The Sydney Journal of Interdisciplinary Engineering

SJIE 1(5) X-X (2025) https://doi.org/XXXX/XXXX

Illicit drug purification: Detection and removal of fentanyl analogues from narcotics supply chains

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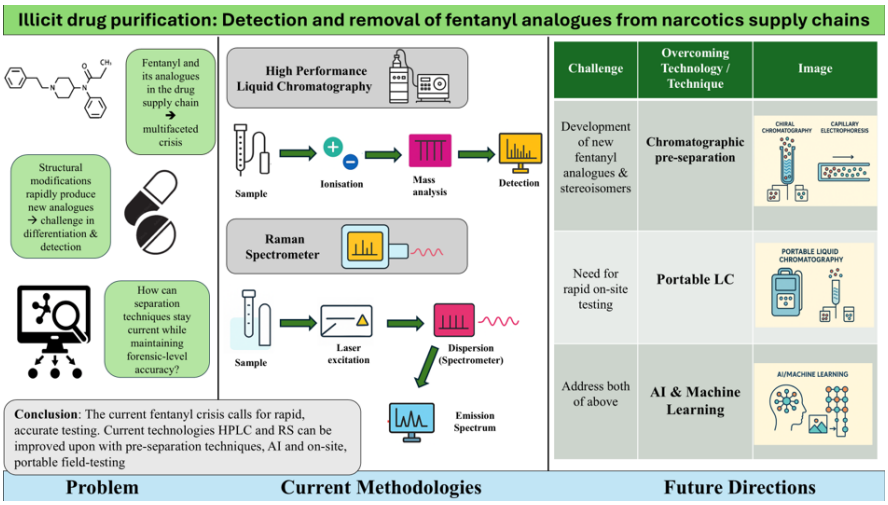
Received xxxxxx
Accepted for publication xxxxxx
Published xxxxxx

Graphical Abstract



Abstract

Recently, the rate of unintentional overdose deaths in Australia has far surpassed that of population growth, reflecting a public health crisis partially driven by illicit production of fentanyl and analogues, which demand rapid and reliable separation for detection and removal. This review critically investigates current and emerging techniques for the detection of compounds, considers social and legal implications, and examines the potential of Artificial intelligence (AI) to transform fentanyl detection from a reactive to predictive science. Presumptive field methods such as Raman spectroscopy and fentanyl test strips provide near-immediate, qualitative results, aiding emergency response, while laboratory techniques, including coupled mass spectrometry and chromatography, offer superior quantitative precision. Fentanyl's structural flexibility enables the continual emergence of novel analogues, challenging identification, which relies on preexisting compound libraries,

SJIE 1(5) X-X (2025) https://doi.org/XXXX/XXXX

making detection impossible in the field and time-consuming in laboratories. AI offers a promising solution, whereby convolutional neural networks and machine learning can identify and predict unknown compounds. Emerging advancements focus on developing accurate algorithms and improving field-deployable chromatographic systems to ensure forensic-level accuracy. By bridging analytical chemistry, AI, and health policy, separation strategies have the potential to enhance detection and removal of fentanyl analogues, mitigating societal and individual harms of synthetic opioids.

Keywords: Fentanyl detection; Fentanyl analogues; Separation techniques; Mass spectrometry; Artificial intelligence; Public health; Illicit drug detection; Machine learning; Mass Spectrometry; High Performance Liquid Chromatography; Immunoassays; Raman Spectroscopy

