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**Technical Report** · March 2023

DOI: 10.13140/RG.2.2.24838.86089

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# Forensic Toxicology of Fentanyl

*An Analytical Study of the Chemical Composition of Fentanyl and Its Application in Forensic Research with Gas Chromatology – Mass Spectrometry (GC-MS) Analysis and the Implications on the British Columbia Opioid Crisis*

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There is no conflict of interest to report

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**Abstract**

This study investigates the function of forensic toxicology in the identification of fentanyl, a potent synthetic opioid that has significantly increased drug overdose fatalities worldwide. In addition to examining the pharmacology and chemical composition of fentanyl, the study also investigates the analytical techniques used to find the drug in biological samples, such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The study underlines the significance of fentanyl detection which is accurate and reliable in forensic toxicology as well as its relevance in examining drug-related mortality. To pinpoint the drug that caused a drug-related fatality, ascertain the cause of death, and present evidence in court, forensic toxicology is essential. Fentanyl is frequently discovered during post-mortem investigations, and the frequency with which it occurs in opioid overdose deaths poses a substantial challenge for forensic toxicology. Fentanyl must be found in biological samples such as blood, urine, or hair to determine the cause of death with accuracy.

Due to its great sensitivity and selectivity, GC-MS is one of the most widely used analytical techniques for the detection and quantification of fentanyl and its analogues. To determine the cause of death in post-mortem examinations, GC-MS analysis can be used to quantify the amount of fentanyl and its analogues present in biological materials.

However, the cost of the necessary equipment and supplies may prevent some forensic labs from using GC-MS analysis due to the specialized training and understanding required. Particularly when analyzing complex biological materials, GC-MS analysis can produce false positives and false negatives, which can lead to erroneous results and poor data interpretation.

Analysts need a strong academic background, certification, and ongoing professional development to perform GC-MS analysis in a forensic lab.

Forensic toxicologists must adhere to strict protocol, exercise extreme quality control, and possess strong analytical and communication skills due to the delicate nature of their work.

This study highlights the value of forensic toxicology in identifying fentanyl, a very strong synthetic opioid that is linked to an upsurge in drug overdose mortality worldwide. To accurately determine the cause of death, fentanyl must be detected accurately and reliably in biological samples utilizing analytical techniques like GC-MS and LC-MS/MS. To work in a forensic lab and conduct GC-MS analysis, forensic toxicologists must have a strong academic basis, certification, and ongoing professional development. Notwithstanding its drawbacks, GC-MS is a potent analytical technique that offers accurate and comprehensive information on the biological sample composition and is essential to forensic analyses and public health programs aimed at addressing the opioid crisis.

**Acknowledgements**

I am honoured to extend my heartfelt appreciation to Suzanna Michener, my dedicated and knowledgeable forensic sciences instructor at Simon Fraser University. Michener's expertise and passion for the subject matter have been instrumental in my academic growth and success in the course CRIM 355: Forensic Science. Her teaching methods and commitment to students' learning have been an inspiration to me, and I feel incredibly fortunate to have had her as my instructor.

I would also like to express my gratitude to Cass Chowdhury, my exceptional teacher assistant for the course. Chowdhury's insightful feedback and guidance have been invaluable to me throughout the semester. Their extensive knowledge in the field of forensic sciences has not only deepened my understanding of the course material but has also sharpened my critical thinking and analytical skills. Moreover, I would like to acknowledge the faculty and staff of SFU's forensic sciences program for creating a challenging and stimulating learning environment. The program's curriculum, with its focus on practical, hands-on experience and exposure to current research, has provided me with a solid foundation in the field of forensic sciences. The courses I have taken in the program have been instrumental in preparing me for a future career in the field.

Last but not least, I am grateful for the unwavering support and encouragement of my family and friends. Their faith in me has been an invaluable source of inspiration and strength throughout my academic journey. Their constant support has motivated me to work harder and strive for excellence in all aspects of my studies. I would like to extend my sincere appreciation to all those who have supported me in my academic journey in forensic sciences. Their contributions have been crucial to my success, and I am deeply grateful for their unwavering support and guidance.

