03

NEW EMERGING TECHNIQUES IN FORENSIC TRACE ANALYSIS AND CANNABIS ANALYSIS

INTRODUCTION

While the majority of the public is aware of and has a general respect for the role of forensics, the wide range of applications within this field is largely unknown. Due to the rapidly advancing nature of forensic technology, even scientists who work in this industry can find it hard to keep up to date on current trends and emerging forensic techniques that can help solve a case.

Fingerprinting is one of the most well-known areas of forensics, thanks to television, film, and crime literature. Despite its value and utility in solving a crime, fingerprinting is just one of many highly specific and sensitive modalities used in forensics today. Forensic evidence – particularly forensic trace evidence – is an integral component of the criminal justice system. Trace evidence, which can include any small particles left at the scene of a crime by either the victim and/or the perpetrator, can help to establish guilt or innocence of possible suspects. This evidence can also help investigators identify the origins of illicit substances or chemicals associated with a crime.

Trace evidence can be found in different environments, and the nature of the evidence can vary. Evidence such as gunshot residue, exogenous chemicals excreted onto fingerprints, lubricants, chemical compounds, blood, hair, and fabrics can all provide insight into how a crime played out, the motives of the crime, and the potential culprit. Paint evidence, another common form of trace evidence, can also be an essential trace component that is rarely discussed in popular culture.

Evidence found at the scene can take a multitude of different forms, creating complexities within the analytical framework of forensics. Due to the variety of potential evidence analyzed in this field, there now exists a range of techniques and technological platforms available to examine them and hopefully bring justice to a case.

The majority of techniques utilized in forensics incorporate physics, chemistry, biology, computer science, and engineering disciplines. Novel analytical methods used in a variety of evidence analyses include mass spectrometry (MS), matrix-assisted laser desorption/ ionization MS imaging, ultraviolet Raman spectroscopy, high-resolution-direct analysis in real time-MS, and 2D gas chromatography (GC) MS, among many others.

Over the past few years, many innovations have been made on these techniques to improve their sensitivity and selectivity for obtaining reliable data from forensic evidence, and in particular, minute quantities of trace evidence. Overall, this improvement in technology in such a short period has increased the efficiency of identifying a guilty party and excluding innocent bystanders. Further research and advancements are still needed. However, the state of forensic science has developed even further in recent years, which has undoubtedly benefited the productivity of the justice system.

Advancements in fingerprint analysis, for instance, has enabled investigators to identify endogenous and exogenous chemicals in latent fingerprints. Exogenous chemicals may help to link certain external substances that the culprit used or came into contact with, which can help narrow down the list of potential perpetrators. Additionally, microbiome analyses of bacterial colonies on the human skin have shown promise in forensics for predicting both the physical traits and lifestyle of a culprit or victim, which can help associate them with trace evidence and the crime scene.

The estimation of post-mortem index (PMI), a measure commonly used in forensics, has also been improved with recent technology. Nuclear magnetic resonance, GC-MS, and 2D GC-MS are all highly sensitive modalities that estimate the PMI, which can ultimately help to gain further insight into a murder case.

Emerging developments in vibrational spectroscopy, including deep ultraviolet resonance Raman and infrared spectroscopy, have supported improvements in the analysis of gunshot residue (GSR), another essential type of trace evidence. The use of 3D scanning technologies can assist investigators in the analysis of markings left on a bullet by a weapon. Many of these devices are portable and handheld, which can facilitate rapid on-scene analysis of evidence.

3.1 ADVANCED BIOANALYTICAL TECHNIQUES HELP SOLVE CRIMES THROUGH THE ASSESSMENT OF TRACE EVIDENCE

Forensic analysis has become increasingly sophisticated over the years, due primarily to the rapid development of technology that is currently being employed in forensic investigations. Techniques often used in forensic sciences include gas chromatography (GC), mass spectrometry (MS), and vibrational spectroscopy.

Forensic analysis, a multicomponent and detailed investigative process, offers insight into the course, perpetrators, and consequences of a crime by analyzing trace evidence left behind. Forensic science specialists gather several different types of information gleaned from electronic analytical devices, both in the lab and on the scene.

Digital, mobile devices, software, and memory forensics are all types of computer forensic tools used during the investigation of a crime. The evidence and data obtained from the scene and these tools, respectively, provide unbiased information that can aid the investigation and the justice system.

Gunshot residue is an example of trace evidence that is frequently assessed in cases where a firearm was the suspected weapon. Other elements, including lubrication, soil, estimated time of death, hair, blood, and fabric, can be analyzed with various platforms to provide insight into a case.

Emerging research and trends have helped to improve the sensitivity and specificity of these tools in the forensics space. GC, for instance, is a standard analytical technique that forensic laboratories rely on for separating molecules before their analysis using MS.

GC can often be used as an on-scene forensic application to detect environmental chemicals, explosive residues, and illicit substances. For these purposes, many forensic scientists will use portable GC devices to assist in the rapid identification of these substances. At Pittcon, PerkinElmer presented its GC-MS system, which features high throughput and high sensitivity as well as rapid results for time-sensitive analyses.

Vibrational spectroscopy is also an emerging spectroscopy field. This area of spectroscopy features techniques such as Raman spectroscopy and infrared spectroscopy, which can identify analyte classes and characterize the structures of organic and inorganic compounds discovered at a crime scene. Handheld vibrational spectroscopy devices are portable, easy to use, and can provide rapid analysis in loco.

While MS is often the method of choice in forensic science, Raman spectroscopy is another modality that offers advantages over its traditional counterpart. Raman spectroscopy is a non-destructive and non-contact technique that can identify compounds using their vibrational energy. Since Raman spectroscopy is non-destructive, compounds can then be subsequently analyzed with additional tools if required. Renishaw featured their Raman spectrometers, including the Virsa™ Raman Analyzer at Pittcon.

The Pittcon Conference and Expo is a central meeting place for forensic scientists and researchers to learn about new developments in their field, including research regarding analytical techniques and technologies to assist in investigations.

This article provides an overview of the latest forensics research that was presented at Pittcon and will discuss emerging techniques and recent advances in analytical technology that are being employed in the field. This article will also provide a deeper discussion into the analysis of trace evidence and why it is important, how to determine the post-mortem interval (PMI) and its value for helping to solve a case, the use of GC-MS in forensic science, and an overview of combined techniques for analyzing gunshot residue.

References

- *National Institute of Justice, "Overview of Trace Evidence," June 21, 2016, nij.ojp.gov: https://nij.ojp.gov/topics/articles/overview-trace-evidence.*
- *GS-MS. PerkinElmer. https://www.perkinelmer.com/uk/category/gas-chromatography-mass-spectrometry-gc-ms.*
- *Applications of Raman Spectroscopy for Trace Evidence Examinations. Microtrace. https://www.microtrace.com/applications-of-raman-spectroscopy-for-trace-evidence-examinations/.*
- *Infrared, Near Infrared and Raman Spectroscopy. Bruker. https://www.bruker.com/products/infrared-near-infrared-and-raman-spectroscopy.html.*
- *Aeris. X-Ray Diffractometer. Malvern Panalytical. https://www.malvernpanalytical.com/en/products/product-range/aeris-range.*
- *Texcan Solaris. Tescan. https://www.tescan.com/product/fib-sem-for-life-sciences-tescan-solaris/.*
- *An experimental study on investigating the postmortem interval in T dichlorvos poisoned rats by GC/MS-based metabolomics. Legal Medicine 36 (2019) 28–36.*
- *Revealing Individual Lifestyles through Mass Spectrometry Imaging of Chemical Compounds in Fingerprints. Scientific Reports volume 8, Article number: 5149 (2018).*
- *Chemical profiling of fingerprints using mass spectrometry. Volume 16, December 2019, 100183.*
- *Vibrational Spectroscopy and Chemometrics in Forensic Chemistry: Critical Review, Current Trends and Challenges. J. Braz. Chem. Soc. vol.30 no.11 São Paulo Nov. 2019 Epub Oct 24, 2019.*
- *Raman Spectroscopic Analysis of Gunshot Residue Offering Great Potential for Caliber Differentiation. Anal. Chem. 2012, 84, 10, 4334-4339.*
- *Attenuated total reflectance-FT-IR spectroscopy for gunshot residue analysis: potential for ammunition determination. Anal Chem. 2013 Aug 6;85(15):7287-94.*
- *A rapid method for detection of gunshot residue using microwave plasma torch-mass spectrometry. Procedia Engineering. Volume 7, 2010, Pages 22-27.*
- *Detection of Gunshot Residues Using Mass Spectrometry. BioMed Research International. https://doi.org/10.1155/2014/965403.*
- *Detection of Organic Components of Gunshot Residue on Carbon SEM Stubs by Raman Spectroscopy. http://www.marshall.edu/forensics/files/CRAWFORDKARYN_08082014_Research-Paper-GSR1.pdf.*

3.1a TRACE EVIDENCE IN FORENSIC SCIENCE

Trace evidence, a common form of evidence analyzed in forensics, typically consists of any evidence that can be transferred between objects, people, or the environment.

Hair, human DNA/bodily fluids, textile fibers and fabrics, soil, and gunshot residue are all examples of trace evidence that can help investigators gain insight into a crime and its possible suspects.

Several new techniques for forensic trace analysis were discussed at Pittcon, and many highly anticipated presentations demonstrated how these techniques are assisting in trace analysis. Researchers from various universities across the United States presented a National Institute of Justice (NIJ) talk titled, 'Advancements in the Analysis of Forensic Trace Evidence.' Speakers discussed how NIJ-funded researchers are currently applying advanced biological and chemical analyses to forensic challenges, particularly in relation to diverse and difficult-to-study samples.

In recent years, the analysis of trace materials for forensic investigations has come a long way, especially in terms of the techniques and platforms available. Raman spectroscopy is one technique that can characterize diverse trace evidence materials without destroying the sample, offering considerable advantages over other intrusive methods.

Renishaw's Virsa™ Raman Analyzer is an example of one of the leading Raman spectrometry devices on the market that traces evidence analysis. Bruker, another leading manufacturer of Raman spectrometers and vibrational analytical devices, was at Pittcon to discuss its suite of devices for trace evidence analysis in forensics.

X-ray diffractometers, such as the ones from Malvern Panalytical, represent a new benchtop device that features rapid data acquisition of trace evidence found at the crime scene. In recent years, 2D and 3D characterization of trace evidence have been increasingly used to assess forensic trace evidence. The Tescan Solaris ultrahigh-resolution scanning electron microscope (SEM) is a device that can be used in forensic laboratories to offer characterizations while enabling variable and filtered backscattered electron detection.

In cases of sexual assault where biological evidence is sometimes absent, forensic analysis of lubricant evidence can sometimes provide clues into a case. The use of GC-MS is an option, but this technique is primarily used for non-silicone-based lubricants as well as illicit substances, limiting the technique's application in this area. However, high-resolution-direct analysis in real time-MS (DART-MS) helps to identify a lubricant base and its minor

components, compared with GC-MS that offers separation of many lubricant peaks. The combination of the two techniques could help to optimize analyses of sexual assault cases. PerkinElmer attended Pittcon to display its several GC-MS systems, including portable platforms and systems that can analyze trace evidence often found in cases of arson.

In a talk at Pittcon, Brooke Baumgarten from the University of Central Florida described a study that examined the strength of forensic lubricant evidence from both DART-MS and GC-MS analytical data. In her research, Dr. Baumgarten and colleagues from the University of Central Florida used DART-MS and GC-MS to analyze multi-component silicone-based and water-based lubricants. A total of 50 samples with and without additives were selected to identify differences between the two analytical methods in terms of differentiation and association. Based on their results, the investigators found stronger evidence for DART-MS for each lubricant data set compared to GC-MS.

Forensic paint analysis is also a common challenge in forensics, one that is sometimes approached with SEM/energy-dispersive X-ray spectroscopy (EDS). Guidance on SEM/EDS in forensic paint analysis is sparse, particularly in the ASTM guide. Therefore, many forensic laboratories struggle to marry both the theoretical and practical understanding of paint analysis methods to compare paints based on their elemental analysis.

In a talk presented at Pittcon, Christopher Palenik discussed a review of the current ASTM approach to the comparison of paint by SEM/EDS. The talk also covered findings from a direct analysis of 300 automotive paints comprising approximately 1,200 layers. Paint layer analysis is often performed with little thought to the impact of analytical parameters. Criteria for including or excluding samples compared by elemental analysis will also often use a subjective comparison marker without attempting to discover the significance of the findings. Findings from the talk led by Palenik at Pittcon have hopefully provided forensic researchers with better guidance for paint analysis by SEM/EDS, while offering an improved understanding of automotive paint constituents.

At Pittcon, several suppliers of GC-MS and DART-MS platforms presented their complete solutions. Representatives from Thermo Fisher Scientific were in attendance to display how its suite of GC-MS solutions can assist forensic laboratories with analyses of lubricants and other evidence samples. JEOL, a developer of DART for ambient ionization, was also present.

Trace Evidence Analysis at Pittcon

Trace evidence is an essential component of any case, and several speakers and companies presented solutions to common challenges of trace analysis in forensics. The use of GC-MS and DART-MS were highlighted during this year's conference and expo. While these tools are helpful and trace analysis is an important component of forensic science, it is not the only component that can help solve or provide insight into a crime. Additional high-quality analytical techniques and devices are needed, some of which are now emerging and being widely adopted in the field.

