A Review of Analytical Methods Used to Identify Fentanyl and Its Analogs

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Writer's Comment: Chemistry goes beyond the general and organic courses that people more frequently remember. So, as a fourth-year chemistry major when Dr. Melissa Bender assigned our UWP class a literature review which would cover a scientific topic of our choice, I immediately knew I wanted to cover a topic in the Chemistry field. I drew inspiration from my chemistry courses and spent several days narrowing down the focus of my paper. I ultimately made the decision to write about the chemical analysis of Fentanyl, which is extremely dangerous and poses a risk to first responders and the general public, in most cases unknowingly. I used the literature review as an opportunity to compare the different analytical methods that have been proposed to identify fentanyl in the field and how the research that has been conducted may not have accounted for factors in the field which cannot be controlled, but are normally controlled in a laboratory setting. I hope this review makes people consider how experiments may be adapted from a laboratory to a field setting.

Instructor's Comment: According to the Centers for Disease Control and Prevention, almost 450,000 people in the U.S. died as a result of opioid overdose between 1999 and 2018. Yet, while significant efforts to control the opioid epidemic have been made recently, and the overall opioid-related fatality rate decreased by 2% from 2017 to 2018, deaths caused by synthetic opioids, such as fentanyl, increased 10% in the same period. A reader need look no further than these statistics to appreciate the significance of Lacey Conlon's literature review topic. To turn this disturbing trend around and save lives, first responders need safe and efficient methods for detecting fentanyl and its analogues, while also protecting themselves. In this review, Lacey presents recent research on three such methods. Never losing sight of *the fact that these methods will be employed by human beings in the field, and under emergency circumstances, Lacey also addresses the crucial issue of training first responders to employ these methods optimally. There is much here for a novice literature review writer to emulate, as Lacey handles two of the most challenging aspects of writing a literature review—evaluating research articles and synthesizing material from various sources—with grace and clarity. Notably, Lacey met the usual, considerable challenges of writing a literature review during a time of additional complications—spring quarter 2020, when UC Davis courses had shifted quickly to online delivery due to the COVID-19 pandemic. Hats off, Lacey!*

—Melissa Bender, University Writing Program

Abstract

Fentanyl is a synthetic opioid similar to morphine, except that it is significantly more potent. Fentanyl has many analogs and all forms of fentanyl have raised concerns due to their involvement in illicit drug use since a few milligrams can cause a fatal overdose. The low lethal dose poses a risk for first responders, medical personnel, and law enforcement. More research needs to be conducted regarding the field instrumentation for the identification of fentanyl and its analogs. The purpose of this review was to identify the weaknesses in the most current instrumentation—Surface Enhanced Raman Spectroscopy, Ion Mobility Spectrometry, and Paper Spray Ionization-Mass Spectrometry—that has been proposed for the identification of fentanyl and its analogs when outside of a laboratory setting. Web of Science and PubMed databases were searched for peer-reviewed pieces that covered "fentanyl identification," "Surface Enhanced Raman Spectroscopy," "Ion Mobility Spectrometry," and/or "Paper Spray Ionization-Mass Spectrometry," and were published after 2010, while precedence was given to articles published in the last five years. In the field, the consistent result found for the three instrumentations was that researchers did not consider the effects that would be encountered outside the controlled setting of a laboratory, which may interfere with the reliability and reproducibility of the instruments' analysis. Lack of research involving possible environmental interference by researchers for the instrumentation in a field setting emphasizes the need for future research that addresses the performance of the instrumentation in the

analysis of fentanyl in the field.