## The Power of Fentanyl: An Analytical Study of Its Chemical Composition for Forensic Investigations

Drug overdose fatalities have increased significantly because of the opioid crisis, which has become a serious public health concern globally, including in British Columbia (BC), Canada. It has been determined that the main cause of these fatalities is the synthetic opioid fentanyl, which is more potent than morphine and heroin (Ciccarone, 2019). To increase its strength, fentanyl is frequently mixed with other narcotics like cocaine, methamphetamine, or heroin. Although establishing the cause of death in these cases is dependent on reliably detecting the presence of fentanyl in biological specimens, the rising prevalence of fentanyl in opioid overdose deaths has become a significant problem for forensic toxicology.

The scientific study of how drugs and other harmful substances affect living things is known as forensic toxicology. To identify the presence of drugs or other harmful compounds, biological materials like blood, urine, or hair are analyzed. When looking into drug-related deaths, forensic toxicology is essential since it helps identify the drug that was used, determine the cause of death, and offer evidence in court.

This study aims to investigate fentanyl's forensic toxicology and how its chemical make-up is used in forensic investigations. The study looks at the pharmacology of fentanyl, its chemical makeup, and the different ways it can be found in biological samples. The study also emphasizes the significance of fentanyl's accurate and reliable detection in forensic toxicology as well as the relevance of forensic toxicology in the investigation of drug-related fatalities.

### Overview

Often used to treat severe pain, fentanyl is a synthetic opioid that is 50–100 times more powerful than morphine. Fentanyl is a powerful narcotic, but it is also abused, and individuals

who use it recreationally or without a prescription run the risk of death. Fentanyl overdose deaths have increased because of the rising prevalence of fentanyl usage, prompting significant public health concerns. In post-mortem examinations, forensic toxicology is essential for finding and identifying fentanyl. (Drug Enforcement Agency, n.d).

Forensic toxicology studies depend heavily on the identification and examination of fentanyl in biological materials. Blood, urine, and hair are just a few of the biological samples where fentanyl can be found. The most typical sample for fentanyl analysis is blood since it offers a snapshot of the drug's concentration at the moment of death. Samples of your urine and hair may provide longer-term drug use information. Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and immunoassays are among the analytical methods used to detect fentanyl. (Valdez et al., 2022).

The most effective method for finding fentanyl in biological materials is GC-MS. The process entails utilizing gas chromatography to separate the components in a sample and mass spectrometry to identify them. This method is frequently used to confirm the presence of fentanyl in a sample since it offers great specificity and sensitivity. (Chen & Shi, 2022). Another method for finding fentanyl in biological materials is LC-MS/MS. In this method, the chemicals in a sample are separated using liquid chromatography before being detected using mass spectrometry. More sensitive than GC-MS, LC-MS/MS is frequently used to calculate the concentration of fentanyl in a sample (Chen & Shi, 2022).

To quickly check for the presence of fentanyl in biological samples, immunoassays are used. Although less sensitive and specific than GC-MS and LC-MS/MS, these techniques are nonetheless effective for preliminary screening. When immunoassay testing yields a positive result, GC-MS or LC-MS/MS confirmation is required (Angelini et al., 2019).

It can be difficult to interpret the findings in cases where fentanyl-related deaths occurred, therefore having a solid grasp of pharmacology and toxicology is crucial (Guerrieri et al., 2017). Fentanyl levels in a sample might not accurately represent the cause of death. Results must be interpreted in light of factors like tolerance, the use of additional medicines, and individual variances in metabolism. Also, the cause of death may not always be determined by the presence of fentanyl in a sample. For instance, the presence of fentanyl in a post-mortem sample may not be significant to the cause of death if it was prescribed for pain management. Nonetheless, the cause of death can be attributed to drug toxicity if fentanyl is found in conjunction with other drugs, including benzodiazepines (Angelini et al., 2019).

**Qualifications and Education for GC-MS Forensic Toxicology**

Gas chromatography-mass spectrometry (GC-MS) measurement of fentanyl is a challenging operation requiring substantial skill and knowledge. To complete the analysis correctly and efficiently, one needs a strong academic background in chemistry or a closely connected discipline due to the technical nature of the work (Han et al., 2017).

To work in a forensic laboratory, one must possess at least a bachelor's degree in chemistry, biochemistry, or a closely related discipline (Alberta Learning Information Service, n.d.). Many companies also prefer candidates with doctoral degrees in forensic science, analytical chemistry, or pharmacology (Alberta Learning Information Service, n.d.). In addition to having a good academic background, analysts need to be skilled in using analytical tools like GC-MS and have had thorough training in handling and interpreting biological materials (Smith et al., 2007).