References

- *National Institute of Justice, "Overview of Trace Evidence," June 21, 2016, nij.ojp.gov: https://nij.ojp.gov/topics/articles/overview-trace-evidence.*
- *GS-MS. PerkinElmer. https://www.perkinelmer.com/uk/category/gas-chromatography-mass-spectrometry-gc-ms.*
- *Applications of Raman Spectroscopy for Trace Evidence Examinations. Microtrace. https://www.microtrace.com/applications-of-raman-spectroscopy-for-trace-evidence-examinations/.*
- *Infrared, Near Infrared and Raman Spectroscopy. Bruker. https://www.bruker.com/products/infrared-near-infrared-and-raman-spectroscopy.html.*
- *Aeris. X-Ray Diffractometer. Malvern Panalytical. https://www.malvernpanalytical.com/en/products/product-range/aeris-range.*
- *Texcan Solaris. Tescan. https://www.tescan.com/product/fib-sem-for-life-sciences-tescan-solaris/.*

3.1b DETERMINING POST-MORTEM INTERVAL (PMI)

In forensic science, there is a diversity of high-quality techniques that can help solve a case. For death investigations, the estimation of post-mortem interval (PMI) is one such technique in helping determine the period of time that has elapsed between death and the discovery of the body. Having this time frame is crucial as it can ultimately help identify the potential causes of death. Additionally, an accurate estimation of PMI can be important for either including or excluding possible suspects. Despite the importance of PMI for forensic casework, it has been historically challenging to achieve an accurate estimation of this metric. Traditionally, post-mortem phenomena have been used to provide an approximate estimate of PMI.

Several studies have suggested that the overall change in the biochemical markers in blood can also help determine PMI. Nuclear magnetic resonance (NMR) and MS are potentially useful tools for investigating metabolic profiles in corpses, subsequently helping to establish a PMI. The utilization of GC-MS is more ideal than NMR for estimating PMI, particularly since GC-MS offers an accessible standard database and features superior abilities in separating complex mixtures and quantifying analytes.

In one study that investigated the use of GC-MS to analyze the metabolic profiling of blood from rodents at the time of death, GC-MS was able to identify 39 metabolites associated with PMI. These metabolites included different amino acids, carbohydrates, and lipids.

Additionally, the combination of these metabolites produced a support vector regression (SVR) model that offered good predictive ability for PMI.

The utility of GC-MS reaches beyond PMI, with some forensic professionals using the approach for on-scene illicit drug analysis. In a talk at Pittcon, Brooke Kammrath from the University of New Haven discussed the use of portable ion trap GC-MS in the study of illicit drug use in an effort to reduce the incidence of wrongful arrests.

Since many forensic laboratories rarely receive evidence to help identify illegal substance unless a defendant goes to trial, and considering some defendants will take a plea deal for a crime they did not even commit, having a highly accurate analysis platform in place that can offer on-scene illicit drug testing is imperative.

According to Dr. Kammrath, GC-MS may improve the reliability of illicit drug testing via confirmatory methods that can achieve very low limits of detection. Dr. Kammrath presented findings from a research study that tested the ability of the GC-MS library in detecting and identifying specific illicit drug substances and their additives in drug samples.

While GC-MS is suitable for determining PMI, it is not the only technique forensic investigators can use. Comprehensive two-dimensional GC-MS (GC×GC-MS) is a secondary approach for determining PMI.

Compared with conventional GC, GC×GC-MS is a technique that can separate complex mixtures for bioanalytical applications. The method uses a pair of GC columns, with each column containing a chemical phase orthogonal to each other. A high-frequency modulator diverts the totality of one-dimensional effluent onto a second-dimension column. Compound mixtures with similar boiling points can be separated based on their polarity, or another physical or chemical property, a feature that is not typically possible with one-dimensional chromatography. The GCxGC-MS technique also offers image patterns that demonstrate compound class structure, a "fingerprinting" capability that can assist researchers in characterizing complex samples, such as essential oils or fatty acid extracts.

In one-dimensional GC of complex samples, the result is typically a chromatogram consisting of a substantial percentage of unresolved components. While MS can help resolve some of this unknown complexity and large differences in concentrations, the presence of structural isomers can often create challenges in the spectral interpretation and data analysis of a sample. Using this technique, forensic

investigators can significantly increase the chromatographic separation space, helping to achieve a drastic increase in the potential peak capacity. The resulting chromatogram becomes structured and the resolution increases. The clustering of chemically and structurally similar compounds provides a beneficial pointer that may assist in peak identification.

At Pittcon, Katelynn Perrault from the Chaminade University of Honolulu presented a talk on the use of GCxGC-MS for analysis of post-mortem microbes. Since volatile organic compounds (VOCs) offer foundational data regarding the patterns of microbial succession, Katelynn and colleagues used the technique to characterize VOC profiles for two newly characterized postmortem microbe species, Ignatzschineria indica and I. ureiclastica. These microbes produce VOCs on human skin remains and can provide additional forensic insight. One of their highlighted findings was the superiority of GC×GC-MS over one-dimensional GC in detecting additional VOCs and improving understanding of longitudinal trends.

Pittcon featured manufacturers of instrumentation for standard GC-MS and GCxGC-MS. Shimadzu displayed their complete 2-dimensional GCxGC-MS solution. The solution comprises the firm's fast scanning, high-sensitivity quad GCMS-QP2010Ultra, and the Zoex Corporation ZX-1 or ZX-2 series of modulators that can be incorporated into forensic laboratories, including PMI investigations.

PerkinElmer was also on-site at this year's meeting with information on their suite of GC-MS solutions, including the Torion T-9 Portable GC/MS and Arson Analysis by Automated Thermal Desorption-GC/MS.

The benefit of estimating PMI is an essential factor in solving a murder case, yet the methods for accurate estimation of this metric can vary. Using GCxGC-MS may offer a potentially effective approach for estimating PMI, among mathematical models currently being employed.

At Pittcon, talks on GCxGC-MS in terms of PMI estimation provided attendees with greater knowledge of using this platform in cases where PMI needs to be factored into a case. The latest innovations in the analysis of illicit drugs were also presented. Pittcon is an ideal venue for forensic scientists to learn about this topic as well as new technologies and techniques being utilized in the field.

References

» *An experimental study on investigating the postmortem interval in T dichlorvos poisoned rats by GC/MS-based metabolomics. Legal Medicine 36 (2019) 28–36.*

3.1c NEW EMERGING TECHNIQUES IN FORENSIC SCIENCE

From DART-MS to GC-MS, there are several techniques currently being used in forensic science. As science, technology and analytical chemistry continue to advance, so does the quality of today's forensic science investigations. Currently, there are new emerging technologies that can impart a substantial impact in forensics, including handheld devices for on-scene identification, 3D facial reconstruction, and Raman spectroscopy. These tools may offer quicker approaches to criminal investigations, helping to expedite conclusions, reducing time and utilization of resources.

Portable handheld spectroscopy devices, for instance, can provide rapid identification of unknown substances found at crime scenes. Raman spectroscopy is a portable technology that, unlike infrared spectroscopy, is not impacted by atmospheric water molecules. Handheld fluorescence spectrometers, in combination with photoluminescent indicators, is a portable alternative to Raman spectroscopy and another potentially useful approach for the on-scene identification of unknown substances that are held in a reference database.

At Pittcon, Frances Scott discussed other emerging analytical methods for chemical and biological forensic evidence. In the talk, Dr. Scott and colleagues discussed novel analytical methods for analyzing biological and chemical samples recovered from crime scenes.

Fingerprint analysis is one of the hallmark techniques that has been used in forensics for over 100 years. Excretions left behind on a surface imparts a fingerprint pattern that can be compared to reference fingerprints, ultimately leading to the identification of a possible perpetrator. Not all fingermarks left at a crime scene can individualize a perpetrator, particularly if that fingermark's ridge detail is of poor quality or if the fingerprint is not in a database. However, various techniques have been developed over the years to assist in improving the analysis of fingermarks for forensic investigations.

In cases of ambiguity and uncertainty, the chemical composition of fingermarks can be targeted by MS. The use of MS modality for fingerprint analysis has a wide range of applications in forensic science, including helping investigators identify age, sex, ethnicity, drug use, and medical conditions of an individual who left the fingermark.

Exogenous chemical compounds in latent fingermarks identified by MS can also provide lifestyle information of an individual, considering products applied, food and beverages consumed, and environmental contacts can "build up" on the latent fingermark.

In one study, an MS classification model for fingerprints helped identify an individual donor's smoking habits at a 90.4% accuracy rate. This study's MS classification model for determining a fingerprint donor's gender also featured a 77.9% accuracy.

One study that analyzed the chemical composition of 1852 fingerprints used MS to compare amino acid and lipid profiles between male and female donors. In the targeted approach, the investigators found a substantial inter-variability in lipid and amino acid content as well as significant differences between males and females in terms of the levels of various amino acids. Full-scan MS data was then used to produce classification models that predicted the sex of donors with 77.9% accuracy.

Forensics may also rely on MS imaging (MSI), a rapidly developing technology within science, for uncovering intricate details of molecular distributions on surfaces or tissues. Combined with matrix-assisted laser desorption ionization (MALDI), MSI may also assist in the chemical imaging of latent fingermarks or a fingermark that is left on a surface via deposits of sweat and oils from an individual's finger.

Chemical compounds contained in the latent fingermark residue provide insight into the individual who deposited this fingermark. While liquid chromatography MS (LC-MS) and GC-MS can help forensic scientists obtain information about a fingermark, these techniques often require several fingerprints with no spatially relevant information. The use of MSI alone may be helpful for fingerprint analysis. However, challenges lie in the modality's lack of chromatographic separation and retention time data.

A multiplex MSI method that combines MALDI and MSI (MALFI-MSI) can target a wide range of exogenous and endogenous fingerprint chemicals. Endogenous compounds can differentiate subsets of people while assisting in the identification of the fingermark's age. Comparatively, exogenous compounds offer insight into the types of chemicals present in the fingermark that are not naturally produced and excreted from the human body, such as illicit drugs or explosives.

In the talk, Dr. Hinners presented the potential use implications of MALDI-MSI analysis of fingerprints for determining the time since deposition. Specifically, the talk's main focus highlighted the potential application of MALDI-MSI for estimating the age of fingermarks using ozone-degradation of unsaturated triacylglycerols in the first few days of a crime.

The estimation of a biological stain's age offers crucial information for an investigation, including when a crime may have occurred or

whether biological evidence (e.g., bloodstain) was deposited at the time of or before/after a crime. There is currently no optimal method for determining the amount of time that has elapsed since bloodstain deposition, which often creates challenges when presenting evidence in court.

Vibrational spectroscopy techniques, another emerging field discussed at Pittcon, features a highly specific vibrational signature that can provide confirmatory class identification of analytes. These techniques, including infrared and Raman spectroscopy, offer numerous advantages in forensics, as they are capable of characterizing the structure of several organic and inorganic compounds found at crime scenes. Vibrational spectroscopy techniques are simple to use, particularly handheld and portable instruments that allow for analysis in loco.

Terahertz spectroscopy is another vibrational technique occasionally used in forensics with the potential to emerge in future research.

Raman spectroscopy is a vibrational spectroscopy technique providing several different advantages in forensic chemistry. Firstly, Raman spectroscopy features an ability to analyze a range of samples independent of the chemical state of the sample and without the need for preparation. Raman spectroscopy is considered non-destructive due to the reliance on low-intensity laser powers. It is also relatively rapid in terms of its ability to obtain spectral information with good signal-to-noise ratio. A limitation of the technique, however, includes the potential for the laser beam to be too intense for a small area of focus, leading to heating and possible burning of the analyzed sample. This can be circumvented with the regulation of the instrument's laser power. Surface-enhanced Raman spectroscopy can help counter fluorescence, another related limitation of Raman spectroscopy.

Infrared spectroscopy, another vibrational technique based on molecular energy absorption, can offer information about overtones, combination bands, and fundamental transitions of a sample. The high level of versatility of infrared spectroscopy for analyzing liquids, solids, and gases has made the technique widely accepted in forensic science. Near-infrared spectroscopy is a type of infrared spectroscopy that is low in cost, simple, robust, and easy to miniaturize. Compared with Raman spectroscopy, instruments that employ infrared spectroscopy are often easier to use and offer a higher speed of spectral acquisition, often over significantly large sample areas. Infrared spectroscopy is also not limited by interference from fluorescence.

In forensics, determining gunshot residue is a principal component for estimating firing distance and connecting a suspect and/or weapon to a crime. The use of Raman spectroscopy can play a useful role in detecting varying metal anions in gunshot residue particles, including lead and iron oxide, barium, as well as sulfate and carbonate mixtures.

While very few studies have been published on the use of Raman spectroscopy in this area of forensics, some studies have shown that Raman spectroscopy and advanced statistics can be effectively used for caliber differentiation as well as differentiation of gunshot residue particles.

Infrared spectroscopy can also help to detect organic gunshot residents from the propellant, due to the highly selective and non-destructive nature of the analysis technique. In one study, the use of attenuated total reflectance Fourier transforms infrared spectroscopy (FT-IR) correctly classified gunshot residue particles to their corresponding calibers.

Pittcon included a talk by Igor Lednev from the University of Albany, which discussed the use of vibrational spectroscopy and advanced statistics for both the detection and characterization of gunshot residue. Dr. Lednev's talk covered a study that demonstrated the ability of vibrational spectroscopy for detecting organic and inorganic constituents of gunshot residue on adhesive tape. Dr. Lednev discussed emerging developments in using vibrational spectroscopy for the detection and identification of organic gunshot residue analytes.

Vibrational spectroscopy, including Raman and infrared spectroscopy, MALDI-MSI, and MS represent essential techniques for the analysis of latent fingermarks, among other applications in forensics science.

References

- *Revealing Individual Lifestyles through Mass Spectrometry Imaging of Chemical Compounds in Fingerprints. Scientific Reports volume 8, Article number: 5149 (2018).*
- *Chemical profiling of fingerprints using mass spectrometry. Volume 16, December 2019, 100183.*
- *Vibrational Spectroscopy and Chemometrics in Forensic Chemistry: Critical Review, Current Trends and Challenges. J. Braz. Chem. Soc. vol.30 no.11 São Paulo Nov. 2019 Epub Oct 24, 2019.*
- *Raman Spectroscopic Analysis of Gunshot Residue Offering Great Potential for Caliber Differentiation. Anal. Chem. 2012, 84, 10, 4334-4339.*
- *Attenuated total reflectance-FT-IR spectroscopy for gunshot residue analysis: potential for ammunition determination. Anal Chem. 2013 Aug 6;85(15):7287-94.*

3.1d GUNSHOT RESIDUE (GSR) WITH MS AND RAMAN SPECTROSCOPY

In forensic investigations, no material left at a crime scene is ever considered too small or insignificant to be examined. The analysis and characterization of trace evidence – including human hair, textile fibers, soil, glass, and fabric – is a major component in crime scene investigations.