1. Introduction

1.1 Context: Illicit Fentanyl Analogues - A crisis

The proliferation of fentanyl and its analogues in the illicit drug supply chain have triggered a multifaceted crisis around the world, fuelling the need for sophisticated separation processes, regulation, healthcare policies, and harm reduction strategies to effectively combat this issue. Fentanyl is a highly potent synthetic opioid (depressant) that is 50-100x more potent than morphine,[1] often used in a pharmaceutical setting for severe pain management, anaesthesia, and analgesia. Fentanyl misuse and illicit manufacturing has been a prevalent issue since the 1970s,[2] and in recent years structural variations on the main drug, otherwise known as analogues, are becoming increasingly apparent due to lower costs and stronger potencies. One such example, carfentanil, a common analogue is 50 times stronger than fentanyl.[2] Fentanyl and its analogues all share the same core structural characteristics, comprising of a piperidine ring, amide group, N-Phenyl group (aniline), and phenethyl group. Disparities between analogues resultingly arise from interchanging functional group substituents at these 4 key bonding sites, driving a significant challenge in specific differentiation between analogues and the identification of new ones, as structural modifications can be easily and rapidly generated. Historically, fentanyl analogues have been 'cut' into other drugs,[1] enhancing drug strength, and creating powerful, potentially lethal biological interactions when being unknowingly ingested. This fuelled the US opioid crisis and today has resulted in majority of deaths from opioids being from unintentional overdose from fentanyl and its analogues cut into heroin, cocaine, Xanax and other drugs. Recently demand for novel psychoactive substances (NPS) in the form of fentanyl analogues in the pure form has arisen, exacerbating existing problems in detection, prevention, harm reduction, and healthcare responses worldwide.[3]

1.2 Relevance: Individual and Societal Impacts - Sizing of the problem

The impacts of fentanyl and its analogues are multifaceted and profound, the effects of their sale and consumption are felt in various social contexts such as crime and violence, environmental sustainability, the economy, health, politics, and economic cost. Currently the opioid crisis is preventing the continuous improvement and achievement of the UN Sustainable Development Goals, the detection and removal of fentanyl analogues from narcotics supply chains is thus indispensable in promoting health and well-being (SDG 3) and reducing inequalities (SDG 10).

On a global scale, the likelihood of fatally overdosing on fentanyl overpasses the likelihood of death from a motor vehicle related accident.[2] Despite signs of a slowing fentanyl market in recent years based of seizure levels,[4] this is still a large societal cost. The use and abuse of fentanyl's, both prescription and illicit, is concentrated in North America, as novel synthetic opioids have become a drug of choice in both manufacturing and consumption patterns, reflecting a generalised trend in the favour of higher potency drugs. As such, overdose deaths are overrepresented in the US, whereby in 2023, 72,000, or nearly 70% of drug overdose deaths were estimated to involve illegally manufactured fentanyl.[5] In 2023, more than 390 million lethal doses of fentanyl were seized in the US alone,[6] with half of all pills containing lethal doses. One of the main issues in the response and reduction of fentanyl use is the tracing of manufacturing origin or where the drug was obtained, due to the evolving illicit drug market, whereby anonymity is enhanced due to cryptocurrencies, e-commerce, and online servers.[1]

Although Australia has not experienced the same scale of fentanyl-driven mortality as North America, the increasing detection of fentanyl analogues amongst the drug market and supply chain has prompted rising concern amongst policymakers, law enforcement, and healthcare. Fentanyl usage and abuse represent a significant social cost, whereby in 2022/23 opioids were projected to have a combined social and economic cost of $18.4bn and contributed to the largest proportion of overdoses, sitting at 43.9% of deaths in 2023.[7] Many studies have concluded that death from overdose is both avoidable and inexplicitly linked to the environment in which it occurs,[5] therefore harm minimisation and preventative techniques achieved through separation processes should be at the forefront of public health policy in Australia to minimize individual, societal, and economic cost, and promote global

sustainable development. A challenge also lies in preexisting legal regulations and societal viewpoints, whereby stigma and punitive policies can prevent harm reduction and individuals accessing support services. As such, Australia must align with the UNs recommendation for a comprehensive approach which places individuals at the centre of policy making, to effectively address the issue.