Most organizations demand certification from the American Board of Forensic Toxicology (ABFT) or comparable certification bodies to hire forensic toxicologists.

Professionals in the field of forensic toxicology who satisfy the eligibility standards, including as education, training, and work experience, can obtain certification from the ABFT, a nonprofit organization (Gluodenis, 2021). The analyst has proven their professional and technical competency by earning their certification in the field of forensic toxicology. Candidates must maintain their certification through continuing education and ongoing professional development to keep it. The certification procedure often entails a written exam, a practical exam, and an oral exam. For employment as a forensic toxicologist, many firms demand certification from ABFT or other certification bodies (Melbourn et al., 2019).

A forensic laboratory job also calls for specific attention to detail, critical thinking abilities, and the capacity to function in a fast-paced setting with rigorous adherence to quality control and safety procedures (National Research Council, 1985). To effectively explain their findings in both written and spoken reports, forensic toxicologists must be able to analyze, interpret, and summarize complex data. Due to the complex nature of fentanyl analysis employing GC-MS, a thorough understanding of chemistry, pharmacology, and forensic science is required (National Research Council, 1985). Forensic toxicologists must have a good academic foundation, certification, and continual professional development. Due to the sensitive nature of their work, forensic toxicologists are required to uphold rigorous protocol, high levels of quality control, and analytical and communication abilities.

## Chemical Composition of Fentanyl

Fentanyl is a member of the opioid medication class, which works by interacting with brain and nervous system receptors to relieve pain and have additional effects. A phenethylamine group coupled to a piperidine ring and an N-phenyl-N-propionamide group make up the

chemical structure of fentanyl (National Center for Biotechnology Information, 2023). Several psychotropic compounds share the phenethylamine group, including amphetamines and phenethylamines. It is made composed of a primary amine group linked to a benzene ring with a two-carbon side chain. It is thought that adding a methyl group to the alpha position of the phenethylamine group in fentanyl will increase the drug's potency (National Center for Biotechnology Information, 2023).

Many pharmacological drugs contain the six-membered nitrogen-containing piperidine ring. The propionyl group is replaced at the nitrogen atom of the piperidine ring in fentanyl (National Center for Biotechnology Information, 2023). This change is expected to make the drug more lipophilic, which makes it easier for it to pass through the blood-brain barrier and have an impact on the central nervous system. Fentanyl has a special N-phenyl-N-propionamide group that is thought to be largely responsible for the drug's potency. This group is made up of a propionyl group and a phenyl ring connected by an amide bond. To relieve pain and have other benefits, it is thought that the phenyl ring interacts with certain receptors in the brain and nervous system.

Being highly lipophilic, fentanyl can dissolve in lipid- or fat-based compounds (Vardanyan & Hruby, 2014). Because of this characteristic, fentanyl can easily pass across the blood-brain barrier and affect the central nervous system. In comparison to morphine, which has a therapeutic dose range of 30–60 milligrams (United States Food and Drug Administration, 2010), fentanyl is likewise quite strong, having a range of 0.5-2 micrograms per kilogram of body weight (Nova Scotia Department of Health and Wellness, 2015).

The liver is the organ that metabolizes fentanyl the fastest, with N-dealkylation turning the majority of the drug into inert metabolites. Norfentanyl, one of the main metabolites of

fentanyl, is still capable of causing opioid effects and can be found in biological samples after fentanyl consumption (Smith, 2009).

Moreover, fentanyl has a short half-life, with an elimination half-life of 2-4 hours. This indicates that fentanyl leaves the body quickly, making it challenging to detect in biological samples after a short time (Huhn, Hobelmann, Oyler, & Strain, 2020).

**Gas Chromatography-Mass Spectrometry (GC-MS) of Fentanyl**

One of the most popular analytical methods for the detection and quantification of fentanyl and its analogues is gas chromatography-mass spectrometry (GC-MS) (Misailidi et al., 2019). For the detection of fentanyl, GC-MS provides great sensitivity and selectivity in addition to enabling the recognition of structurally similar compounds in complicated matrices (Piechocka et al., 2020). In-depth information about the technical steps, chemical make-up, and formulae used in the GC-MS analysis of fentanyl will be provided in this section.