Introduction

Fentanyl is a man-made opioid that was first synthesized in the late 1950s and is known to be significantly more potent than morphine. In the past few years, fentanyl and its analogs have raised concern for its illicit drug use (Kuczyńska, Grzonkowski, Kacprzak, & Zawilska, 2018). The concern stems from the low lethal dose of fentanyl since only a few milligrams can cause an overdose. The threat of an overdose from a trace amount of fentanyl or its analogs is hazardous for first responders, medical personnel, and law enforcement officers since illicit drugs have become more commonly contaminated with fentanyl and its analogs. This hazard concern has brought researchers to present effective analytical methods that can be made portable and that could identify fentanyl and its analogs in the field so that sufficient precautions can be taken to protect first responders, medical personnel, and law enforcement communities (Goodchild, et al., 2019). Traditionally implemented analytical methods, such as gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry, are generally used because of their known reliability and reproducibility. However, they require large amounts of the samples being tested, take a longer time to produce results, are cumbersome, expensive instruments that require a laboratory setting, and are not available in the field to help reduce the risk fentanyl poses (Haddad, Comanescu, Green, Kubic, & Lombardi, 2018).

More research is being conducted to find effective and court-admissible methods to identify fentanyl and its analogs when in the field (Haddad, Comanescu, Green, Kubic, & Lombardi, 2018). This review describes the research involved in three promising field identification methods: Surface-Enhanced Raman Spectroscopy, Ion Mobility Spectrometry, and Paper Spray Ionization-Mass Spectrometry. The research behind all three methods confirms the successful identification of fentanyl and its analogs. While the methods can successfully identify fentanyl and its analogs, there are cases where the analogs cannot be uniquely identified, or the portable method proposed does not account for the variability of the environment in the field. Further research needs to be conducted to improve field identification methods for fentanyl and its analogs.

Surface-Enhanced Raman Spectroscopy

Raman Spectroscopy utilizes laser light excitation that results in a Raman scattering of photons that reveal a molecule's vibrational energy states. Vibrational energy states are unique to a molecule, and their detection creates a spectral fingerprint for the molecule or molecules being examined that allows for the identification of molecules as well as its characteristics (Hoppmann, Yu, & White, 2014). Raman spectroscopy is known to be an effective method of analysis for illicit drugs. However, it is a weak method for distinguishing the materials when there are trace amounts. In order to compensate for the weak Raman scattering of photons, Surface-Enhanced Raman Spectroscopy (SERS) is used to permit the identification of pure fentanyl as well as fentanyl mixed with other drugs. When the AgPaper substrate was used in SERS, it successfully identified fentanyl by itself in addition to successful identification when it was in a mixture in trace amounts. This experiment was performed in a laboratory, but the portable Raman instrumentation has become more available, which would allow swabbing to facilitate the identification of fentanyl when in the field. Raman instrumentation is classified as paper-based, so it is considered to be a low-cost sampling and analysis method that is able to identify and confirm drugs of abuse at a rapid speed, which makes it ideal for usage in the field (Haddad, Comanescu, Green, Kubic, & Lombardi, 2018).

In a study, SERS was used to analyze fentanyl from common surfaces that would need to be examined in the field, including the inside of the plunger on a syringe, the inside of a plastic bag, the inside of a glass vial, the surface of a glass slide, the inside of a needle, and the surface of a razor blade. These surfaces were selected to best represent the reality of where fentanyl may be found in the field, and the analysis was proven to be effective (Fedick, Pu, Morato, & Cooks, 2020). This method provides a quick and effective analysis of illicit drugs, including fentanyl and its analogs, whether they be pure or in mixtures. While studies have proven SERS can be a successful analysis method for the identification of pure fentanyl and other fentanyl compounds, there are some obstacles the method faces that must be addressed. The method can produce false negatives due to the light in the environment where the sample is being tested. The study that was completed to validate the method was only completed in a controlled laboratory setting where interference of the environment can be eliminated within the setup of the experiment (Chen, et al., 2019). Further studies in SERS should be completed to improve the portability of the instrumentation and take into account where the methods may be used and what would enhance the effectiveness of the identification.