1.3 Literature review on existing and emerging methods

Separation processes act as the bridging force between law enforcement, policy makers, and healthcare providers in the fight to address and prevent fentanyl-based overdose, whereby both laboratory and in field methods are utilised to match different analytical requirements and separate fentanyl analogues from other narcotics. Majority of methods used are not specific to fentanyl or its derivatives, rather they are used among most drug separation situations. Due to the complex mixture of compounds often found in illicit substances, sensitive and selective processes are used. Laboratory processes consist of comparatively higher sensitivity and accuracy as opposed to field methods, evolving from the use of gas chromatography (GC) aided with mass spectroscopy (MS) to liquid chromatography (LC), aided with in tandem mass spectroscopy (LC/MS).[8] In field detection is primarily achieved through portable spectroscopic devices, namely Fentanyl testing strips, and a Raman spectrometer, which are made intended to inform emergency operators immediately, allowing for actionable results to be produced in time-sensitive situations.[9] While these devices are not comparable in terms of sensitivity and performance with laboratory techniques, these is growing recondition that due the proliferation of unintentional fentanyl use and overdose, these methods should be the priority for future development and implementation.

Artificial Intelligence (AI) and Machine Learning (ML) offer a unique opportunity to transform responses to clandestine manufacturing of fentanyl and its use from reactive to proactive. By leveraging data processing techniques, deep learning and generative models, AI can be trained on current fentanyl analogues, chemical properties of drugs, and mass spectrometry outputs to identify new substances and predict potential further analogues.[10,11] Thus, the proficient and strategic implementation of AI has the potential to enhance drug discovery, strengthen detection capabilities, and reduce testing time.

Current research on fentanyl detection faces several limitations. Laboratory methods are forensically reliable but can be slow especially with rapidly emerging analogues. AI shows some promise for screening and prediction but remains underutilised and unvalidated for routine forensic use. Portable devices enable fast field testing but can be unreliable and potentially legally inadmissible.

This raises key questions:

- How can separation methods be adapted to stay current with the constant development of new fentanyl analogues while maintaining forensic-level accuracy?
- What strategies will allow ML/AI models to not only be helpful in fentanyl detection but still be robust, explainable and admissible in forensic workplaces?
- What innovations in microfluidic or portable separation technologies could provide rapid, reliable, and admissible on-site screening for fentanyl analogues?
- How should separation processes balance harm-reduction goals (field testing for public safety) with evidentiary standards in criminal justice?

1.4 Ethical removal, purification, and end-of-life handling (forensic & harm-reduction)

Effective separation in this context does not end at identification, ethical practice requires safe handling after analytical processes to prevent secondary exposure, and other consequences. Implementing ethical and sustainable methods for removing and disposing of fentanyl analogues is crucial to prevent negative environmental impact and ensure long-term safety of users and first responders, directly supporting SDG15 by promoting the protection of ecosystems from pollution, and aligning with ESG principles.

Separation workflows should focus on containment, this includes closed preparations, appropriate personal protective equipment (PPE) and minimal sample size.[12] Biological Safety Cabinets (BSCs) have been recommended however this only applies to laboratory settings and not field testing.[13] During such a process, it is important to keep evidence logs to preserve chain-of-custody and prevent diversion.

Decontamination post analysis is also crucial. Decontamination must avoid aerosolisation and use controlled, wet cleaning with clear clean and dirty zones within the lab. PPE should be disposed into sealed tamper-evident waste.[14] Field testing should have a focus on consistency and simplicity, this could include sealed containment for wipes and PPE, pre-moistened wipes, return-to-lab protocols along with measures to respond to symptoms of overdose such as having ready access to naloxone.[15] These practices reduce risk without compromising on courtroom admissibility or downstream laboratory confirmation.

The final obligation is disposal. Opioid containing wastes such as used sorbents, PPE, residual extracts should be segregated and labelled as high-risk material until they reach an endpoint, usually high temperature incineration. Discharge to drains or general waste streams should be avoided unless

consistent with environmental and controlled-substance regulations.[16]

Organisations should implement policies and training to comply with such measures. Removal, cleanup and disposal can be integral features of testing while complying with analytical quality, public-health protection and legal defensibility.

1.5 Scope and objectives

This review examines separation methods for fentanyl analogues with a focus on laboratory methods, AI approaches and portable technologies, while considering social and legal implications. It covers experimental methods such as chromatography, electrophoresis, and sample preparation; computational strategies for optimisation, anomaly detection, and analogue generation; and field-deployable technologies for harm-reduction and forensic use.