In cases where a suspected firearm was used, gunshot residue can be an insightful marker that can link a weapon with a suspect or specific bullet. Gunshot residues are produced when a weapon is fired. These residues consist of small particles measuring less than 500 µm.

Organic and inorganic gunshot residues are assessed during forensic investigations, with inorganic residues being primarily assessed using microscopy, and X-ray spectroscopy being used to identify particle size and residue composition. However, in these samples, there is typically a limited number of elemental analytes, and the abundance of these analytes in the environment can sometimes result in false-positive identifications. Comparatively, organic gunshot residue particles feature a more chemically unique structure due to the propellant's use of various stabilizers, explosives, and other materials.

One of the most powerful tools for the analysis of forensic materials is Raman spectroscopy. There are several advantages to using Raman spectroscopy in forensics, including the instrument's non-destructive and non-contact nature, the ability to pair the instrument with a microscope to assess small pico-mole quantity samples, the platform's chemical specificity, and the ability to add a fiber optic probe for remote sampling.

Regarding gunshot residue, Raman spectroscopy can identify organic components in the residence and then provide a characteristic spectrum of each compound. Since Raman spectroscopy is considered non-destructive, it allows compounds to be tested further by other analytical techniques.

The MS modality also provides excellent detection abilities, particularly for heavy metals. This technique, when combined with a chromatographic system, can be highly sensitive and specific in identifying gunshot residue components. In one study, a microwave plasma torch-mass spectrometer method simultaneously detected lead, barium, and antimony, which are three heavy metals commonly found in gunshot residue. The microwave plasma torch, in addition to MS, was used as a source of ionization. The combined approach featured high sensitivity and specificity for element detection.

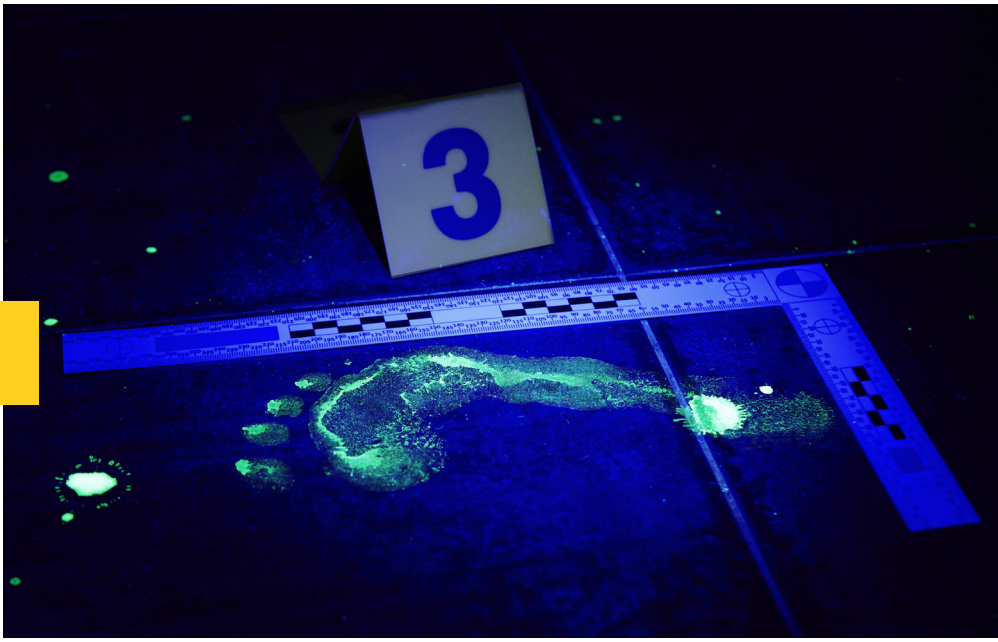
At Pittcon, Kaiser Optical, an unparalleled leader in Raman spectroscopy, demonstrated its analytical, process, and research Raman instruments currently used in forensics. Kaiser's RamanRxn2™ Analyzer is deployed for in situ reaction analysis at crime scenes.

Advion was also at Pittcon to discuss its suite of MS products that analyze large and small molecules. The SOLATION® Inductively Coupled Plasma Mass Spectrometer, one of Advion's MS platforms, might be a useful tool for forensics due to its ability to analyze trace elements from urine, serum, plasma, soil, whole blood, and tissue.

The role of Raman spectroscopy and MS in the analysis of gunshot residue is well known. However, many professionals in forensics fields may not know about the published evidence related to these solutions.

References

- *A rapid method for detection of gunshot residue using microwave plasma torch-mass spectrometry. Procedia Engineering. Volume 7, 2010, Pages 22-27.*
- *Detection of Gunshot Residues Using Mass Spectrometry. BioMed Research International. https://doi.org/10.1155/2014/965403.*
- *Detection of Organic Components of Gunshot Residue on Carbon SEM Stubs by Raman Spectroscopy. http://www.marshall.edu/forensics/files/CRAWFORDKARYN_08082014_Research-Paper-GSR1.pdf.*



CONCLUSION

Forensic science represents a rapidly growing field that is experiencing innovative transformations in the way it performs crime scene analytics. Advancements in technology have offered forensic professionals the ability to analyze trace evidence in a new way, using emerging platforms that protect the sample and facilitate greater visualization and separation of analytes.

Advancements in MS, Raman spectroscopy, infrared spectroscopy, GC, and portable analytical equipment have helped to reduce laboratory workloads and assist in efficient crime scene investigations.

At Pittcon, a variety of relevant presentations, short courses, exhibits, and symposia focused on essential and newly emerging techniques for forensic professionals. Speakers covered topics such as analyzing trace evidence, residue analysis, latent fingerprints, gunshot residue, illicit substances, explosives, and many more. The role of PMI, and the applications used to estimate this metric in murder cases, is another topic that was discussed extensively throughout the meeting.

Pittcon also featured exhibitors from all over the globe. Exhibitors presented their suite of standard and new laboratory and portable technologies for forensic investigations. These include the GC-MS devices from Thermo Fisher Scientific and Shimadzu, MALDI-MSI devices from Bruker, Renishaw's Raman spectrometers, DART-MS from JEOL, among numerous other analytical platforms from leading manufacturers.

3.2 ANALYSIS OF POSTMORTEM MICROBES USING GAS CHROMATOGRAPHY

In the search and recovery of human remains during mass disasters, homicides, and missing persons investigations, the odors released from decomposing bodies is crucial in the search process. One contributor to the odor released from remains are microorganisms, which contribute to a portion of the chemicals released into the air. New technology is being used to profile the odor from postmortem microbes to improve techniques that rely on the detection of decomposition odor.

In this interview, Katelynn Perrault, from Chaminade University of Honolulu talks to News-Medical and Life Sciences about her research into using Multidimensional Gas Chromatography to achieve odor profiling of postmortem microbes.

How do novel separation methods help us to solve critical challenges in bioanalytical applications?

We use separation science across numerous life science fields to perform chemical analysis (e.g., biomedical diagnostics, environmental monitoring, food regulation, forensic science). The main advantage of using a separation technique is that we can take complex samples and figure out which chemicals are present and how much of each chemical is present. Those two things allow us to provide a chemical profile of the sample. This may not sound too difficult, but when dealing with incredibly complex

samples, these two jobs become significantly more challenging.

Consider a small room with only a few people inside. It is probably relatively easy to know the number of people present and the identity of each person. Now think about a large crowd of people at a concert. That job becomes much more difficult. In analytical chemistry, we have to resolve each compound from one another if we want to know the number of compounds present and their respective quantities.

Novel multidimensional separations such as comprehensive two-dimensional gas chromatography (GC×GC) or comprehensive two-dimensional liquid chromatography (LC×LC) allow us to be more successful when analyzing samples with high complexity because we can develop a more accurate chemical profile.

Accurately determining postmortem interval (PMI) is important in forensic investigations. Can you explain why?

In many cases, the estimation of postmortem interval (PMI) is required to understand the series of events in a forensic case. Knowing accurate information about when someone died helps to confirm or refute suspect alibis and establish the order of events leading to an individual's death. The family of a victim also has a right to know how, when, and where their loved one died to assist in the grieving process.

While we have some methods for estimating postmortem intervals when someone has recently died, this becomes more challenging with longer postmortem intervals. Many research studies have been conducted to improve our understanding of how a body decomposes to enhance our understanding of which chemicals could potentially be used to estimate the postmortem interval accurately.

One potential category of chemicals that have been proposed in the past is the use of volatile organic compounds (VOCs), which contribute to the odor evolved from decomposing remains. By understanding a microorganism's contribution to the VOC profile, we can potentially improve our ability to predict trends in VOCs and use those as indicators to estimate postmortem interval.

In forensic science, how can postmortem microbial succession be used to determine PMI?

There is a predictable pattern of microbes on a body at different time intervals following death. This pattern is referred to as the microbial succession. A large body of work in forensic microbiology demonstrates the potential to use these patterns to estimate PMI.

Therefore, to understand the VOCs evolved by a cadaver, linking it with the VOCs originating from microbes on a body would be very beneficial. The challenge lies in the fact that numerous microbial species are competing and interacting on a decomposing body.

We are investigating isolated bacterial species that we know are associated with decomposing remains to improve our understanding of the individual contribution of VOCs from each species. Over time, we will build a database of VOCs that are evolved from a postmortem microbial species, and we can eventually use that data to interpret the evolution of decomposition odor.

What does the study of decomposition odor contribute to death investigations?

The purpose of understanding volatile emissions from microbes is to improve understanding about decomposition odor. Decomposition odor is essential for several applications in a death investigation.

In cases of homicides, missing persons, or mass disasters, scent detection canines are often brought to search for and locate human remains. They do so based on their ability to orient towards the source of a decomposition odor. If we can improve our understanding of this odor, we can provide a scientific foundation for the success of the canines, as well as provide other recommendations about effective training aids that will mimic the target odor.

There is also a lot of interest in developing portable sensors that could be used in a complementary manner to the canines or in scenarios where canines are not available. It is essential to understand the profile of decomposition odor through separation science to figure out which targets a sensor device would need to target. Each study we perform helps us to build an improved understanding of decomposition odor so we can provide information for these applications.

Why did you choose to use a comprehensive two-dimensional gas chromatography-mass spectrometry (GC×GC-MS) technique in particular? What were the benefits of this research?

Volatile organic compound profiles collected from decomposing remains or microbial metabolism tend to be very complicated. We have to be able to separate hundreds of compounds from one another, determine each of their identities, and in some cases, quantify the compounds. The compounds we target exist across almost all known compound classes. In addition, they are present in a broad concentration range, with some present in trace levels and others present in very high abundance.

All of these challenges mean that the use of a multidimensional technique such as GC×GC-MS gives us the best chance of being able to provide an accurate and reliable chemical profile. One-dimensional analyses, such as gas chromatography-mass spectrometry (GC-MS), often lack the resolution and range required for the samples we analyze.

When applying this method, did you uncover additional information about the profile of postmortem microbes than you would have using conventional methods?

The multidimensional chromatographic technique we use, GC×GC-MS, allows us to separate more peaks and be more successful in providing an accurate profile. However, this also means that we end up with a significantly larger number of compounds. This is, in part, because compounds contributed by background and/or chemical interference are separated from the compounds of interest. In this study, we were able to increase the number of compounds we were targeting from approximately 30 (using conventional methods) to around 70 (using multidimensional methods). This is a lot more information than we had about our samples previously, and we are using that to inform the next stages of the work.

What benefit did Fisher Ratio (FR) filtering provide in this study?

When we work with multidimensional separations, we are fortunate to get more information about our samples. Unfortunately, this also means we have more information to sift through. It is challenging to determine which compounds are important for differentiating your sample from other types of samples. We have to figure out which compounds are the most important.

The Fisher Ratio filtering step allows us to probe our data and determine which compounds are significant rather than being contributed by background or chemical interferences. We get the luxury of limiting our data set to the compounds that are the most important, rather than having to "take what we can see" with conventional methods. This often means we end up with a shorter list of compounds in our profile, but it will not be the same list of compounds we would have had if we used a conventional technique with lower resolution.

How are you going to define FR filters further to provide guidelines for future research?t

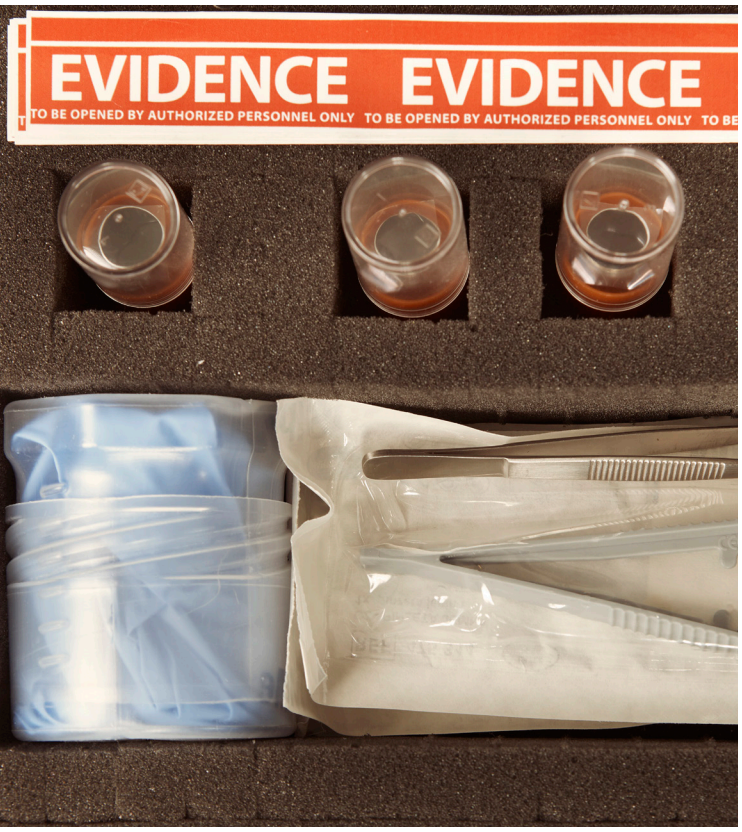
We are working with chemical standards and creating simulated challenges that might impede the efficacy of the Fisher Ratio filtering step. This essentially means we are creating mock samples with the problems we encounter in real samples so we can see if we are falsely reporting compounds as being significant when, in fact, they are not.