There are many strengths to the GC-MS of fentanyl. Even in small levels, fentanyl and its analogues can be detected and identified using GC-MS analysis, which is a very sensitive and selective technology (Krauss et al., 2019). This is especially helpful in forensic investigations because fentanyl can be identified in trace levels in biological samples (Krauss et al., 2019). GC-MS analysis can offer quantitative information on the quantity of fentanyl and its analogues present in biological samples, which can be helpful in establishing the cause of death in post-mortem investigations (Valdez, 2022).

The United States Drug Enforcement Agency (DEA) and other regulatory agencies promote the use of GC-MS analysis as an analytical technique in forensic toxicology (Drug Enforcement Administration, 2019). But with so many advantages, there are several

disadvantages which I will outline.

The price of the necessary tools and consumables for GC-MS analysis might be prohibitively high (LabioAnalytics, n.d.), which may restrict its use in some forensic labs. Accurately performing and interpreting the results of GC-MS analysis, a difficult analytical technique, calls for specific training and knowledge. GC-MS analysis can only recognize recognized fentanyl analogues (United Nations Office on Drugs and Crime, 2018), which may limit its applicability in identifying novel and emerging analogues ("*Identification of Novel Psychoactive Substances*," 2012). In addition, the analysis of complicated mixtures with GC-MS can take a long time, which could limit its applicability in emergency situations. False positives and false negatives can occur during GC-MS analysis (Wu, 1995), especially when examining complex biological materials, which can result in unreliable results and incorrect data interpretation.

Due to its great sensitivity and specificity, GC-MS is a powerful analytical technology that enables the precise identification and quantification of chemical constituents in complicated matrices (Kałużna-Czaplińska, 2011). This technique has emerged as a crucial tool in the forensic examination of fentanyl (Valdez, Leif, Corzett, & Dreyer, 2022), a strong synthetic opioid that has been linked to an increase in overdose fatalities (National Institute on Drug Abuse, 2021). Due to the great strength of fentanyl and its analogues, detecting them requires very sensitive and focused analytical methods (Milone, 2012). Trace levels of fentanyl in biological samples can be analyzed by GC-MS (Wei & Su, 2022), giving crucial data for post-mortem examinations and monitoring of illicit drug use (Lehrmann et al., 2008). Even in the presence of interfering substances, the GC-MS combination allows for the precise separation and detection of fentanyl molecules (Valdez et al., 2022).

GC-MS is a potent analytical method for the investigation of fentanyl and other complex chemical compounds due to its sensitivity, specificity, and adaptability (Fayed, Kohder, & Soliman, 2022). It is a useful tool in forensic investigations and public health initiatives to combat the opioid issue since it can give precise and in-depth information about the biological sample composition (Ciobanu et al., 2015).

A separation method called gas chromatography divides volatile chemicals based on how they are distributed between a stationary phase and a moving gas phase ("*Gas Chromatography*," 2020). The chromatograph is used to vaporize and insert the analyte-containing material into the column ("*Gas Chromatography,*" 2020). A stationary phase coated on a long, narrow tube makes up the column. As the sample components move through the column, they encounter the stationary phase. The polarity and boiling points of the analyte components are used to separate them, and the strength of their interactions with the stationary phase determines the order of elution ("*Gas Chromatography*," 2020).

Typically, a capillary column coated with a nonpolar stationary phase, such as polydimethylsiloxane (PDMS) or polyethylene glycol (PEG), is used to separate fentanyl and its analogues (Chan et al., 2020). The exact analytes and sample matrix under investigation will determine the column and stationary phase to be used. Due to its low polarity and high volatility, fentanyl analysis calls for a nonpolar stationary phase (Chan et al., 2020).

**Mass Spectrometry (MS) Analysis of Fentanyl**

A powerful analytical method called mass spectrometry ionizes substances and separates them according to their mass-to-charge ratio (m/z) ("*What is Mass Spectrometry*," n.d.). By using an electron beam to ionize and evaporate the sample's constituent parts, the produced ions are

then accelerated by an electric field and separated by a magnetic field ("*Mass Spectrometry*", n.d.). A mass spectrometer then detects the ions and logs the ion current at each m/z ratio. Molecular ions [M]+ and fragment ions [M-CH3]+ and [M-CH2CH2]+ are the main products of electron ionization (EI), the most popular ionization method for fentanyl analysis (Nan et al., 2020). Fentanyl and its analogues have well-characterized EI spectra that can be utilized to locate and measure these substances in intricate matrices. Fentanyl's normal ionization energy is 70 eV, which allows for good molecular fragmentation while limiting thermal degradation (Feeney, Moorthy, & Sisco, 2020, Wichitnithad et al., 2010).