Ion Mobility Spectrometer

Ion Mobility Spectrometers (IMS) utilize the ionization of a sample to analyze the ions formed at the sample's environmental temperature and pressure. In the instrument, gas-phase ions are made through radioactive ionization, commonly by 63Ni. After the sample has been ionized, the resulting ions are separated by their different speeds in the instrument's drift gas, which is positioned in an electric field. The separation achieved by the IMS is based on the ion's mobility through the instrument and that depends on the weight of the ion, the charge of the ion, and finally its shape (Mäkinen, Anttalainen, & Sillanpää, 2010). IMS is a commonly used technique for the identification of illicit drugs because it is a rapid and sensitive technique that can be adapted for portable instruments (Chen, et al., 2019). IMS is not only an instrument that can efficiently be used in the field, but it is designed for a non-technical user to be able to detect trace amounts of illicit drugs, including fentanyl and fentanyl present in heroin (Verkouteren & Staymates, 2011).

IMS often faces challenges in analysis when there is an absence of a reference compound, and when samples that must be taken are complex mixtures that have a trace amount of fentanyl or its analogs. While the absence of reference compounds presents an obvious challenge for the detection of the analyte of interest, complex mixtures raise concern for the loss of signal for an analyte due to competitive ionization of compounds in the mixture. A study was conducted to determine if IMS analysis is a reliable method for detecting a single substance in a complex mixture of illicit drugs and determined that IMS was a reliable method for many commonly found controlled substances, which included fentanyl (Verkouteren & Staymates, 2011). Further studies were completed regarding IMS with a sole focus on fentanyl and its wide range of analogs. One study confirmed the successful identification of fentanyl and its analogs and suggested the sensitivity of IMS may allow wiping a bag for collection of a mere 10 nanograms of substance or less for successful identification of fentanyl (Sisco, Verkouteren, Staymates, & Lawrence, 2017). The presented possibility would allow the personnel trying to identify the substance to detect if fentanyl or its analogs are in a bag without having to open the bag and put themselves at greater risk of accidental exposure.

A subsequent study found that IMS could successfully identify fentanyl and its analogs through detection of trace amounts of samples that were taken from the exterior of a container, and established that the trace amounts found on the exterior of the bag were what was being kept in the container. This study established successful identification of fentanyl and its analogs using IMS with a sample size as small as 100 nanograms. However, the study also ran into an obstacle in the identification of the fentanyl compounds that were chosen for analysis. The study found that IMS could not uniquely identify all the fentanyl compounds outside of establishing the presence of fentanyl. Certain instrumental steps were proposed to improve the resolution of the instrument and updates that could be made to the library of data that provided the basis of analysis to improve the instrument's unique identification of the many existing fentanyl compounds (Verkouteren, Lawrence, Verkouteren, & Sisco, 2019).

Paper Spray Ionization-Mass Spectrometry

Mass Spectrometry (MS) is a well known and defined analytical technique that can identify nearly every element in the Periodic Table by measuring the mass-to-charge ratio of the ions produced from a sample that is introduced to the instrument (Skoog, Holler, Crouch, 2018). Paper Spray Ionization (PS) is a variant of electrospray ionization in which a paper substrate is loaded with the analyte of interest, sprayed with a solvent, and then a high voltage is applied to the paper substrate to yield ions (Meher & Chen, 2017). Paper Spray Ionization and Mass Spectrometry are coupled together for cost-effective and rapid detection of illicit drugs. PS is one of the simplest techniques for the introduction of a sample for a portable MS since it eliminates the need for time-consuming sample preparation and the use of a portable MS implies the need for quick and efficient identification of the substance in question (Fedick, et al., 2018). PS is one of many ambient ionization methods that can be coupled with MS, but it is the simplest to couple with a portable MS.

A study in that last few years validated PS-MS analysis for drug analysis because this form of ambient ionization mass spectrometry has high sensitivity. Surface swabbing is the preferred method of sample collection since the limit of detection ranges from a low- to mid-nanogram range, and although it is susceptible of user error, the error was found to be minor (Lawton, et al., 2016).