When we use simulated mixtures, we can control the concentration of compounds, so we know if the compound should be labeled as significant. This will inform how the Fisher Ratio filter should be used with multidimensional VOC data in scenarios where those challenges are present, meaning that it will eventually help us to be more accurate in the chemical profiles we report.

Why would being able to improve data reduction strategies improve the adoption of comprehensive two-dimensional gas chromatography (GC×GC) in biological sciences?

The biggest benefit of working with multidimensional chromatography is that you can get more data about your sample. It is also one of the most significant challenges. When we increase the amount of data we have about a sample, the challenge is how we handle that data.

Data reduction is not performed to make the dataset smaller. The purpose is to limit the dataset to the variables that are important and remove the data that is not. The ability to focus on the most significant components of a sample means that we can use the most important information in answering questions of biological importance.



Where could further research into this field take us?

We are currently working on individual microbial cultures on standard nutrient agar. This is far from the scenario in which postmortem microbes exist in communities on decomposing remains. As the work evolves, we hope to gradually introduce higher levels of complexity, such as combinations of species and different nutrient sources.

This will build our knowledge gradually and help us to relate data back to data we have on decomposing remains. We have to start simple and build layers of knowledge. Microbial contributions are the biggest missing link to what we currently know about decomposition odor, and it will require many controlled studies before we can fully understand these complex interactions.

What do you hope to gain from Pittcon with your research and presentation?

I hope to communicate the importance of incorporating fundamental analytical research with rigorous application-based research. The work we do with GC×GC is exciting, but ultimately, I want to use my knowledge to help people to adopt the technique so they can accomplish their own analysis goals.

I would love to contribute to this technique being adopted in new areas and to provide data on how it can be beneficial for different end-users. I also hope to learn more. At conferences, I am always eager to interact with people who approach similar problems to my work but use different techniques, statistical approaches, or interpretation strategies. I am always looking for new ideas that we can apply to our work.

3.2a USING TWO-DIMENSIONAL GAS CHROMATOGRAPHY TO STUDY DECOMPOSITION ODOR

The below article is a summary of Katelynn Perrault's talk at Pittcon titled 'Comprehensive Two-Dimensional Gas Chromatography for the Analysis of Postmortem Microbes'.

Developing new technologies and introducing these into routine use in forensic laboratories takes time. Most new technologies begin with a proof of concept or a refined hypothesis before work is published, which leads to a dialog in the wider scientific community. Studies will also take place where researchers aim to replicate, optimize, validate, and standardize their approaches before disseminating their work via further publications.

In the forensic sciences, new technology must be robust and reliable enough to be used in a courtroom scenario, as well as being widely accepted by the relevant scientific community. Publications help us achieve this. When developing new tools for forensic science, there are steps to consider which are not always applicable to other fields.

We need to consider error rates and how confident we can be that our results are accurate enough to stand up in court. We must ensure we have appropriate analytical controls in place, so we can defend what we have done from an analytical standpoint. We must also think about practical considerations in forensic laboratories because there are often restrictions in terms of budgets for new equipment or how many people can be taken off a specific analysis job to learn a new system.

Decomposition Odor Profiling

Decomposition odor is essentially a collection of volatile organic compounds (VOCs). These compounds prefer to exist in a gaseous state, produced by the human body throughout the decomposition process. The compounds produced towards the beginning of decomposition are typically different from those in the middle and towards the end of decomposition. It is a process.

Decomposition odor is a piece of evidence that we can exploit in different scenarios, for example, homicides or missing person investigations. However, this approach also has potential uses in humanitarian scenarios such as natural or humanmade disasters, where we can improve the way that cadaver detection dogs are trained.

Analyzing this odor presents a lot of analytical challenges. These compounds create a very complex mixture, existing in a variety of different compound classes and across a broad dynamic range – meaning that some compounds are highly saturating. In contrast, some are present at very trace levels. We need to conduct longitudinal analysis because decomposition odor changes over time, and when we introduce time as a variable, this adds an additional level of complexity. Variables such as weather and a body's internal composition can complicate things further. Finally, we are performing a non-targeted analysis, where we want to understand every chemical that is present in the profile.

Two-Dimensional Gas Chromatography

Comprehensive two-dimensional gas chromatography can help us address a lot of these challenges. This is a multidimensional chromatographic technique, meaning a sample undergoes at least two separations.

With gas chromatography (GC), a hot inlet vaporizes a sample before introducing it onto the column. Analytes travel through this column based on their affinity for the stationary phase, separating out and arriving at the end of the column at different times. The column is inside of an oven, keeping everything increasing in temperature, moving forward throughout the analysis. Unfortunately, analytes do not always arrive at the detector individually, making analysis difficult.

Two-dimensional gas chromatography (GCGC) employs a secondary column and a modulator at the junction between the columns. The modulator collects for a few seconds, then releases this over and over across the whole run, effectively taking the output from the primary column and splicing this into pieces.

At the secondary column, this short plug undergoes a mass separation with a different stationary phase possessing a different selectivity, resulting in more separation. This way, compounds arrive at the detector individually. The detector's speed is important here, as this must be capable of rapid acquisition.

"Overall, this approach offers significant benefits. We obtain more information about the sample, as well as increased visibility when there are small differences between samples."

Katelynn Perrault

Microbial Communities and Decomposition Odor

GCGC has been incredibly beneficial to the study of decomposition odor, mainly due to the sheer number of different variables that impact this process. GCGC has been employed to study the impact of many of these variables, including subject age and sex, sampling methods, and environmental conditions such as weather.

However, one largely untouched area is the microbial community on the body. Microbes have a significant impact on decomposition, and while we know these grow in predictable patterns on a body, we do not know what they are contributing to decomposition odor.

We have focused on analyzing three bacteria so far: bacillus subtilis (found in many environments and on remains in the later stages of decomposition), ignatzschineria indica, and ignatzschineria ureiclastica (associated with earlier stages of decomposing tissue, but not often found in other environments). Our goals are to understand the roles these bacteria play in decomposition odor and to establish VOC trends in ignatzschineria.

We approach this using a technique known as the Fisher Ratio, where we group samples and then investigate similarities between groups. This lets us calculate the Fisher Ratio for each compound, using a threshold called BF critical to decide which value is significant. However, we need to know if this process is reliable, even in problematic scenarios. To address this, we have been developing mixtures of compounds that we know the concentrations of and comparing these to one another. We calculate the Fisher ratio, apply a filtering step, then verify that the approach works. Our overall goal is to establish the error rates mentioned earlier.

GCGC affords us the accuracy needed to effectively limit which compounds we focus on while we work on verifying our approach. We are continuing to take this work forward into developing a comprehensive microbial VOC library, which will help us better understand the full decomposition profile and the effects of microbial communities on decomposition odor.

3.3 USING ANALYTICAL CHEMISTRY TO UNDERSTAND CANNABIS ANALYSIS

A growing number of states and jurisdictions have legalized cannabis in some form over recent years and each year seems to bring new milestones in this phenomenon. In the USA, Illinois became the first state to legalize cannabis sales without a ballot[1] and, on 1 January 2020, customers lined the blocks outside dispensaries to be among the first to purchase the drug legally.

Meanwhile, the Farming Act, which was passed toward the end of 2018, legalized hemp and CBD, resulting in an explosion of CBD and hemp-containing products coming to the market in 2019.

To date, fourteen US states and territories have legalized recreational cannabis sales for adults[2], and many states have now made medicinal cannabis available.

This burgeoning new industry brings with it many challenges, especially with its speed of development. How can we ensure that cannabis products reaching consumers are safe? Most cannabis products receive no oversight from the US Food and Drug Administration, regulation is a patchwork, varying from state-to-state, and consensus on testing standards has not yet been achieved.

The Importance of Testing

There are several components of cannabis that require analysis to ensure product efficacy and consumer safety. Potency is particularly important because it is linked to the therapeutic effects of the drug. Of special interest is the ratio of tetrahydrocannabinol (THC) – the main psychoactive component of cannabis - to cannabidiol (CBD), which is not psychoactive. The ideal ratio in medicinal cannabis depends on the condition it is intended for, with THC being beneficial for some while better avoided in others. In recreational cannabis, THC is associated with a greater "high", but high-THC varieties are also linked to an increased risk of psychosis.

Another essential dimension to testing is to measure and exclude contaminants. These include pesticides, residual solvents from extraction processes, mycotoxins, and heavy metals[3]. Such contaminants can be harmful to health if consumed and could be particularly dangerous to immunocompromised patients in a medical setting.

Could Cannabis Ease the Opioid Crisis?t

The opioid crisis is one of the United States' most pressing public health issues. In 2017, it was declared a national emergency by the President and has now overtaken road traffic accidents as a cause of death[4].

Medical opioids for acute or chronic pain are the initial source of opioid exposure for many individuals who then develop an opioid use disorder. Pain is also the primary indication for medical cannabis use. Could medical cannabis help to decrease reliance on opioids for pain relief and help fight the opioid crisis? There is anecdotal evidence to suggest that this is true.

At Pittcon, the Plenary Lecture was presented by Ziva Cooper, a researcher trying to find out more definitive answers through randomized controlled trials. Dr. Cooper is an associate professor at the University of California, Los Angeles, where she is also the Research Director for the University's Cannabis Research Initiative. Her talk looked at the potential role cannabis constituents could play in dealing with the opioid epidemic.

A Rapidly Changing Industry

The cannabis industry is expected to grow to $22 billion by 2022[3], which represents an extremely fast turnaround from it being an illegal substance to a major commodity. However, this carries the challenge of ensuring the safety of products when they are coming to the market faster than they can be assessed. To complicate matters, this is coupled to a lack of standardized testing and regulations.

At Pittcon, speakers Christopher Hudalla and James Roush of ProVerde Laboratories, and Marian Twohig of Waters Corporation, discussed these issues and asked the question: how is it possible to keep up with all these developments?

They discussed the growth of the cannabis market, its impact on consumers, risks from "derivative" products such as vapes, and strategies for analytical labs to cope with increasingly demanding methodologies in this area.

References

- *Berke J & Gould S (2020) Legal marijuana just went on sale in Illinois. Here are all the states where cannabis is legal. Available at: https://www.businessinsider.com/legal-marijuana-states-2018-1?r=US&IR=T#illinois-4 Accessed: January 2020.*
- *National Conference of State Legislatures (2019) State Medical Marijuana Laws. Available at: https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx Accessed: January 2020.*
- *Nie, B., Henion, J. & Ryona, I. The Role of Mass Spectrometry in the Cannabis Industry. J Am Soc Mass Spectrom. 2019; 30: 719. https://doi.org/10.1007/s13361-019-02164-z.*
- *Mazzei, P (2019) Opioids, Car Crashes and Falling: The Odds of Dying in the U.S. Available at: https://www.nytimes.com/2019/01/14/us/opioids-car-crash-guns.html Accessed: January 2020.*

3.4 THE IMPORTANCE OF QUALITY CONTROL ON CANNABIS-RELATED PRODUCTS

In this interview, Joshua Crossney talks to News-Medical Life Sciences about the recent advancements in the Cannabis industry, and why quality analysis and quality control are more important now than ever for cannabis medicinal products.

Please could you give us an update on your efforts as an advocate for improving the quality of medicinal cannabis products?

The cannabis industry is such a fast-moving industry from one year to the next. So much can change and happen. Last year, we launched the East Coast edition of our Cannabis Science Conference, and now we are hosting a Spring edition on the East Coast of the U.S. and our Flagship Show in Portland, Oregon, in the late summer.

It has been great to see the need for more education, and obviously, we have seen a medical cannabis revolution, in a sense, on the East Coast markets. When we launched the show in 2016, it was mostly a West Coast conversation.

It was also great to launch the show in Baltimore, which is my hometown. We are

getting ready to head back to Baltimore again for our upcoming show. It has just been great to work together with the advocates and leaders of this industry and continue to bridge the gaps between traditional science and medicine and the cannabis industry.

The relationship between cannabis and the analytical chemistry industry is ever-changing and evolving. Why is it important that these two markets work hand in hand?

Bridging the gaps between the analytical science community and the cannabis industry was one of the main driving factors that started the Cannabis Science Conference. When we spoke last year at Pittcon, I mentioned how at the 2014 or 2015 Pittcon, talking about cannabis was very taboo. A lot of people did not want to talk about it, but now we have seen such a revolution.

It was our primary goal to bring these communities together, and I think we have done a great job in doing so. We are seeing a lot more involvement from the analytical instrument manufacturers, the sample prep providers, and even academic cannabis. We are also seeing programs all over the U.S. that are starting to implement cannabis in their education for university.

Illinois recently legalized recreational marijuana, and more recently, Kentucky passed the legalization of medicinal marijuana. How are you helping combat the stigma against marijuana?

Stigma is at the core of this entire industry and community. Cannabis has been used and accepted as a medicine in society and the world at large for far longer than it has been prohibited.

This goes back to ancient Chinese medicine, ancient Indian medicine, and ancient Egyptian medicine. This is something that was brought about from propaganda and reefer madness if you will, and the monopolization of America and industries such as pharmaceuticals and paper. I always say that cannabis has been stigmatized because of this misinformation and propaganda over the past 50 or more years, and it is great to see that we are starting to dig our way out of that long era of misinformation.