## Technical Procedure of GC-MS Fentanyl Analysis

Preparation of the sample, injection, separation, and detection are all phases in the GC-MS analysis of fentanyl (Malkawi et al., 2008). A thorough explanation of each step in the process is provided below:

### Sample Preparation

An essential stage in the analysis of fentanyl using gas chromatography-mass spectrometry (GC-MS) is sample preparation (Malkawi et al., 2008). Before being injected into the GC-MS instrument, the analyte must be extracted from the biological matrix and purified (Baumer et al., 2020). This step is essential to getting exact and accurate findings. The purpose of sample preparation is to concentrate the target analyte while removing interfering elements ("*Analytical Sciences Digital Library*," n.d.). When analyzing fentanyl, the biological matrix may contain a variety of endogenous and exogenous chemicals that could obstruct the results. They include any possible proteins, lipids, salts, and other medicines in the sample (de Campos et al., 2022).

Several sample preparation methods have been developed to remove fentanyl from the biological matrix (Wei & Su, 2022).. They include protein precipitation, solid-phase extraction (SPE), and liquid-liquid extraction (LLE) (Wei & Su, 2022). Fentanyl can be extracted from biological matrices using the widely used technique of liquid-liquid extraction (Tikhomirov et al., 2021). The procedure entails mixing an organic solvent with the sample, which causes the analyte to partition into the organic phase. After being separated from the aqueous phase, the organic phase is dried by evaporation before being reconstituted in a suitable solvent for injection into the GC-MS system. For the liquid-liquid extraction of fentanyl, common organic solvents include hexane, ethyl acetate, and chloroform (Hammad et al., 2022).

Another popular technique for removing fentanyl from biological matrices is solid-phase extraction (Mulder & Halquist, 2021). Using a solid-phase sorbent, this technique selectively preserves the analyte while removing the interfering elements. After applying a suitable solvent to elute the analyte from the solid-phase sorbent, the eluate is dried before being reconstituted in a suitable solvent for injection into the GC-MS instrument (D'Ovidio et al., 2022). C18 and Oasis HLB are typical solid-phase sorbents used for SPE of fentanyl (Gao, Guo, Du, & Gu, 2015). Fentanyl is easily and quickly removed from biological matrices using protein precipitation (SCIEX, 2019). The procedure is mixing a sample with an organic solvent, like acetonitrile or methanol, which causes proteins to precipitate (Polson et al, 2003). After centrifuging the sample to remove the protein precipitate, the supernatant is dried by evaporation before being reconstituted in an appropriate solvent for injection into the GC-MS instrument (Liakh et al., 2019).

To get the best recovery and the fewest matrix effects, it is crucial to optimize the extraction conditions regardless of the sample preparation technique utilized. The kind and

quantity of the organic solvent, pH, and the presence of salts and other interfering compounds are all variables that can affect how effective the extraction is (Jiang et al., 2012).

**Injection**

As the material is introduced into the gas chromatography column during injection, it is a crucial step in the GC-MS analysis. The fundamental goal of injection is to minimise sample deterioration or contamination while introducing the sample into the GC column as a narrow, repeatable band. The use of the proper injection technique is essential for attaining consistent and precise outcomes (Wei et al., 2010).

Split injection is the technique used to inject samples most frequently. Split injection involves sending part of the sample into the GC column and the rest to the waste port. In order to avoid overloading the column, which may result in peak distortion or resolution loss, this is done (Chasteen, 2000).