A recent study confirmed that PS-MS can directly identify fentanyl, its analogs, and its presence in a mixture. The study evaluated the quality of the paper substrate that is used in the paper spray ionization portion of the method and determined that it plays a role in the quantitative analysis of the substance. Since the paper substrate is used to load the miniature mass spectrometer in this method, the positioning of the paper also affects the quantitative analysis of the sample of interest (Vandergrift, Hessels, Palaty, Krogh, & Gill, 2018). Another study also confirmed that PS-MS is a high-resolution method that can screen, identify, and quantify fentanyl and its analogs. The study was performed in a laboratory and determined that high-resolution mass spectrometry and tandem mass spectrometry can identify drugs when references are not available (Kennedy, Palaty, Gill, & Wiseman, 2018). Although that is possible in a laboratory setting, the study does not establish if a reference is necessary for the portable method. Few research projects have been completed for PS-MS with a solitary focus on the identification and quantification of fentanyl and its analogs. Further research needs to be conducted on the effectiveness of the PS-MS analysis of fentanyl, the characteristics that affect the quality of the analysis in the laboratory, and whether more reference materials are needed to improve the number of fentanyl analogs that can effectively be analyzed. In addition, research should be conducted on the portability of PS-MS and how the environment affects the results of the analytical method.

Conclusion

Fentanyl is a synthetic opioid that was first created for medical use in treating pain and has since become a serious contributor to overdose in illicit drug use because of the minuscule amount that is necessary for the lethal dose. Since its development, fentanyl is now commonly found in mixtures of illicit drugs and it poses a risk to anyone who is coming into contact with it, especially since the contact is often accidental. The biggest communities that this drug puts at risk are first responders, medical personnel, and law enforcement communities. Several identification techniques for fentanyl and its analogs have been designed for field use to help reduce the risk of death for those who are coming into contact with substances that are suspected to contain fentanyl. Recent studies have suggested the use of Surface-Enhanced Raman Spectroscopy, Ion Mobility Spectrometry, and Paper Spray Ionization-Mass Spectrometry. These three methods have been established as effective methods of analysis for fentanyl and its analogs in pure form, as well as identification of them in the more commonly found mixtures of illicit drugs.

The three methods covered in this review are adaptable to portable instrumentation for analysis of fentanyl. This is an important characteristic of the method for it to be successful in the identification of fentanyl in the field. These methods are all sensitive and able to analyze the suspected fentanyl compound by swabbing or wiping the outer surface of the container that is storing the substance, rather than requiring the user to open the container and put themselves at greater risk of toxic exposure. SERS, IMS, and PS-MS need further research that considers how testing in the field may cause false-negatives or false-positives. While each method can successfully identify fentanyl and its analogs, they each need further research that considers the effect of testing in the field, the education and training of the instrument operator, and improvements that can be made to the portability of the instrumentation as well as the efficiency of the instrumentation in the analysis of fentanyl.

References

Chen, H., Chen, C., Huang, W., Li, M., Xiao, Y., Jiang, D., & Li, H. (2019). Miniaturized Ion Mobility Spectrometer with a Dual-Compression Tristate Ion Shutter for On-Site Rapid Screening of Fentanyl Drug Mixtures. *Analytical Chemistry*, 91(14), 9138–9146. https://doi.org/10.1021/acs.analchem.9b01700

Fedick, P., Fatigante, W., Lawton, Z., O'Leary, A., Hall, S., Bain, R., . . . Mulligan, C. (2018). A Low-Cost, Simplified Platform of Interchangeable, Ambient Ionization Sources for Rapid, Forensic Evidence Screening on Portable Mass Spectrometric Instrumentation. *Instruments*, 2(2), 5. https://doi.org/10.3390/

instruments2020005

Fedick, P. W., Pu, F., Morato, N. M., & Cooks, R. G. (2020). Identification and Confirmation of Fentanyls on Paper Using Portable Surface Enhanced Raman Spectroscopy and Paper Spray Ionization Mass Spectrometry. *Journal of the American Society for Mass Spectrometry*, 31(3), 735–741. https://doi.org/10.1021/jasms.0c00004

Goodchild, S. A., Hubble, L. J., Mishra, R. K., Li, Z., Goud, K. Y., Barfidokht, A., . . . Wang, J. (2019). Ionic Liquid-Modified Disposable Electrochemical Sensor Strip for Analysis of Fentanyl. *Analytical Chemistry*, 91(5), 3747–3753. https://doi.org/10.1021/acs.analchem.9b00176