This review aims to assess current methods, including laboratory separations such as chromatography, electrophoresis and sample prep strategies with performance benchmarks (e.g. sensitivity, specificity, turnaround time) and admissibility notes. Field deployable screening such as immunoassay strips, Raman and emerging microfluidics is also studied with an emphasis on triage accuracy, potential false positives and how they can be confirmed in a lab downstream. This review also explores AI/ML approaches including model design for analogue detection and prediction in ways relevant to broader societal challenges.

This review does not cover detailed synthetic routes for fentanyl or any of its analogues, clinical pharmacotherapy such as dosing regimens, supply chain and markets for fentanyl nor any consumer level handling for “DIY” or “at home” processing. Where referenced, these topics are only included to provide context or frame analytical requirements.

2. State-of-the-Art in Advanced Separation Strategies

2.1 Current Methodologies

Historically, GC coupled with MS (GC/MS) was the predominant method for separation and identification of fentanyl and fentanyl analogues, yet this has been replaced primarily by LC coupled with MS (LC/MS) or often high-performance liquid chromatography (HPLC). This is due to the necessity of the analyte in GC needing to be volatile and thermally stable, yet many fentanyl analogues have a relatively low volatile and are thermally labile (decompose under high temperatures) thus not suitable for separation via GC.[3] LC relies on the same principles as GC, where the separation occurs due to the level of interaction between the analyte and the stationary phase, yet the mobile phase is a solvent that carries the analyte, and it is driven through the stationary phase by a high-pressure pump. Within the analyte, the molecules that have a higher interaction with the stationary phase have a higher retention time and the molecules separate into elution peaks.[17]

Whilst HPLC is the primary method for separation of fentanyl and its analogues, mass spectroscopy is the method used to analyse the sample as it has very high sensitivity (can detect very small amounts), requires low purity for analysis and is highly specific. Mass spec is primarily used as it allows for the identification of the molecular mass of the molecules and the fragmentation patterns (especially in MS/MS). Since these analogues often vary via addition of side chains, replacement of hetero-atoms in cyclic structures, substitution of different atoms with halogens and functional group alteration, often fragmentation occurs at identical points for between analogues. Zhang, 2022 found that fentanyl forms fragments either side of the piperidine ring structure (N-αC and N-4C) forming MS peaks at 188.14 and 105.07. Between fentanyl analogues, depending on side chains added, the analogues formed peaks at 188.14 +R1 +R2 and 105.07 +R1 where R1 and R2 denote side chains.[18] Furthermore, fentanyl analogues with similar retention times were able to be differentiated via fragmentation patterns providing a heavy advantage to the coupling of HPLC and MS. The usefulness of these fragmentation patterns leads to the usage of MS/MS where the precursor ion is separated with an initial mass spectroscopy, then collided with an inert gas to further fragment the ion, then these fragments are analysed.[19]

Samples however can not be directly placed into GC,HPLC or MS as often contain impurities that are insoluble and can lead to damage of the instruments or tamper results and thus need to be prepared for analysis. Often solid phase extraction is done such as microextraction by packed sorbent as it offers the best selectivity in comparison to methods such as liquid-liquid extraction as well as its small sample and solvent requirements.[20] The sorbent is chosen to interact well with the target molecule so that it can be adsorbed to the surface of the sorbent. The analyte material is passed through carried by a weak solvent (a solvent that does not strongly interact with the sorbent). It is then washed with a strong solvent do desorb the analyte from the sorbent and collect it essentially purifying the sample and allowing for accurate analysis.[21]

Raman spectroscopy relies on the stokes and anti-stokes scattering of light depending on the vibrational modes of the molecules. Unlike Rayleigh scattering where the emitted light has the same wavelength as the absorbed, in stokes and anti-stokes, the emitted light has a larger or smaller wavelength depending if it increases or decreases the vibrational energy of the bonds. This produces a characteristic Raman emittance

pattern of a molecule that can be used to determine the molecule.[5] Raman spectroscopy (RS) can compare the spectrum produced by the compound to a library of spectrums to determine if fentanyl analogues are present in the sample however this raises some key issues with (RS). Often drugs are laced with fentanyl and its analogues rather than purely fentanyl and thus exists in very small amounts. The detectable limit of lab grade Raman spectrophotometer's is 25 mcg/ml and thus for small traces of fentanyl or its analogues, this method will unable to detect it.[5] Depending on the other compounds present within the sample tested, they can also interfere with the spectrum observed and further reduce the detectable limits of RS. Furthermore, as (RS) must compare the measured spectrum to a library and search for a match within that library to identify the compound, it is only able to identify compounds with measured Raman spectrums. Resultantly, it can not detect fentanyl analogues that have not previously been measured and detected in a laboratory.