Splitless injection is a different injection technique in which the complete sample is injected into the GC column. For trace-level analysis, this approach is favoured since it can raise the limit of detection. However, if the sample concentration is too high, it may result in column overload. Prior to injecting the sample, the injection port temperature is normally held at a high temperature, often between 250 and 300 °C, for several seconds. This is done to ensure full vaporization of the sample and to stop the sample solvent from condensing in the injection port. ("*Benefits of Split-Splitless Injection*," 2014)

The injection volume should also be considered. A volume that is either small or too great can overwhelm the column or result in a poor signal-to-noise ratio (Gathungu et al., 2020). The sample matrix may influence the parameters and injection technique selection because some matrices may call for more delicate or exact injection methods (Cortese et al., 2020). To increase

the signal-to-noise ratio, for instance, a large-volume injection may be employed if the sample matrix contains a lot of interfering substances (SWGTOX, 2013). The injection port needs to be properly maintained in addition to the injection technique and settings. To avoid the accumulation of sample residues or other impurities that can compromise the repeatability of the injection, the injection port should be cleaned often. To avoid sample contamination or adsorption, the septum, which closes the injection port, should also be changed on a regular basis (Marsol-Vall, Balcells, Eras, & Canela-Garayoa, 2016).

**Separation**

In GC-MS analysis, the separation stage is crucial because it divides the various chemical components of the sample mixture, enabling precise identification and quantification. Due to the existence of other chemical components that may hinder the detection and identification of fentanyl, separation is crucial in the study of fentanyl (Harper et al., 2017). In GC-MS analysis, a stationary phase—typically a coated capillary column—and a mobile phase—typically a carrier gas—are used to separate the components (Khan Academy, n.d.). The sample components interact with the stationary phase, a solid or liquid coating on the inside of the column, as they move through it. The sample is forced through the column by the mobile phase, which is the carrier gas, and the separation is completed by a combination of adsorption, partitioning, and/or size exclusion (Bioglobax, 2018). For the separation of the various components of the sample mixture, the selection of stationary phase and mobile phase is crucial (Coskun, 2016).

The choice of stationary phase and mobile phase in a fentanyl analysis will depend on the sample matrix, the anticipated fentanyl concentration, and any additional chemical components that may be present in the sample (Harper, Powell, & Pijl, 2017). Polydimethylsiloxane (PDMS), phenyl, and cyano columns are frequently employed as stationary phases for the study

of fentanyl (Greyhound Chromatography and Allied Chemicals, 2017), whereas helium, nitrogen, and hydrogen are frequently utilised as carrier gases (Peak Scientific, 2021). For each individual analysis, the column and carrier gas should be tuned because they might have a big impact on the separation's sensitivity and resolution ("*Gas Chromatography*," 2020).

Upon injection, the sample is propelled through the column by the carrier gas, where it is divided into its constituent parts based on how they interact with the stationary phase ("*Analytical Toxicology*," 2017). The characteristics of the stationary and mobile phases will influence the typical retention time at which fentanyl and its metabolites will elute from the column (Strayer et al., 2018). The components of fentanyl in the sample mixture are identified and measured using retention time (Strayer et al., 2018).

The column temperature, the flow velocity of the carrier gas, and the injection volume are some additional variables that may influence the separation process in addition to the stationary and mobile phases (New Jersey Institute of Technology, n.d.). A crucial factor that affects how the sample components interact with the stationary phase is the column temperature (New Jersey Institute of Technology, n.d.). To make sure that every component is eluted from the column throughout the analysis, the temperature is often ramped up (Chromatography Online, 2010). The speed at which the sample components pass through the column depends on the flow rate of the carrier gas, which is also significant. The analysis's sensitivity can also be influenced by the injection volume; bigger injection volumes often result in greater sensitivity but also increased danger of column overload and loss of resolution (Wilson, Groskreutz & Weber, 2016).

**Detection**

In addition to the stationary and mobile phases, other factors that may affect the separation process include the column temperature, the carrier gas's flow velocity, and the

injection volume (Zhang, Zhang, Zhou, & Qi, 2020). The column temperature has a significant impact on how the sample components interact with the stationary phase (Hao, Xiao, & Weng, 2008). The temperature is frequently cranked up to ensure that every component is eluted from the column throughout the study. The flow rate of the carrier gas is also important since it affects how quickly the sample components go through the column. The injection volume can affect the sensitivity of the analysis; larger injection volumes increased sensitivity but also increase the risk of column overflow and loss of resolution (Zhang, Zhang, Zhou, & Qi, 2020).

Electron impact ionization (EI), which involves bombarding the molecules of the analyte with high-energy electrons to create ions, is the most popular ionization technique used in GC-MS. Each analyte produced by EI ionization exhibits a distinctive fragmentation pattern that can be utilised to identify it (McIndoe, 2018).