Combating the stigma is difficult. But I think that what we do is combat stigma with evidence in research and science. What we do in bringing together the world's leading researchers and scientists and medical professionals to share their knowledge and data is the best way to combat stigma and normalize cannabis further.

In the cannabis industry, the standards and benchmarks for drug delivery, analytics and testing, consumer reporting, and packaging are currently of a basic nature, and there is substantial room for more sophistication and technology. What role can analytical chemistry play in this development of sophisticated technology?

Analytical chemistry is already playing a huge role in the cannabis community. In regards to cannabis testing labs for quality control, this is one of the fastest-growing and most profitable segments of the cannabis industry. Sometimes that gets overlooked when you think of cultivation and owning dispensaries, but testing is such an essential part of it.

We continue to see these leaders in science play a role in the advancement of this, and one of the most significant issues is that cannabis testing is not standardized. Therefore, we are trying to work towards getting cannabis testing standardized as much and as fast as possible, because this is something that people are using as a medicine. It is essential that people can stand by this medicine.

I am very passionate about quality control in general, and we are here at Pittcon, which is an analytical science show that caters to not only food but pharmaceuticals. It now also caters to the cannabis industry. You can see that we are seeing a marriage of analytical professionals getting together with the community and trying to advance technologies and standards.

Can industries such as the pharmaceutical industry help the cannabis industry leverage technology for further development? If so, how? Are there other industries or applications that the cannabis industry can leverage to help with this?

I think that there is a lot of room for the pharmaceutical industry and the cannabis community to collaborate and work together. Again, that ties into bridging the gaps. I think they are two communities that can learn a lot from each other.

I know that a lot of people that are just cannabis-industry-specific people do not have their foot on both sides of the conversation as I do. I always say I spend half of my time at science shows and the other half at cannabis shows, but to the general cannabis community, some are scared of pharmaceutical involvement because they fear that pharmaceuticals will come and take over the entire industry.

I always advocate and push for it being almost a marriage of knowledge sharing and information because the leaders of this industry have developed the cultivation techniques and the processing, and turned this into a medicinal, high-grade medicine. It is so different than it was in the '60s or '70s, and sometimes I jokingly say, "It's not your parent's weed anymore." What I mean by that is that it is not the level of quality of cannabis that has been developed over the past few decades. It is just incredible.

I feel that there is a lot of room for involvement from pharmaceutical industries, but I strongly feel that both communities need to respect each other, their position, and their knowledge, and learn from each other and then grow this industry together. I always tell people in

the industry that have reservations about pharmaceuticals that you cannot expect to have legalization and normalization without regulation on a federal level.

If this is legalized federally, I think we will see a lot more of that. But I think preparing for that and getting these communities together to network and learn from each other before that happens is probably the best idea.

Some states in the U.S. do not perform quality analysis or quality control on cannabis-related products. Why is this troubling? Why is the testing of cannabis essential?

One of the things that piqued my interest in getting involved with this industry was the fact that I realized that California, at the time, did not require cannabis testing at all. There were no requirements at all to test cannabis for quality control. Coming from a background of analytical science recruiting and working in this field, I found this troubling because I did not understand how a product that people are using in medicine is not being tested for quality control. Anything that is regulated and comes into human contact should be quality controlled.

It was troubling to me, and I connected early on with people that were using cannabis to treat their children that had cancer. I was associated with a lady in California who had an eight-month old daughter who had an optic pathway glioma brain tumor and was going through chemotherapy and other dangerous treatments that can help but are also detrimental to the body. They were using cannabis as an option to treat their baby in a market where it was not required to have cannabis tested for quality control.

> "I think it is extremely troubling to see that some of these markets are still not requiring cannabis testing because when you look at cancer patients and other patients that are immunocompromised from conditions or treatments that they are going through, it is very important that the cannabis that they are using is contaminant-free and does not contain molds, heavy metals, pesticides, and solvents."
>
> *Joshua Crossney*

The issue with pediatrics and geriatrics and cannabis use is that children are not smoking cannabis. They are using concentrated tincture oils. The danger in that is when you take contaminated cannabis and you put it through an extraction process, you could potentially not only be extracting cannabinoids to a more potent, higher level, but if there is contamination present, you could potentially be extracting the contamination to a higher level as well.

This could be especially troubling in immunocompromised individuals. Therefore, I feel that cannabis testing is hugely important and every market that has this should require it. We are still trying to figure out the standardization of cannabis testing, and that is an issue because we are seeing that not everyone is using the same rule book. Different labs are using different instrumentation and different techniques to do their testing, and that can obviously cause variables in results.

If you take a cannabis sample and send it to five different laboratories, you are likely to get five different results just because not everyone is using the same techniques or instrumentation. Requiring cannabis testing in all these markets, and furthermore, standardizing this process, is a big passion of mine.

What further advancements in the cannabis industry do you foresee happening over the next coming months and years?

I feel we are going to see a lot more research coming out of the U.S concerning cannabis. A friend of mine, Dr. Sue Sisley, has a Schedule 1 research license to study the effects of the potential therapeutic effects of cannabis for PTSD in veterans. One of the issues that she faced in her research was that there is currently only one source of cannabis for medical research purposes, and that is coming from the University of Mississippi, which is being cultivated with standards from the National Institute of Drug Abuse.

The standards that they are cultivating with are quite archaic and they are not going back to what I said before about creating high-grade products; it is more in line with what I said about your parent's weed than what is actually on the market now.

However, one of the great promising things with that is, after her first trial, Dr. Sisley opened a federal lawsuit against the DEA and won her case, based on the federal monopoly of cannabis for research. It is not going to be tomorrow when she gets access to better cannabis to use for her research, but it is a step in the right direction.

I honestly feel that we are going to see the legalization of this plant within the next year or so, if not sooner. I think that this is a plant that, as I said before, has been stigmatized for so long, but really could help our country and our world in so many ways. If you look at the numbers of how much money and taxes that have come in from cannabis markets all over the country and world, it is astonishing. We currently have financial crises all over the world, and I think that this could help the job market.

I believe there are a lot of ways that this could positively impact human culture, and I feel that we are closer than ever to that. We must speak as one collective voice as much as possible. I know that can be difficult, especially in an emerging industry such as cannabis, but the more that we can get together and be on the same page, the more chance we have of helping define the rules and the way that this industry will go when it is a federal industry.

How are you helping to educate and improve technical cannabis science at this crucial time?

"We have been at the core of an analytical science show that caters to the science behind cannabis, cannabis testing, and everything with that. We have played a role in bringing people together, helping to advance this industry, and connecting the genius cultivators with the genius analytical professionals by networking and building collaborations between them."

Joshua Crossney

We had one individual that attended our show who met Dr. Dedi Meiri from the Technion Institute in Israel, who is one of the world-renowned researchers on cannabis and cancer. He went from meeting Dr. Dedi at our show to going to Technion in Israel and studying under him for three years.

We have been excited to see the advancement in academia. For instance, the University of Maryland School of Pharmacy just recently rolled out a Cannabis Science Master's Degree program. We have also seen that Northern

Michigan University is now doing a Medicinal Plant Chemistry Program.

We are going to have talks from the National Institute of Standards and Technology (NIST) about the standardization of cannabis, and we are going to hear from the U.S. Pharmacopeia about the work that they are doing with cannabis and standardization. At the core, we are an analytical science show, but we do also cater to medical canvas topics, such as doctors and nurses, who talk about different topics in cannabis such as pediatric care, geriatric care, and routes of administration.

We are just excited to continue to work together with the industry to bridge the gaps between science and medicine and cannabis.

Why do you think events such as Pittcon are important for educating the scientific community on cannabis and the research and work you are doing?

Events such as Pittcon are crucial because when I come to these events, I get to network with more and more analytical professionals. Over the years of coming to Pittcon, interest has gradually grown, but it is exciting to see the level of interest that folks have now.

It has been great to see the evolution of that at Pittcon, and I think that Pittcon is a leader in analytical sciences and bringing people together.

3.5 GC-MS IN THE ANALYSIS OF CANNABIS EXTRACTS

Cannabis consists of the dried parts of the cannabis plant, also known as hemp or marijuana. It is the most used mind-altering (psychotropic) drug in the US after alcohol. Active compounds in cannabis include tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN). There are many different strains of the cannabis plant, and each has different concentrations of each active compound.

Users can smoke cannabis or add it to food. Resins extracted from the plant contain very high levels of THC and range from oils to waxes and hard solids. Edible forms take longer to digest and produce a 'high', so this can mean people consume more to feel the effects faster. There is also a growing trend to 'vape' products containing cannabis extracts. The FDA has warned that vaping is associated with severe lung illnesses and has caused some fatalities. Tests have shown that vaping products containing THC may be to blame.

A total of 11 states and Washington DC have legalized cannabis for recreational use, and 33 states have legalized it for medical use. However, the US FDA has not recognized or approved cannabis as medicine as they do not have enough evidence to show that the benefits of cannabis (rather than cannabinoid ingredients) outweigh the risks.

The cannabis plant contains more than 100 cannabinoids, but medical researchers are mainly interested in THC and CBD. THC can reduce nausea, pain, inflammation, and muscle control problems, and increase appetite.

The FDA has approved two THC-containing drugs called dronabinol and nabilone in pill form. They treat nausea after chemotherapy and increase appetite in patients with extreme weight loss caused by AIDS.

CBD can help decrease pain and inflammation, control epileptic seizures, and possibly aid mental illness and addictions. Unlike THC, CBD does not produce 'highs'. The FDA has approved a CBD-based liquid medication called Epidiolex® for treating two types of severe childhood epilepsy (Dravet syndrome and Lennox-Gastaut syndrome).

Sativex® (nabiximols), a mouth spray containing THC and CBD, has been approved to treat muscle control problems in multiple sclerosis (MS) sufferers in the UK, Canada, and several European countries. However, it is not approved in the US. Many researchers are investigating THC, CBD, and other cannabinoids as potential treatments for diseases such as MS and HIV/AIDS.

However, using cannabis can have serious health implications. For example, it has been shown to have short-and long-term effects on the brain. It may impair movement or memory or alter senses. In high doses, it can produce hallucinations, delusions, and psychosis. In the long-term, cannabis can affect brain development. Researchers are currently investigating if some changes to the brain are permanent.
Cannabis can produce a range of other health problems ranging from breathing issues, increased heart rate, problems with child

development in and after pregnancy, and intense nausea and vomiting. Long-term use has been linked to mental illness in some people, for example, worsening symptoms in patients with schizophrenia and depression.

Over the past decade, cannabis has been found to contain significantly higher levels of THC, which increases the risks of harmful reactions and addiction. Some researchers attribute higher THC levels to the rise in the number of users visiting emergency rooms.

The demand for analytical techniques

Thanks to the legalization of medical and recreational cannabis use in several US states, the prevalence of cannabis-containing products has grown dramatically in the past few years. However, while pharmaceuticals require rigorous testing to meet regulations governing quality and safety, this does not apply to cannabis and its related products. As medical uses of cannabis increase, this leaves quality control in the hands of the dispenser, manufacturer, and even the consumer, who require the best analytical methodologies to screen for a variety of factors.

For example, plants could retain potentially toxic chemicals used to cultivate and store cannabis plants, such as pesticides, herbicides, and fungicides. Another concern is that solvents leftover from when cannabis components are extracted from the plants, such as organic solvents, could be left in the final product. Analysis can also help determine particular strains of the plant, flavor profiles, and potency, as well as provide information to help prescribe the correct dosage for patients.

Before analysis, the plant must be processed (extracted). Many solvents can be used for this, including organic solvents such as n-Hexane, methanol, and ethyl acetate; hydrocarbons such as butane or propane; or supercritical carbon dioxide. Some extraction methods are 'solventless', that is, they use only heat, pressure, and water. Extraction allows impurities to be removed and helps prepare the sample for analysis.

GC-MS has long been used for the analysis of cannabis and its components. It is simple, fast, and provides quantitative information on component concentrations.

Effectively, GC reproduces what happens when plant material is smoked. That is, acids in the plant are converted to free cannabinoids. Combining GC with MS allows cannabinoids to be identified as well as pesticides and other compounds of interest.

Terpenes

While THC and CBD have attracted significant research and commercial interest, attention is now turning to terpenes. Cannabis contains over 140 terpenes, the main component of aromatic oils found in the plant. They are responsible for flavor and smell. Different terpenes, and ratios of terpenes, can be found in different cannabis strains.

The recent proliferation of new terpene profiling methods has been fueled by several factors: legislative changes, including the legalization of cannabis; possible synergistic properties of cannabinoids and terpenes; and potential health benefits of terpenes.

The main terpenes in cannabis include β-myrcene, which has antibiotic properties and enhances the THC muscle relaxant effect; α-pinene, which has anti-inflammatory properties and enhances the THC bronchodilator effect; and β-caryophyllene, which also has anti-inflammatory properties and enhances the THC protective gastric effect (amongst other benefits).

At Pittcon, speakers from Entech Instruments Inc discussed the importance of analyzing terpenes as well as less well-known cannabinoids. In their talk 'Beyond THC and CBD: Solvent-Free Headspace Extraction and GC-MS Profiling of Terpenes and Less-known Cannabinoids in Cannabis', the speakers explained the advantages of effective and efficient chemical profiling. For example, plant growers can use unique terpene profiles as a 'fingerprint' to help identify a specific strain for cultivation or to develop products with interesting aromas.

The team at Entech has developed a flexible technique that allows chemists to manage highly abundant volatile terpenes while still maximizing the signal for lower volatility cannabinoids. It involves a solvent-free headspace extraction approach – vacuum-assisted sorbent extraction (VASE) – coupled to thermal desorption GC-MS (TD-GC-MS) in a unique dual-column split/spitless setup. They will report how the technique produced complete chemical profiles for monoterpenes, sesquiterpenes, and cannabinoids with an extraction time of five minutes. For analysis of raw material to the final result takes less than an hour. The study of several cannabis strains revealed different terpene and cannabinoid compositions and allowed measurements at relevant sample quantities.