Irrespective of these drawbacks, handheld RS still appears to be one of the major emerging technologies in drug detection. The most primary of its advantages is its capability of determining the percentage composition of a sample not just detecting if compounds are present.[22] Its ability to detect samples through packaging material (plastic, glass, etc.) makes it highly advantageous in comparison to immunoassays which must come into physical contact with the substance.[22]

Fentanyl test strips are immunoassays which are able to detect the presence of trace amounts of fentanyl or its analogues in a sample. They utilise specific antibodies for the compound for detection by stimulating an immune response to the compound within animals which will change colour on a test strip if the antibody binds.[22] Since these antibodies are proteins, they function primarily through structure where the overall structure of the protein creates amino acid motifs at specific locations to selectively bind with the fentanyl and its functional groups. Whilst these immunoassays are very simple to use not requiring any expert opinion and are quick and relatively cheap to implement, they have a few drawbacks. They can lead to false positives as similar narcotics that are not fentanyl can bind to it due to structural similarities and lead to a false positive detection of the drug.[23] However, this can be advantageous in the case of fentanyl analogues detection where even if the immunoassay is not designed for a specific analogue, its structural similarity can still cause the antibody to bind and thus trigger detection. This leads to the development of broad range immunoassays that are not built for any specific molecule rather attempting to bind to the structural similarities between analogues however this is not foolproof and can cause no detection of the compounds.

Through the implementation of technologies, especially in a field context, methods such as the fentanyl testing strip and Raman spectrometer have the potential to reduce overdose deaths and help users and responders make informed decisions, overall improving health outcomes and aligning with UN SDG 3. Furthermore, these methods actively protect vulnerable communities, whereby structural vulnerabilities have been found to increase exposure to adverse health outcomes.[24] Cost-efficient and portable instruments will extend the capabilities of scientistic and health care professions in regional and resource limited settings, where communities who can be disproportionately affected by drug addiction and unsafe substances. These efforts align with UN goal 11 (reduced inequality).

2.2 Emerging Technologies

Artificial intelligence provides a potent opportunity to the separation field as it provides the capability to accelerate pattern and anomaly identification and ability to identify insights, patterns and knowledge without human interaction.[25] AI utilises machine learning, deep learning and neural networks to automate analysis of large data sets, improve the consistency and accuracy of analysis and to complete optimisation of experiments, particularly through the identification in noise in datasets.[25] The predominate strategy for separation classification is convolutional neural networks (CNN), allowing for an input ‘neuron’ to be connected to an output ‘neuron’, allowing for a more wholistic approach to data classification.[26]

Most prominently, CNNs have been used in tandem with LC-MS to identify components due to CNN’s improved sensitivity and selectivity.[25] CNN models such as ‘MST Tracer’, act as a machine learning model for peptide feature identification.[27] Peptides each produce multiple trails after signal peaks, collectively labelled a peptide feature. In instances that noise to signal ratios are minimal, peptide feature identification is difficult and poses a challenge. This iteration of CNN conducts a comparative analysis on past databases and using CNN, identifies the new ‘input’ neurons to decipher the most correct output neurons. Similarly, other models have been similarly generated for other medicinal purposes with 100% sensitivity and 93.18% specificity.[28] The development of successful models is promising for separation processes in the identification of fentanyl analogues as similar models may be applied to the fentanyl analogue dataset. However, a limiting factor to creating a model for fentanyl analogue identification may lie within a lack of data. AI may address this through the ability to generate an expansion on current databases.