Chemical derivatization is frequently employed to increase the volatility and stability of the analytes in order to improve the sensitivity and selectivity of GC-MS analysis (Halket et al., 2005). For instance, by creating more stable and volatile derivatives, a derivatizing agent like trifluoroacetic anhydride (TFAA) can enhance the detection of fentanyl and its analogues (Vardanyan & Hruby, 2014). The goal of the derivatization reaction is to create a more stable, volatile, and GC-MS-compatible derivative by reacting the analyte with the derivatizing agent (Halket et al., 2005).

The choice of ionization mode is a crucial factor in GC-MS detection. Fentanyl and its analogues can be found using soft ionization techniques in addition to electrospray ionization (ESI) and chemical ionization (CI) (Sekuła et al., 2022). Soft ionization techniques yield fewer fragments than EI does, which can enhance the analysis's sensitivity and selectivity (De Vijlder et al., 2018).

**Conclusion**

Investigations into drug-related deaths require forensic testing, especially when fentanyl is present (Morgan, 2017). Overdose deaths from fentanyl, a synthetic opioid that is substantially more potent than morphine, are a major cause of death worldwide, including in British Columbia, Canada (Belzak & Halverson, 2018). By analyzing biological substances including blood, urine, and hair, forensic toxicologists can detect and check for fentanyl (Salomone et al., 2020). The main analytical techniques to find fentanyl in biological samples are gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and immunoassays (Zhang et al., 2022). The opioid problem is a serious public health issue, and the usage of fentanyl has significantly increased in recent years, contributing to a considerable rise in overdose deaths (Health Canada, n.d.). Investigating these cases, identifying the drug used, confirming the cause of death, and producing evidence in court are all made possible by forensic toxicology (Argo et al., 2022). It is crucial to identify fentanyl accurately and consistently in biological materials, and GC-MS is the most potent technique for doing so (Wei & Su, 2022). The results can be difficult to interpret, though, and the presence of fentanyl in a sample may not always indicate the cause of death. It is important to consider tolerance, other drug use, and individual metabolic variations.

Since novel fentanyl analogues and derivatives are found and there is a growing need for more sensitive and focused analysis techniques, the science of fentanyl analysis is continually changing. The goal of current research is to create analytical techniques that can quickly, accurately, and reliably detect even minute amounts of fentanyl and its analogues in biological samples. (Vardanyan & Hruby, 2014).

The creation of alternate sample preparation techniques that can speed up and simplify analysis is one area of focus. One such technique is the use of microextraction techniques, which, in comparison to conventional sample preparation techniques, can extract fentanyl and its equivalents from smaller sample volumes and with fewer steps (Kataoka et al., 2014). The creation of more specialized and sensitive detection techniques is another area of focus. Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) is one such technique that can offer more precise identification and quantification of fentanyl and its analogues (Vincenti et al., 2021).

Another expanding field of fentanyl analysis research is the creation of portable analytical tools (U.S. Department of Homeland Security, 2022). In cases of suspected fentanyl overdoses, these tools, including handheld mass spectrometers, can provide speedy on-site analysis and speed up decision-making (U.S. Department of Homeland Security, 2022). But, as more fentanyl analogues are found, it's possible that present analytical techniques won't be able to detect them. Forensic laboratories will have a difficult time fast adapting and creating new techniques to find these analogues (National Institute of Justice, 2019). In addition, law enforcement and forensic scientists are faced with a new dilemma due to the growing availability of fentanyl and its analogues on the illicit market (Australian Institute of Criminology, 2021). As a result, there is a drive for increased cooperation between forensic laboratories and law enforcement in order to share information and boost the success of fentanyl detection attempts (U.S. Department of State, 2018).

It is impossible to overestimate the importance of forensic toxicology in examining drug-related mortality (National Institute of Justice, 1992). To detect and check for the presence of fentanyl, numerous analytical procedures are used to analyse biological materials such blood, urine, and

hair (Guerrieri et al., 2017). A thorough knowledge of pharmacology and toxicology is required for the interpretation of the results, which can be difficult. As the opioid crisis shows no indications of ending, it is crucial to keep researching and developing new and improved forensic toxicology techniques to effectively address this serious public health issue (Public Health Ontario, 2021). In order to address the opioid crisis, the future of fentanyl analysis is likely to require the development of more discerning and sensitive analytical techniques as well as more cooperation between law enforcement and forensic labs (Canada. Parliament. House of Commons. Standing Committee on Health, 2019).

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