Speakers from Restek discussed the role of GS-MS in terpene analysis at Pittcon in their talk, 'Comparison of Techniques for Terpene Analysis via Gas Chromatography-Mass Spectrometry.'

While terpenes in cannabis are studied mainly for their aromas and flavors, they also play a significant role in the 'entourage' effect. This is when terpenes interact with cannabinoids and other naturally occurring compounds, giving each cannabis strain its unique 'user experience'. The speakers reported on research to compare the terpene content of cannabis flowers using headspace (HS)-GC-MS using HS-Syringe, an HS-SPME Arrow-GC-MS, and direct immersion (DI)-SPME Arrow-GC-MS.

Terpenes are also one of the application areas under development at Shimadzu. Teams at Shimadzu have demonstrated how terpene levels can fall over time, depending on different strains and different storage conditions. In one study, they analyzed several strains of cannabis for 41 terpenes in a study using its GCMS-QP2010SE single quadrupole mass spectrometer with headspace injection.

Shimadzu offers a comprehensive series of GC-MS and GC-MS/MS instruments. Recently, it launched the NX Series of GC-MS. This exceptionally sensitive triple quadrupole GC-MS comes with a new, highly efficient detector and three forms of noise reduction technologies. The GCMS-TQ8050 NX can

detect and quantify trace amounts of ions down to femtogram levels.

Perkin-Elmer also has a range of GC-MS systems that can be used for identifying and quantifying volatile and semi-volatile compounds. The integrated Clarus™ SQ 8 GC MS delivers high throughput and sensitivity, while the AxION® iQT™ GC/MS/MS is well-suited for unknown samples.

Perkin-Elmer has also developed the portable, easy-to-operate Torion® T-9 GC MS, ideal for rapidly identifying environmental volatiles and semi-volatiles. Its capillary GC is equal to any benchtop system in chromatographic resolution and performance.

Restek experts were at Pittcon to provide scientific support and to showcase their comprehensive line of products. The company offers chromatography columns, accessories, and sample preparation supplies needed for fast, accurate, and reliable cannabis analysis. It also boasts a wide range of cannabinoid-related certified reference materials (CRMs), manufactured, and QC tested in ISO-accredited laboratories.

In summary, GC-MS is a key player in the cannabis industry, aiding the analysis of cannabinoids and terpenes.

References

- *Chromatography Today, The Analysis of Medicinal Cannabis for Pesticides and Residual Solvents using a Portable GC (2017) https://www.chromatographytoday.com/article/columns-gc/52/ellutia-chromatography-solutions/the-analysis-of-medicinal-cannabis-for-pesticides-and-residual-solvents-using-a-portable-gc/2209*
- *National Institute of Drug Abuse, Marijuana (2019) https://www.drugabuse.gov/publications/drugfacts/marijuana*
- *National Institute of Drug Abuse, Marijuana as medicine (2019) https://www.drugabuse.gov/publications/drugfacts/marijuana-medicine*
- *News Medical Life Sciences, GC-MS Analysis of CBD and THC (2019) https://www.news-medical.net/life-sciences/GC-MS-Analysis-of-CBD-and-THC.aspx*
- *Pittcon 2020, Beyond THC and CBD: Solvent Free Headspace Extraction and GC-MS Profiling of Terpenes and Less-known Cannabinoids in Cannabis https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=1527&SessionDateID=1*
- *Shimadzu, Simplified Cannabis Terpene Profiling by GCMS https://www.shimadzu.fr/sites/shimadzu.seg/files/gcms-1604-terpeneprofilingcannabis.pdf*
- *Shimadzu, GCMS-TQ8050 NX https://www.shimadzu.com/an/gcms/8050/index.html*
- *Perkin-Elmer, Gas Chromatography Mass Spectrometry https://www.perkinelmer.com/uk/category/gas-chromatography-mass-spectrometry-gc-ms*
- *Pittcon 2020, Comparison of Techniques for Terpene Analysis via Gas Chromatography-Mass Spectrometry (GC-MS) https://s23.a2zinc.net/clients/pittcon/pit20/Public/SessionDetails.aspx?FromPage=Sessions.aspx&SessionID=1531&SessionDateID=1*
- *Restek, Growing Analytical Solutions for Cannabis Labs https://www.restek.com/cannabis*

3.6 THE ADVANCEMENT AND FUTURE OF CANNABIS

In the last decade, the laws surrounding cannabis have become more lenient and relaxed in several states and countries. These include outright legalization, as seen in Canada, state-by-state legalization of recreational and medical cannabis in the USA, and national legalization of medical cannabis, such as in the UK. As a consequence, the emerging cannabis industry requires support from the analytical chemistry industry. In recent years, this has led to the development of new approaches for characterizing cannabis, extracting, and excluding contaminants, as well as ways to make testing faster and more accessible in new settings.

Fourteen US states and territories have legalized recreational cannabis sales for adults, and a total of 33 states have legalized cannabis for medicinal purposes. Illinois began 2020 with the legalization of cannabis, the 11th state to do so.

However, as a lucrative new industry – expected to be worth $22 billion by 2022 – and with incoming players drawn through commercial interest, it becomes critical to ensure the safety of products reaching the market. There is currently a lack of standardization when it comes to cannabis testing and regulation, but these are likely to evolve in the coming years.

3.6a CURRENT TESTING METHODS IN CANNABIS

As cannabis becomes legalized in various states and countries around the world, there is a need for better analytical methods to characterize products reaching the market and ensure their safety. Unfortunately, there is currently a lack of standards for cannabis testing compared with those employed in the food and pharmaceutical industries.

The Importance of Analysist

There are several components of cannabis that require analysis to ensure product efficacy and consumer safety.

Potency

Cannabis contains over 100 different cannabinoids, but these can vary in their concentration and potency between plants and at different times in the growth cycle. In a therapeutic context, it is particularly important to be able to identify and characterize the potency of the cannabinoids present in cannabis. Of particular interest is the ratio of the psychoactive component THC to CBD, but due to the so-called “entourage effect” of the many 100s of compounds (including cannabinoids) present in cannabis, it looks as if it is relevant to also characterize these to ensure a consistent therapeutic effect.

Pesticides

Pesticide residue is a major concern for the cannabis industry due to the number of chemicals that farmers may use on their crops to ensure a good yield. These include insecticides, fungicides, and rodenticides.

There have already been several recalls in the US and Canada due to high pesticide levels in cannabis products. In 2018, a cannabis sample from one producer tested by Health Canada contained high levels of the pesticides myclobutanil and bifenazate. Myclobutanil is banned for use on plants that will be inhaled as burning produces hydrogen cyanide.

Studies have shown that samples of medical cannabis are frequently contaminated with pesticides. According to a report, the Washington State Department of Agriculture detected banned pesticides or unacceptably high pesticide levels in 43% of samples tested during a 16-month period. In the US, each state has a list of pesticides that must be monitored in cannabis samples.

Terpenes

Along with cannabinoids, terpenoids are another class of compounds that determine the medicinal effects of cannabis. These compounds, which are also responsible for the flavor and smell of cannabis, are, therefore, essential to quantify. There are over 200 terpenes in cannabis, which can be present in varying ratios that influence their effects.

Heavy metals

Cannabis plants are extremely effective at absorbing heavy metals from the soil, with no negative effects on the plant itself. Although, when consumed by humans, these concentrated heavy metals can pose a risk to health. The metals can include lead, mercury, cadmium, and arsenic, which are toxic to humans.

Mycotoxins

Another safety concern is to exclude the presence of mycotoxins. These are toxic metabolites produced by mold and must be tested for in both medical and recreational cannabis. Several of these, such as aflatoxin B1, are especially harmful to humans and can be dangerous to immunocompromised patients even at very low concentrations.

Residual Solvents

Solvent-based extraction techniques are frequently used with cannabis to produce concentrates or extracts such as oils, waxes, and tinctures. A downside to this approach is that the residual solvents need to be removed and their absence from the sample confirmed, as some of these residual solvents can be harmful to health. GC and static headspace GC are often used to concentrate volatile components for analysis to allow identification and quantification of residual solvents.

Analytical Cannabis Techniques

There are several approaches that have been commonly used for cannabis analysis. These include:

Chromatography

Liquid chromatography (LC) and gas chromatography (GC) are well-known techniques used in the cannabis industry. HPLC using a photodiode array detector is commonly used in the cannabis industry for determining the potency, and is relatively easy and inexpensive to employ, as well as being highly accurate.

GC/flame ionization detector (FID) is the most commonly used approach for profiling the complex mixture of terpenes present in cannabis samples. Headspace GC/FID is frequently used for residual solvent testing, which is required by many US states. This approach has the advantages of simple sample preparation and relatively low equipment costs compared with alternative methods.

GC/MS is commonly used to screen for pesticides and residual solvents as it can accurately detect traces of volatile compounds

Mass Spectrometry

Mass spectrometry is not as well established in the cannabis industry as other modalities. However, research has shown its potential applications and reasons to suspect its utility include greater specificity and sensitivity than alternative techniques.

Gas Chromatography-Mass Spectrometry (GC/MS) is increasingly used to test for residual solvents and pesticides in cannabis samples. This approach has an advantage over GC/FID in being able to identify unknown peaks corresponding to impurities in the sample, as opposed to purely quantitative analysis.

Extraction techniques

In addition to the methods mentioned above for analyzing cannabis samples, there are also several approaches to cannabis extraction. There are currently four main approaches to extraction: alcohol, carbon dioxide, hydrocarbon, and solvent-free extraction.

In lab settings, the most popular approach is hydrocarbon solvent extraction. This technique is well established from use in other industries, such as pharmaceuticals and cosmetics. It offers the benefit of being highly efficient in terms of cost, time, and extract purity. However, a key disadvantage is that it requires further purification steps to remove any residual solvents, which could be harmful to consumers.

More recent methods include, which is more expensive but gaining more widespread use, supercritical fluids chromatography, or CO2 extraction, which uses carbon dioxide as a solvent and therefore does not require as much post-processing.

The method involves cooling carbon dioxide gas to take its liquid form before manipulating the pressure and temperature so that it exists in a supercritical state i.e., between a liquid and a gas. In this state, the carbon dioxide easily dissolves and extracts compounds when passed through a cannabis sample. Subsequently, the carbon dioxide is allowed to return to a gaseous state, causing the extracted compounds to precipitate. The approach is particularly useful in the cannabis industry as it can be customized to the compound of interest as well as produce a wide range of cannabis end products. However, it is more expensive than organic solvent extraction.

Cannabis analysis at Pittcon

Pittcon is fortunate to be joined by companies who have spent many years invested in cannabis testing. One of these is Restek, a company that has been involved with the emerging cannabis industry since its earliest days. Restek offers a complete line of LC and GC products, including chromatography columns, accessories, and sample preparation supplies.

CEM Corporation offers two systems to simplify sample preparation ahead of analysis. The systems help to ensure accurate results even when analyzing the variety of samples in which cannabis can present, such as oils, edibles, and plant material. Its product range is suitable for sample testing for potency, pesticides, metals, terpenes, and mycotoxins.

References

- *Nie, B., Henion, J. & Ryona, I. The Role of Mass Spectrometry in the Cannabis Industry. J Am Soc Mass Spectrom. 2019; 30: 719. https://doi.org/10.1007/s13361-019-02164-z.*
- *Global News (2019) Health Canada data reveals roughly 95% of pot samples tested negative for pesticides. Available at: https://globalnews.ca/news/6233169/health-canada-testing-data-pesticides-in-pot/ Accessed: January 2020.*
- *The Stranger (2018) WSDA Tests Show Frequent Illegal Pesticide Use on Cannabis. Available at: https://www.thestranger.com/slog/2018/10/29/34697776/wsda-tests-show-frequent-illegal-pesticide-use-on-cannabis Accessed: January 2020.*
- *Medical Cannabis Network (2019) Present day use of solvent extraction in cannabis extraction. Available at: https://www.healtheuropa.eu/cannabis-extraction-2/91341/ Accessed: January 2020.*
- *Waters. Supercritical Fluid Extraction (SFE) Systems. Available at: https://www.waters.com/waters/en_US/Supercritical-Fluid-Extraction-(SFE)-Systems/nav.htm?cid=10146521&locale=en_US Accessed: January 2020.*

3.6b SIMPLE AND FAST DETERMINATION OF CANNABINOIDS

As the major psychoactive component in cannabis, tetrahydrocannabinol (THC) has received a lot of scientific attention. However, it has emerged that cannabis consists of nearly 500 compounds, with over 100 of these coming from the same family as THC – the cannabinoids.

The other best-studied cannabinoid in cannabis is cannabidiol (CBD). By contrast to THC, CBD is not psychoactive. These two compounds have opposing actions within the brain, and the balance between them strongly influences the effect of the cannabis plant or extract.

When it comes to the expanding interest in medical cannabis, the relative ratios of THC to CBD become particularly important. For example, in some illnesses such as mental health disorders, low THC varieties could be preferable. Meanwhile, in other conditions, such as multiple sclerosis (MS) related spasticity, it is thought that the presence of some THC is beneficial. The MS treatment nabiximols, which is not yet approved by the US FDA, contains a 1:1 ratio of CBD to THC, and has been shown in placebo-controlled trials to improve spasticity.

Dronabinol, which is approved to treat AIDS-related weight loss and chemotherapy-induced nausea and vomiting, is a synthetic form of THC. Epidiolex, which is purified CBD from cannabis, is approved by the FDA in two forms of severe childhood epilepsy and contains no THC. In clinical trials, the drug reduced the frequency of seizures when added to existing antiepileptic medications, compared with a placebo.

Terpenes are another essential family of compounds in cannabis. These aromatic oils are responsible for its distinctive flavor and aroma. The significance of terpenes and their ability to interact with THC and modulate its psychoactive effects has become apparent and is known as the “entourage effect”.

As well as testing samples of cannabis products and plants, another area of current interest is the testing of blood plasma for recent cannabis use as it remains a challenge for forensic toxicologists to determine the time elapsed since cannabis consumption.