Integration of ML frameworks must be conscious of dataset bias and design considerations that must consider the nature of drug analysis. Cost sensitive modelling should suggest a penalty matrix for design that outweighs false negatives in comparison to false positives.[29] The detrimental consequences

of undetected fentanyl presence; possibly leading to overdoses, deaths or the movement of lethal drugs in a community and in contrast with a false positive that may trigger further testing and waste resources; however, the potential detriment is at a greater cost than the cost of additional resources.[30]

It should be noted that emerging advancements with AI and Machine learning do result if significant water and energy usage, thus diverting from sustainable ESG practices and global decarbonisation efforts. To mitigate these adverse effects, it is strongly recommended that these technologies should be run using renewable energy and recycled water to minimise the effect on overall sustainability.

2.3 Comparative Analysis of Current Methodologies

The widespread integration of traditional methods that rely on LC-MS/MS, often in tandem with SPE or LLE for extraction, is well documented and often cited for laboratory and clinical adoption.[3] However, emerging technologies in Raman spectroscopy are gaining attention due to the high true positive and accuracy rates.[5,22]

Table 1: Comparison of Fentanyl Separation and Detection Methods

Method	Setting	Purpose	Speed	Sensitivity	Cost
Fentanyl Test Strips	Field	Presumptive, consumer, single use	Instant (seconds)	Qualitative, dependent on brand. Requires at least 1µg/mL [Rodriguez, 2023; green, 2020].	Low (~$1 each)
Raman Spectroscopy	Field	Presumptive/Screening, clinical and law enforcement	Fast (minutes)	Qualitative/Semi-quantitative/. Requires at least 25µg/mL [Green, 2020].	High ($35,000+)
GC-MS / LC-MS/MS	Lab	Confirmatory/ Quantitative, research, identification	Slow (hours)	Quantitative. Requires at least 3.1µg/mL [green, 2020].	High (lab equipment)

Novel approaches to drug testing are first outlined in Kranenburg et al.'s findings, using a chemometric-based cocaine detection using a handheld Raman spectrometer.[22] A barrier to implementation is the lack of previous work on chemometric based detection, however it is apparent that the strength of existing studies supersedes reluctance as implementation of the Raman spectrometer is suggested as a solution in clinical drug detection settings as a quick and accurate method of drug detection.[22,31] Despite the high specificity and reliability of handheld devices, a primary drawback is poor detectability at lower concentrations or in more structurally complicated analogues.[5,22,23] A primary benefit to machine learning is the ability to identify analogues which were not present in the training set, particularly the improvement in discrimination comparative to manual spectra comparison.[32] The implementation of Cooman et al's CNN model demonstrated overall accuracy of 98.4%, expanding the capabilities of existing technologies.[32]

Fentanyl test strips are a promising frontline tool due to high accessibility, low cost, portability and high analytical reliability.[33,34] Modern FTS exhibit high sensitivity at concentrations at 1µg/mL, and up to 200ng/mL, dependent on the brand of fentanyl test strip,[35] whilst maintaining minimal cross reactivity in the presence of other substances.[33] Although the reliability and accessibility of these tests are pertinent for harm reduction for people who use drugs (PWUD), most FTS are designed for laboratory matrices such as urine rather than substances in aqueous assays.[35]

Further usability difficulties including confusing instructions, poor packaging design and hard to open foil strips remain limitations to further widespread implementation of FTS within PWUD.[36] These limitations emphasise design flaws which limit the interpretability and accessibility of such techniques by the user. The integration of machine learning methods such as CNN algorithms that interpret lateral flow assays may be similarly applied to FTS.[37] These CNN based algorithms automatically read and quantify test lines using smartphone camera, compensating for factors such as poor lighting, user error or other environmental factors.