Haddad, A., Comanescu, M. A., Green, O., Kubic, T. A., & Lombardi, J. R. (2018). Detection and Quantitation of Trace Fentanyl in Heroin by Surface-Enhanced Raman Spectroscopy.*Analytical Chemistry*, 90(21), 12678–12685. https://doi.org/10.1021/acs.analchem.8b02909

Hoppmann, E. P., Yu, W. W., & White, I. M. (2014). Inkjet-Printed Fluidic Paper Devices for Chemical and Biological Analytics Using Surface Enhanced Raman spectroscopy. *IEEE Journal of Selected Topics in Quantum Electronics*, 20(3), 195–204. https://doi.org/10.1109/jstqe.2013.2286076

Kennedy, J. H., Palaty, J., Gill, C. G., & Wiseman, J. M. (2018). Rapid Analysis of Fentanyls and Other Novel Psychoactive Substances in Substance Use Disorder Patient Urine Using Paper Spray Mass Spectrometry. *Rapid Communications in Mass Spectrometry*, 32(15), 1280-1286. https://doi.org/10.1002/rcm.8164

Kuczyńska, K., Grzonkowski, P., Kacprzak, Ł., & Zawilska, J. B. (2018). Abuse of Fentanyl: An Emerging Problem to Face. *Forensic Science International*, 289, 207–214. https://doi.org/10.1016/j.forsciint.2018.05.042

Lawton, Z. E., Traub, A., Fatigante, W. L., Mancias, J., O'Leary, A. E., Hall, S. E., . . . Mulligan, C. C. (2016). Analytical Validation of a Portable Mass Spectrometer Featuring Interchangeable, Ambient Ionization Sources for High Throughput Forensic Evidence Screening. *Journal of The American Society for Mass Spectrometry*, 28(6), 1048–1059. https://doi.org/10.1007/s13361-016-1562-2

Mäkinen Marko A., Anttalainen, O. A., & Sillanpää Mika E. T. (2010). Ion Mobility Spectrometry and Its Applications in Detection of Chemical Warfare Agents. *Analytical Chemistry*, 82(23), 9594–9600. https://doi.org/10.1021/ac100931n

Meher, A. K., & Chen, Y.-C. (2017). Electrospray Modifications for Advancing Mass Spectrometric Analysis. *Mass Spectrometry*, 6(2). https://doi.org/10.5702/massspectrometry.s0057

Sisco, E., Verkouteren, J., Staymates, J., & Lawrence, J. (2017). Rapid Detection of Fentanyl, Fentanyl Analogues, and Opioids for On-site or Laboratory-based Drug Seizure Screening Using Thermal Desorption DART-MS and Ion Mobility Spectrometry. *Forensic Chemistry*, 4, 108–115. https://doi.org/10.1016/j.forc.2017.04.001

Skoog, D. A., Holler, F. J., Crouch, S. R. (2018). Atomic Mass Spectrometry. In *Principles of Instrumental Analysis*. Singapore: Cengage Learning Asia Pte Ltd.

Vandergrift, G. W., Hessels, A. J., Palaty, J., Krogh, E. T., & Gill, C. G. (2018). Paper Spray Mass Spectrometry for the Direct, Semi-quantitative Measurement of Fentanyl and Norfentanyl in Complex Matrices. *Clinical Biochemistry*, 54, 106–111. https://doi.org/10.1016/j.clinbiochem.2018.02.005

Verkouteren, J. R., & Staymates, J. L. (2011). Reliability of Ion Mobility Spectrometry for Qualitative Analysis of Complex, Multicomponent Illicit Drug Samples. *Forensic Science International*, 206(1-3), 190–196. https://doi.org/10.1016/j.forsciint.2010.08.005

Verkouteren, J. R., Lawrence, J., Verkouteren, R. M., & Sisco, E. (2019). Method for Evaluating Ion Mobility Spectrometers for Trace Detection of Fentanyl and Fentanyl-related Substances. *Analytical Methods*, 11(47), 6043–6052. https://doi.org/10.1039/c9ay02174d