HPLC for Cannabinoid Analysis

In the past, the primary cannabinoid of interest was THC, but now that the biological and potentially therapeutic activities of other cannabinoids are apparent, new approaches are needed to study these compounds. GC, an established method for analyzing THC, is unsuited for many other cannabinoids due to their thermal lability.

At Pittcon, researchers led by Dennis Polite of Axion Analytical Labs will present a series of experiments they used to explore the use of HPLC/UHPLC for identifying and quantifying several cannabinoids, including THC, THC-A, CBD, cannabinol (CBN), CBD-A, and CBC. The first part of their study focuses on optimizing HPLC separation conditions while reducing analysis time. The research also demonstrates the use of diode array detection (DAD) for cannabinoid analysis and the creation of a UV library to assist and accelerate cannabinoid identification.

An LC-MS Setup to Characterize Hemp

Hui Guo of Shimadzu Scientific Instruments will present data showing the efficacy of LC-MS in identifying and determining cannabinoid concentrations in hemp samples.

This has become particularly important since the US Farm Act of 2018 made hemp legal where THC concentrations fall below 0.3%.

> With such small margins, Guo says that MS could provide an advantage over HPLC with UV and photodiode array detectors due to its quantitative nature and greater sensitivity and selectivity.

This study used an LC-MS method using a single quadrupole mass spectrometer with an integrated LC front end. The set-up was used to quantify 16 cannabinoids, including Δ9-THC, Δ8-THC, and CBD, as well as cannabigerol (CBG), CBN, and cannabicyclol (CBL). Guo will present data to support the use of MS in cannabis analysis to achieve greater accuracy in quantitative cannabinoid determination.

Cannabis at Pittcon

Axion is a US-based company that specializes in training, consulting, and method development within chromatography for clients in the pharmaceutical, chemical, and petroleum industries. Its experience includes HPLC, GC, and ion chromatography (IC) training. They also deliver short courses and seminars from their training institute in Chicago, Illinois.

The company's research team, which presented its developments in new chromatographic methods at Pittcon, also look for ways to improve the sensitivity, reliability, and analysis times associated with existing approaches.

Shimadzu also attended Pittcon. Along with a range of analytical solutions, Shimadzu offers a dedicated cannabis analyzer that allows for a complete workflow within one device. Simple to use, the analyzer can be operated by those with even limited experience. It also has several preset modes to tailor the analysis to your needs. Results are displayed in a clear and quickly produced report.

A recent study by Mara Mandrioli and colleagues showed that a method of reverse-phase HPLC and UV detection allowed them to quantify 10 cannabinoids in eight minutes from a sample of cannabis following organic solvent extraction. The set up creates a fast, easy, and affordable process that the researchers say could be particularly suited to smaller laboratories and day-to-day testing, such as in a quality-control setting.

References

» *Mandrioli M, Tura M, Scotti S, et al. Fast Detection of 10 Cannabinoids by RP-HPLC-UV Method in Cannabis sativa L. Molecules 2019; 24: 2113; doi:10.3390/molecules24112113.*

3.6c CANNABIS AND THE OPIOID EPIDEMIC

The opioid epidemic has its origins in the 1990s when, in an attempt to better control the pain of non-cancer patients, the medical community expanded its use of opioids. Under the belief that medical opioids rarely induce addiction, and with encouragement from the pharmaceutical industry, doctors began to become increasingly reliant on opioids for chronic pain management.

Between 1999 and 2008, deaths from opioids increased fourfold, and by 2017, the opioid epidemic was declared a national emergency by the US government.

Opioid deaths have now overtaken road traffic accidents as a cause of death, and the average life expectancy of an American has also declined as a consequence of the epidemic.

What is the impact of growing cannabis legalization on the opioid epidemic? In the USA, there are already four states that have legalized the use of cannabis for treating opioid use disorders. However, the evidence to support this is mostly anecdotal. The majority of medical cannabis users take the drug for pain-related reasons, and there is some evidence that introducing cannabis can help those with

opioid abuse disorders to lower their dosage of opioids while still managing their pain.

A recent study in Canada issued surveys to 2,032 patients receiving medical cannabis and gathered data on their use of the drug to substitute other prescribed or illicit substances. The researchers, led by Philippe Lucas from the University of Victoria, British Columbia, found that 69% of patients were substituting prescribed medication with medical cannabis, and, of these, around a third were for opioid substitution (n=610). The team found that almost 60% of these patients had completely stopped taking opioid medication.

The key question is: could medical cannabis or cannabinoids help us to reduce our reliance on prescribed opioids?

The Pittcon Plenary Lecture

Ziva Cooper will discuss these issues in her Pittcon Plenary Lecture 'Cannabis Constituents as Novel Strategies to Tackle the Opioid Epidemic'. Dr. Cooper is the Research Director for UCLA's Cannabis Research Initiative and Associate Professor at the Semel Institute for Neuroscience and Human Behavior and Department of Psychiatry and Biobehavioral Sciences at the University of California, Los Angeles. Her current work focuses on conducting controlled trials to explore factors that influence the therapeutic effects and abuse liability of cannabis, cannabinoids, and opioids.

Dr. Cooper received her Ph.D. from the University of Michigan in Biopsychology in 2007 in the field of preclinical psychopharmacology. She served on the National Academies of Sciences Committee on the Health Effects of Cannabis that recently published a comprehensive consensus report of the health effects of cannabis and cannabinoids. She is also President of the International Study Group Investigating Drugs as Reinforcers, a Board Director for the College on Problems of Drug Dependence, and an Associate Editor of The American Journal of Drug and Alcohol Abuse.

Spex Sample Prep, a company that produces a range of solutions for labs working in the cannabis and hemp industries, also attended Pittcon. These include its cryogenic grinder Freezer/Mill, which freezes and pulverizes samples from a whole range of cannabis products ready for analysis. Applications include THC extraction, pesticide residue extraction, screening for residual solvents, pesticide residue, and heavy metals, as well as potency testing. The sample is placed in a grinding vial, where it remains sealed from start to finish, eliminating the risk of cross-contamination, preserving the integrity of your results.

Another offering from Spex is their line of certified reference materials, which are backed by ISO 17034, ISO 17025, and ISO 9001 certifications and accreditations. They offer single and combination reference materials for many substances that can contaminate samples, including pesticide residues, DEA-controlled substances, and residual solvents.

Glas-Col, who also attended Pittcon, offers a range of laboratory equipment for use in cannabis sample prep and analysis, including heaters, evaporators, and mixers. Potential applications include cannabis extraction and quality control. For example, the company offers the StirMantle, a heating mantle with an in-built electromagnetic stirrer, which is suited to carrying out short-path distillation of CBD from cannabis. This allows the CBD fraction to be extracted without being degraded due to excess heat. Glas-Col can provide this as a standalone piece of equipment or deliver a customized, turn-key solution for your lab.

References

» *Jones MR, Viswanath O, Peck J, et al. A Brief History of the Opioid Epidemic and Strategies for Pain Medicine. Pain Ther 2018; 7: –21. doi:10.1007/s40122-018-0097-6.*

» *Lucas, P., Baron, E.P. & Jikomes, N. Medical cannabis patterns of use and substitution for opioids & other pharmaceutical drugs, alcohol, tobacco, and illicit substances; results from a cross-sectional survey of authorized patients. Harm Reduct J 2019; 16: 9. doi:10.1186/s12954-019-0278-6.*

3.6d EMERGING DEVELOPMENTS IN CANNABIS

Cannabis is a rapidly expanding industry, and it is thought that by 2022 it will be worth $22 billion. This brings with it a flood of new players interested in capitalizing in on this burgeoning market. However, this presents challenges, particularly regarding consumer safety.

Canada will see the emergence of new formats of cannabis consumer products in 2020, including edibles, vapes, and beverages. There are concerns over the safety of some of these so-called "derivatives", particularly vaping products.

The new regulations that permit the sale of such cannabis-containing products also include product formulation restrictions, quality standards for ingredients, and testing requirements to protect consumer health.

Pittcon features a session that includes speakers such as Andrew Hiser from Holistic Industries, Christopher Hudalla from ProVerde Laboratories, and Jack Henion from Advion, Inc., who discuss the development of cannabis as an industry from multiple perspectives.

With the industry growing at such a rapid pace, how can we keep up with the analytical developments and protect consumer safety? The rapid evolution of the cannabis industry and the accompanying pace of the analytical cannabis industry are so fast that it can be a struggle to keep up. This talk will discuss the impact that it has and the risks to consumers.

These include recent events, such as the emergence of vaping-related illness, due to contamination that resulted in the deaths of 60 people in the USA. At the time of writing, the Centers for Disease Control and Prevention say that there have been 2,668 hospitalizations for e-cigarette (vaping) product use-associated lung injury (EVALI) in US states and territories. The latest evidence indicates that the outbreak is related explicitly to THC-containing vaporized products.

A study found that most hospitalized patients obtained a THC-containing product from an informal source, such as friends, family, or online, as opposed to a commercial source such as a dispensary or vaping shop. Another line of inquiry is into the role of Vitamin E acetate in the outbreak, which has been found in lung fluid samples from affected patients.

The presenters say that producers are creating new cannabis products at a faster rate than their safety can be evaluated. One area of interest is the stability of these products. Product breakdown could lead to the formation of isomers or degradation products with unknown, potentially harmful effects within the human body. For example, in consumer CBD products, CBD degradation could lead to an unintended dosage. The breakdown products of CBD are also poorly characterized, and therefore any potentially toxic effects are unknown.

The team will also discuss how developments in cannabis analysis produce pressure on the skillset of laboratory staff, who are increasingly required to use demanding and highly sophisticated techniques. The researchers will discuss whether automation and robotics could help to address some of these challenges.

Cannabis Developments at Pittcon

ProVerde Labs, a Massachusetts-based laboratory, which is one of the state's first ISO 17025 accredited labs dedicated to the hemp and cannabis industry, provides analytical and consulting services to clients in the pharmaceutical industry, including dispensaries, the food industry, cannabis product development, and research. Their mission is to be an environmentally sustainable business, and they say that their analytical testing produces 100-500 times less hazardous waste than conventional methods.

References

- *CNN (2019) Canada's Cannabis 2.0: Edibles, beverages, vapes on deck. Available at: https://edition.cnn.com/2019/10/17/business/canada-cannabis-legalization-2-0/index.html (Accessed January 2020).*
- *CDC. Available at: https://www.cdc.gov/tobacco/basic_information/e-cigarettes/severe-lung-disease.html*
- *CNN (2020) 2020 could be a defining year for the cannabis industry. Available at: https://edition.cnn.com/2020/01/09/business/cannabis-2020-legalization/index.html (Accessed January 2020).*
- *Nie, B., Henion, J. & Ryona, I. The Role of Mass Spectrometry in the Cannabis Industry. J Am Soc Mass Spectrom. 2019; 30: 719. https://doi.org/10.1007/s13361-019-02164-z.*
- *Waters (2016) Application Note: Forced Degradation of Cannabidiol. Available at: https://www.waters.com/webassets/cms/library/docs/720005766en.pdf (Accessed January 2020).*
- *Lachenmeier DW, Habel S, Fischer B, et al. Are side effects of cannabidiol (CBD) products caused by tetrahydrocannabinol (THC) contamination? F1000Research 2019; 9: 1394. doi: 10.12688/f1000research.19931.*

CONCLUSION

Illinois state has just legalized sales of cannabis, the first to do so purely through legislation. This demonstrates the changing attitudes to cannabis, not only socially but among lawmakers too. With a presidential election on the horizon, it will be an interesting time to observe where cannabis features in the campaigns. Could federal legalization be next?

There is also a growing awareness of the potential medical value of cannabis, as seen in the recent popularity of CBD products. Thanks to the Farming Act 2018, people without a prescription can now seek out the purported benefits of CBD and hemp legally. However, even though these are now legal, they remain unregulated. The FDA has only assessed one cannabis-related product – the drug Epidiolex for childhood epilepsy. Challenges remain to ensure the safety of cannabis and cannabinoid-containing products.

There is also a surprising lack of data to back up many of the medical uses for which exponents recommend cannabis. A recent meta-analysis in the Lancet Psychiatry found that evidence to support the use of cannabinoids in mental health conditions, including PTSD, ADHD, depression, and anxiety, was scarce. The authors concluded the current evidence base was insufficient to inform the use of cannabis within any kind of regulatory framework. Developments in clinical research – in particular, randomized controlled trials – will be vital in establishing cannabis' role in medicine and determining whether treatment guidelines will be able to recommend the drug as a standard medication.

The current importance of cannabis in the field of analytical chemistry was exemplified by the Pittcon Plenary Lecture, presented by Ziva Cooper of UCLA. The topic brings together the discussion of cannabis with one of the most pressing public health problems in the USA – the opioid epidemic. This epidemic claims the lives of 40,000 individuals each year, and for many of these people, medical prescriptions will have been their first exposure to opioids. What role could cannabinoids play in reducing our dependence on opioids for pain relief, and could they treat or prevent opioid use disorder?

In the analytical cannabis program at Pittcon, topics will include current and emerging testing modalities, the rapid determination of cannabinoids, as well as companies hoping to keep up with such a rapidly evolving industry.

In addition to a filled schedule of presentations and panels, the Pittcon Expo also draws many of the major players in the analytical chemistry industry. Exhibitors include Waters, Restek, CEM Corporation, Shimadzu, and Spex, who, between them, can offer analytical cannabis solutions from sample prep to spectroscopy.

References

» *Black N, Stockings E, Campbell G, et al. Cannabinoids for the treatment of mental disorders and symptoms of mental disorders: a systematic review and meta-analysis. Lancet Psychiatry 2019; 6: 996-1010. Doi: 10.1016/S2215-0366(19)30401-8.*

3.7 THE ROLE OF CANNABIS IN THE INCREASING OPIOID EPIDEMIC

The below article is a summary of Ziva Cooper's talk at Pittcon titled 'Cannabis Constituents as Novel Strategies to Tackle the Opioid Epidemic'.