Beyond improving the interpretation for the user, the integration of machine learning may allow for data aggregation that could further contribute to general geographic data and supply trends which could contribute to predictive modelling. However, the integration of machine learning must be tightly monitored to ensure the anonymity and privacy of users is respected. Ultimately, the success of novel technologies is contingent on the practical ability for implementation; ensuring that operator proficiency, chain of custody and maintenance of equipment are rigorously monitored. Traditional separation methods are reliant on highly trained analysts, routine calibration and adherence to established laboratory operating procedure.[38] The high barrier to entry testing using traditional laboratory methods means adequate training of operators may take years. Although simpler testing devices such as Raman spectroscopy reduce operator training needs and processing complexity, calibration records of these devices should be maintained to ensure the reliability of results. Similarly, FTS are designed to be inherently simpler than a laboratory scale method but must be scaled to ensure that the methodology of results garnered are conclusive the experience of PWUD.[36]

3. Challenges and Future Perspectives

3.1 Identified Challenges

In field and laboratory detection is paramount for addressing the proliferation of illicit manufacturing, and consumption of fentanyl and its analogues; however

significant challenges exist in the application of separation methods in an efficient and cost-effective way. While laboratory methods such as LC-MS/MS are often considered the gold standard for identification of fentanyl and analogues, they still have drawbacks due to the rapid pace at which new analogues are being produced, with slow turnaround times, limiting the ability of these processes to act in a preventative way.[23] While favoured due to quick turnaround time and low cost, in field methods do not come without drawbacks, whereby efficiency and response time improvements come at the cost of lost precision, reliability, and quantitative methods. Further, as these methods rely on a library of identified compounds, this complicates and increases lead time in the identification of novel compounds.

Extending from the physical methodology of separation, legal regulation and societal acceptance of in field drug testing and harm prevention methods remain a key challenge, whereby in Australia statewide discrepancies exist. An inherent tension exists within the optimal policy response as disagreements exist between the prioritisation of reducing the size of the drug market verse reducing the harm associated with their use.[2] From a social standpoint, there is growing recognition that harm reduction methods such as drug checking facilities and safe consumption spaces should be at the forefront of policy where in field testing methods would be employed. However, legal issues arise in navigating liabilities of allowing this occurrence, as well as managing perceptions of condoning and supporting drug use, both of which inhibit the application of on-the-spot drug testing. This has been seen recently in Queensland, as of September 2025 the government passed legislation banning pill testing despite evidence in favour of doing so in terms of harm reduction.[39] Another significant challenge is the complexity of the illicit fentanyl market, whereby trade and production of precursor chemicals being extremely hard to regulate and monitor as they are used in a variety of other pharmaceuticals.[2]

There is growing recognition that buyer-seller relationship predominately occurs via online black market, significantly obscuring the ability of law enforcement to trace back to manufacturing origin.[1] This issue is compounded due to the global complexity of the supply chain, whereby chemicals and different stages of production occur in different countries worldwide. Resultingly, separation processes is unable to address some of the main drivers of the fentanyl and analogue challenge, although it can play a preventative role in detection, other policies, technologies, and regulations are required to adequately address the issue at the source.

3.2 Role of Separations in Addressing These Challenges

Advanced separation techniques are critical to overcome challenges surrounding the rapid emergence of new analogues and balancing this through cost-effective but efficient methods. A present issue concerns novel fentanyl analogues with added chiral centres that evade MS detection, as MS alone cannot distinguish between stereoisomers.[40] An advanced separation technique, chiral chromatography such as LC with chiral stationary phases or capillary electrophoresis are able to resolve the stereoisomers prior to MS detection.[40] Although more intensive, both these methods enhance the capacity of accurate, early detection and appropriate responses.

Further to this, current in field-testing can incur more false positives due to lower precisions and reliability from a short turnaround time. A chromatographic pre-separation of the sample before conducting MS is sometimes required. For example, the compound of cyclopropylfentanyl; crotonylfentanyl, methacrylfentanyl all have the same mass and similar fragmentation patterns.[39] Chromatographic separation was demonstrated by Lee et al., 2019 to be necessary for absolute confirmation and certainty of identification, hence this method can similarly be considered for other fentanyl analogue isomer series.[39] These pre-separation techniques have the potential to increase reliability and reduce false positives with field testing, whilst also addressing the rapid emergence of new fentanyl analogues and stereoisomers.

