to efficiently analyze matrix types that are difficult to interrogate by conventional methods. Furthermore, as new matrix types emerge in the licit and illicit *Cannabis* markets, the developed DART-HRMS triage approach and validated quantification protocols can be readily adopted without the need for major adaptations to be made to the procedures. The developed methods will have both immediate and long-term impacts towards assisting crime laboratories, and subsequently the criminal justice system. In summary, the developed techniques circumvent numerous challenges encountered during the analysis of complex *Cannabis* matrices by current methods. Among the expected advantages for forensic practitioners are: (1) reduction in sample testing backlogs; (2) streamlining of sample analysis protocols; (3) reduction of chemical reagent costs; (4) re-deployment of laboratory equipment such as GC- and LC-MS instruments for other necessary types of analyses; and (5) more timely completion of sample analyses.