The legalization of cannabis for recreational and/or medical use is rapidly spreading across the United States of America. Cannabis is still federally illegal, and this presents several regulatory hurdles to anyone studying the impact of cannabis on behavior and physiology. In 2017, there were 72,000 drug overdoses, 50,000 of which were related to opioid overdoses. A total of 30% of these opioid overdoses are due to prescription opioids. Meanwhile, 12% of people that are prescribed an opioid for pain may develop a disorder related to opioid use. As many as 80% of heroin users started off using prescription opioids.

The work presented in this article work focuses on the intersection between cannabis and opioids, investigating whether cannabis could help alleviate the opioid epidemic. There have been self-reported decreases in opioid use among people who are using medical cannabis, and there are compelling state-level findings that suggest that states with medical cannabis laws see a reduction in opioid use.

Cannabis contains more than 140 unique chemicals called phytocannabinoids, the most well-known being tetrahydrocannabinol (THC), which causes intoxication and is responsible for the abuse-related effects of cannabis. However, the therapeutic effects of cannabis come from this molecule. Alongside phytocannabinoids, several terpenes found in cannabis plants are thought to have therapeutic effects.

There is considerable evidence to support the use of cannabis and cannabinoids for chronic pain, but more studies need to be conducted into cannabis's potential for reducing reliance on opioids by providing an alternative pain relief option.

Studies have provided mixed results, but there is evidence to suggest that opioids and cannabinoids (specifically THC) work synergistically to offer robust pain-relieving effects. Studies have indicated that, in states where medical cannabis is available, this could be why there are fewer opioid prescriptions dispensed.

To reconcile this mixed data, studies have been in controlled laboratory settings where researchers can adequately examine the dose-dependent effects of cannabis and cannabinoids in addition to opioids. One of the studies worked with 18 current heavy cannabis users who did not report adverse effects related to their cannabis use. We administered either a placebo, a low dose drug, or a high dose drug under very controlled conditions, examining the therapeutic effects of cannabis and cannabinoids, as well as the adverse effects while looking at pain thresholds.

The study found that after smoking active cannabis (containing THC), participants reported a 20% increase in their pain tolerance. As expected, there was not a significant difference between oxycodone and the placebo when it was given by itself. However, when cannabis and oxycodone were combined, there was a robust increase in pain tolerance, echoing the synergy that has been seen in animal models.

The researchers looked at adverse effects such as high intoxication and abuse liability, asking participants at several points during the session to rate their subjective feelings about the drug experience. Under placebo conditions, they did not report feeling intoxicated or the desire to retake the drug, which is expected because this cannabis did not contain THC. With the cannabis that included THC, there was intoxication and a desire to retake cannabis.

The study also found that, while oxycodone did not further increase cannabis's abuse liability or intoxication, there were still significant ratings. This affirmed the existing preclinical evidence for this cannabinoid-opioid synergy, as well as evidence from population and state-level findings. It is uncertain if THC can replace opioids for pain management. However, it is clear that they can be used adjunctly. It is also evident that there are side effects when used together.

A similar study was conducted using cannabidiol (CBD). Animal models have shown that cannabidiol helps to alleviate certain types of pain without the psychoactivity that was seen with THC, but how CBD interacts with opioids is unknown. In a recent study, cannabidiol's pain-relieving effects matched THC, and when participants were asked about their intoxication, they reported that neither cannabidiol or oral THC produced a measurable intoxicating effect.

There have been no double-blind rigorous studies investigating whether cannabidiol can potentially be a substitute or an adjunct to opioids for pain, but, currently, there is a first-of-its-kind study underway.

Terpenes are the main constituent that the researchers are working with, particularly beta caryophyllene (BCP) and myrcene. Like CBD, in animal models, BCP and myrcene have been shown to alleviate pain with no behavioral side effects. The team has also seen that BCP can act synergistically with opioids such as THC. The study found that, if we take a dose of BCP that is not generally analgesic, alongside a dose of opioids that is usually analgesic, they will produce analgesia together.

One-third of patients choosing to use cannabis varieties with high levels of BCP and myrcene were able to substitute their cannabis for opioids. However, there have been no double-blind placebo-controlled studies that look at myrcene and BCP, and its pain-relieving effects, or in combination with opioids.

Ultimately, we need to reduce these intoxicating effects, potentially using oral administration. There is strong evidence that THC might be able to reduce opioid use, but the effects of cannabidiol by itself, without any THC, are still unknown. Terpenes have the potential to reduce pain, and these could help to reduce the THC dose and the opioid dose needed to achieve pain relief.

In summary, cannabis constituents certainly have the potential to be alternative pain management strategies to opioids, but there is a long way to go if we are to confirm this.

Control studies in humans are necessary if the research team are to understand the pharmacological interaction between cannabis constituents and opioids fully and if they are to determine their clinical viability as potential therapeutics to help treat pain without abuse liability and intoxication.

3.8 DETERMINING CANNABINOIDS IN MARIJUANA-INFUSED EDIBLES

Marijuana-infused edibles, or medibles, have increased immensely over the last 10 years. With products such as brownies, gummy bears, and chocolate infused with marijuana, it is more important now than ever to test these cannabis-related products.

In this interview, Dr. Carl Wolf talks to News-Medical Life Sciences about his work and research into determining cannabinoids in marijuana-infused edibles.

In the last decade, cannabinoid use in the US has increased tremendously. Marijuana-infused products are one of the cannabis products that have seen the most significant increase. Can you explain why?

For a long time, marijuana has been considered to have medicinal properties. I think we are always looking for something new that is better. People say it is organic. I say there are lots of things that are organic, and we should not be eating or taking them.

The real push now is that as the US population gets older, we have more ailments, and we are under the philosophy that there should be something out there that can fix us.
We are looking for more ways to reduce pain, to make ourselves feel better, and to get ourselves back to the way we felt 20 or 30 years ago.

Cannabis-infused food products or medibles are sold for medicinal and recreational activities. They contain the psychoactive drug delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) and other cannabinoids. Why is it important to test for these products?

It is because there are many other components also, and there are no regulations, guidelines, or standards and you do not know what is in these products.

As it is a natural product, no two plants are the same. No two parts of the plant are the same. Even if you are just looking at the buds of the plant, no two buds are precisely the same.

You are getting a variety of different concentrations of different drugs and different compounds. Some of the effects, the reported effects, or the expected effects, sometimes do not just come from one compound. They come from a combination and then from the right ratio. If you have no idea what is in there to start with, then testing is essential.

For the medibles aspect, we have done testing on several things. You are under the assumption that it was made to what the labeling states on the package, and as we have tested several things, we found that that is not always the case.

There is a high incidence in probably about two-thirds of the products, not just from our research but in published research, that shows that it is either too high, too low, or it is not even there.

Accurate methods to determine THC and CBD content in edibles is a high priority. What role does analytical chemistry play in determining these contents?

We are the ones doing the work. We are the chemists. We are considered as the experts on how to do testing through analysis. I conduct analysis on biologicals, mainly blood, urine, liver tissue, and other things. That is where the importance of our work comes in because we are the ones with the knowledge to understand that there are cannabinoids and other constituents, where other people say marijuana as a singular term.

Food is a wide variety of things that you can see by just walking into the grocery store, and we know that each one acts a little bit differently. Having standardized methods or at least guidelines on how to deal with testing is very important.

Especially in our work, we have seen significant issues with that. Sugar matrices are not the same as fiber matrices, which are not the same as chocolate or fat matrices. Brownies, gummies, and dark chocolate do not act the same. You can see that by just looking at them physically, they may need different testing schemes to analyze them.

THC is the primary psychoactive compound in marijuana that gives the 'high' sensation. Accurate methods to determine its content is a high priority. What are the best analytical methods to determine THC?

That depends on which matrix you are talking about. That is the true question as we have seen it. Depending on what the substance or basic substance you are looking at is, it has a significant effect on whether you can recover it, and if you can accurately quantitate how much is present. As I said, brownies, gummies, and chocolate or high fiber foods, high sugar foods, and high-fat foods, all act differently in analysis.

If you try to use the same extraction method, it may work for one but not the other. If you have not done any method development or validation, assuming that it works for them all is not a good thing.

Last summer, we spent time analyzing and testing beverages. What we found was that the method we developed for doing food products does not work for beverages. In beverages, you had high sugar content foods that acted completely differently than brewed things such as tea and coffee, or alcohol such as beer and wine, for example. There is no single method for doing cannabinoids in all matrices.

Cannabinoid testing is supported by the National Justice (NIJ) Research and Development in Forensic Science for Criminal Justice. Why?

They are great because this gives us an opportunity that other agencies do not. NIJ supports law enforcement, which has different priorities in the United States than the National Institute of Health (NIH) or some of the other federal agencies that people receive grant money from. The NIH is not going to pay for Schedule 1 substance testing methods because the substances are restricted and not readily available for use by a wide variety of laboratories.

On the NIJ side, you are talking about law enforcement, real, daily life things that are going on and are becoming more prevalent throughout communities. We have seen increases in people taking medibles and other products, and drugs that are out there, and these are very relevant to public safety as a whole.

Can you tell us about your work into this NIJ funded research?

We originally were awarded a grant at the end of 2016, and which started in 2017. At the height of the opioid epidemic going on here in the United States, we were looking at the best way to analyze opiates in postmortem liver tissue because there were several reported methods out there that people said, "Oh, we did this, and we got a result." But we were like, "So does the method even work for the same class of drugs?"

We looked at 12 different ways to analyze liver tissue. We found out that even though opiates are a generally simple class and are very similar compounds, they do not act the same way with each extraction method. Some of the methods which were designed for doing urine or blood did not work well for liver tissue or liver homogenous, as we would expect.

As we went forward with the current project, I call it the Medible Project or the Brownie Project, we said, "Okay, let's look at this first: does the method work for extracting cannabinoids?"

We were looking at nine different cannabinoids with three different isotopically labeled standards for quantitation, and we found out that each of the methods we looked at was not quite the same.

"As you would expect, we saw a difference chemically between each cannabinoid for extraction efficiency. We also saw issues with different methods that just did not seem to work very well across all the three matrices we were looking at."

Dr. Carl Wolf

Can you tell us about the work that you presented at Pittcon in your presentation 'Evaluation of 25+ Techniques for the Determination of Cannabinoids in Marijuana-Infused Edibles'?

"This was 25 plus ways. It should have said 25 ways not to analyze cannabinoids in marijuana-infused products. My student and I did a beverage project last summer, and she said she did 40 ways not to analyze cannabinoids in beverages, but this one was submitted first. The negative is not always well received in science."

Dr. Carl Wolf

People say, "You can't publish negative data." And I respond, "But the problem we run into is that people don't publish." People have methods that they present, but they never really show you, or they never publish what did not work or how they got from point A to point B? There is no reason to reinvent the wheel in many of the things we do in science. That is why we have publications and literature for that.

What I am presenting is 25 plus ways not to analyze cannabinoids in marijuana-infused products. We wanted to look at different techniques that were available from manufacturers and publications to see what worked and did not before we came up with anything on our own. We wanted to use what is out there in the literature for people to describe ways we can analyze cannabinoids.

We looked at many manufacture's methods and we evaluated in many different ways. We basically could not find anything that worked. We got to around 20 methods, and it was like, "Okay, this is not working."

We had some collaboration with a couple of manufacturers who said, "Well, try this with our product. Try that. Try changing the pH. Try changing the dilution solvents." We got results that were a little bit better, but it was not really what we wanted. We were not impressed.

Then another manufacturer called and said, "Hey, I'm the new sales guy. What can I do for you?" I said, "Well, send me a quote and I want to buy some. Send me a method that you have." We tried their method and said, "No, it doesn't work just as well as the other ones."

We used the knowledge that we had learned from other manufacturers and said, "Okay, now we can play with things and we have an understanding of how the QuEChERS method works." After tweaking, we were like, "Okay, this looks a whole lot better than anything else we've done." That was what I was presenting today, what works, and what does not work.

As the world and society continue to evolve, so will the role of cannabis. Looking into the future do you see medibles evolving further?

I see them becoming, I do not want to say more mainstay, but more accepted. Food products are more accepted than the other formulations. You do not see people smoking that much on the street.

You are not going to be carrying a joint around with you, and with perception, people are not going to do this as part of their home life. Food is a lot easier to transport and conceal. It is just easier to transport cannabinoids in a food product as it is part of our lives. We live for food. I do not see these products going away.

Why are events such as Pittcon important for the work you are doing?

Pittcon is the largest laboratory exhibition in North America, with a wide variety of manufacturers and individuals presenting.

"My job is running a forensic and clinical testing laboratory, but that also involves research. New drugs come out and new things come out. We need to look at new ways of doing analysis."

Dr. Carl Wolf

We need to look at new ways of doing analysis. We also carry out pharmaceutical testing in the same way. It is a question of what is the best thing out there currently that we can get our hands on?

We may not be able to buy it, but we may be able to borrow it from someone else or benefit just by knowing what is going on and being cutting edge is one way to say it. Maybe not the correct way but being cutting edge or at least knowing what is going on in the world of laboratory science is very important, and Pittcon, to the best of my knowledge, presents that very well.

The Pittsburgh Conference is to bring people together from various laboratory facets. This is to get you outside of your box. I go to a couple of other meetings that are more forensic in nature, forensic toxicology or forensic science in nature, and you are only dealing with people who are in your discipline, so you do not get to see outside of the box, which is good, but a hindrance at times.

As I say, you go through with horse blinkers on saying, "What's in front of me?" Maybe you can see a little bit to the side, but these are places where you can go and talk to people who are doing other things that may be relevant to what you are doing or maybe in a couple of years down the road may be relevant, which is very important and keeps us diversified.

When I have students, and they come to conferences, I say, "Go talk to everybody, because you don't know where you're going to be in five years or you don't know what projects you will be working on once you leave me." They might say, "Oh, so-and-so has this. We could get it from there." Or someone may be willing to collaborate with you and say, "Let's run some samples. Let's see how this works," which moves the project forward and advances science.