Furthermore, separation techniques alongside with emerging models, for example, a linear algebra-based algorithm developed at Johns Hopkins University, has demonstrated an ability to analyse spectra from unknown substances.[41] From a mass spectrum of fentanyl analogues and 300,000 non-fentanyl compounds, it was able to find combinations of spectra that best reconstruct mass spectra of unknown substances.[42] These models and advanced separation techniques have a great role in overcoming challenges associated with illicit drug purification.

3.3 Future Directions

As previously outlined, there is a societal need for efficient and accurate testing, particularly concerning overdose prevention whereby immediate drug identification is needed. Current implemented LC-MS/MS methods are typically slower and more resource intensive. Emerging technology, miniaturised, and field-portable LC have potential to provide robust on-site analysis that allows for rapid decision making.[43] These would reduce issues arising from sample degradation, contamination, or long-term sample storage, significant financial savings as well as reduce reagent consumption and waste generation.[43,44] Past and present application of this technology has shown potential in the field of nutrient monitoring. More recently, improvements to open-tubular

columns and pillar array columns have been made to enhance extraction selectivity and produce ultra-high-resolution separations.[45] The integration of artificial intelligence and machine learning has been an increasing trend in research, to optimise column design, separation conditions and data analysis to ultimately enable faster method development.[45] The primary obstacle facing this technology is the need for proper training with regards to handling small volumes and flow rates.[41] This would allow for a more widespread adoption and rapid development of miniaturised technologies that are crucial to the proliferation of fentanyl and its analogues.

In conjunction with the above technologies, an integration of AI for optimisation and analysis is a critical direction for future separations workflow, particular to overcome the constantly evolving fentanyl analogues. Machine-learning is platform with the potential for this. Through training algorithms on data and patten recognition from a data base of known fentanyl analogues would allow for the identification of novel ones with high accuracy.[41] These systems, alongside developing in-field technologies, assist with driving rapid and portable testing to prioritise the wellbeing and safety of the wider population.

4. Conclusion and Recommendations

Current separation techniques such as HPLC, LC–MS, and Raman spectroscopy provide robust means of detecting fentanyl analogues, acting as cornerstones for drug policy and harm reduction worldwide. However, the rapid emergence of novel compounds starkly outpaces the discovery of in field and laboratory methods. This discrepancy emphasises the necessity for advancements in reliable novel analogue testing methods. Overall, whilst the established methods provide a robust scientific basis for further advancements, successful harm reduction is ultimately contingent on legal regulations, government policy and public health professionals; out of the scope of the discussed scientific methods. Effective regulation, destigmatisation, education, and data-driven policy and implementation are essential for translating analytical findings into tangible social benefit. The integration of AI predictive methods into current analytical findings will further strengthen current models whilst simultaneously aiding in the discovery and analysis of novel analogues. The implementation of current and future technologies support SDG 3 (Good Health and Wellbeing) and SDG 10 (Reduced Inequalities) by promoting equitable access to harm-reduction technologies. Alignment with furthering SDG goals further supports the development of a sustainable future.

Avenues for future research should prioritise the feasibility of implementing novel methods and developing the most effective regulatory framework that supports emerging technologies. A potent barrier to innovation includes the outdated legislation and the persistent stigma towards drug users. Focusing on maintaining individual autonomy, minimising environmental impact, removing specific regulatory barriers, and ensuring scalable solutions is essential for successful harm reduction, improving health outcomes, and saving lives.

Acknowledgements

Chloe Norman investigated the social impacts, literature review, and evaluated challenges of implemetation; Sally Webster constructed the graphical abstract, reviewed the role of separations in adressing the identified challenges and investigated future studies and directions; Madeleine Ly performed the background literature review, assessed gaps in the research and proposed key questions and outlined the scope for the review. Zachary Simmons investigated current technologies both in laboratory and in field regarding fentanyl detection and separation.Angelina Khun contributed to State-of-the-Art in Advanced Separation Strategies and assessed emerging technologies, constructed the comparative analysis of current methodologies, referencing and bibliography and general formatting; Jordan Kambanis, Zihan Zhou and Bishwjeet Binwal provided task direction and project assistance, and Dr. Gobinath Rajarathnam provided conceptual direction, research and writing guiding frameworks, facilitated project resources, and direct project supervision**.**

Acknowledgement of AI.

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