FORENSIC INSIGHTS AND HEALTH SCIENCES BULLETIN

Volume 1: Issue 1: 2023

https://doi.org/10.56770/fi20231111

A SCOPING REVIEW OF FORENSIC DRUG TESTING METHODS

Ayesha Eman*

Department of Biotechnology, University of Karachi, Pakistan

Submitted 18th January 2023, Accepted 22nd February 2023, Published 30th April 2023

ABSTRACT

In light of the ongoing global opioid crisis, harm reduction agencies are working to help individuals who use drugs do so more safely. In order to help users make educated decisions, many of these organizations are researching methods for testing medications to identify and, when feasible, quantify its constituent parts. The purpose of this paper is to list the most popular drug substance testing methods and, after a thorough examination, suggest the best approaches for point-of-care testing. According to our review, mass spectrometry is still the gold standard for forensic drug analysis, while handheld infrared spectroscopy, Raman spectroscopy, and ion mobility spectrometry are the most successful techniques for point-of-care drug testing. Agencies or clinics with the necessary funding should consider reaching out to manufacturers of these devices to explore their application in harm reduction settings. Additionally, lower-tech alternatives, such as spot/color tests and immunoassays, although limited in capability, are affordable and straightforward to use.

Keywords: Drug evaluation, Sophisticated techniques, Review.

***Corresponding Author.** E-mail: ashiaeman_183@gmail.com

INTRODUCTION

Various approaches to drug testing have been established for decades, even in North America. Recently, in Canada, drug testing has gained traction at music festivals. While legal barriers to drug testing exist, there are three primary advantages to testing drugs before consumption: it can help avoid short- and long-term adverse effects (including overdose and fatalities); it enables hospitals and public health authorities to be informed of circulating lethal or novel substances; and it contributes to a global understanding of drug circulation [**1–3**].

The purpose of this paper is to outline the most popular techniques for testing chemicals in both laboratory and point-of-care settings. It ends with suggestions for testing drugs at the point of care. The forensic examination of drug compounds in their intended mode of consumption is referred to as "drug testing" in this context.

Subject-matter experts work together in Scientific Working Groups to create consensus standards and best practices. These techniques are therefore appropriate for identifying unknown compounds since they have demonstrated efficacy in the forensic examination of unknown chemical substances. Since certain techniques necessitate specialized technical knowledge and/or a laboratory setting, not all of them are easily available in a point-of-care context. As a result, the suitability of each approach may differ depending on the circumstances. Some clinics may have access to more advanced testing methods through direct funding or industry partnerships, while others may need to rely on less precise methodologies and equipment due to limited resources [**4-6**].

Discriminatory methods for drug testing often require significant investments in specialized equipment. This may involve forming community partnerships, conducting thorough cost-benefit analyses, or both [**7**]. The approaches are arranged

FIHSB is published by AMMANIF Publisher. The authors retain the copyright without restriction. This article is an open access article published under the terms and conditions of Creative Commons Attribution 4.0 International License.

under distinct subheadings to provide a clear presentation of the information and handle some of the related technical aspects. Three sections are included in each method: 1. A synopsis of the method's underlying concept; 2. A list of compounds that can be detected and their accuracy; and 3. Usability. The best techniques or tools for point-of-care scenarios will be the exclusive subject of a recommendation section at the conclusion of the paper. Our review identifies handheld IR and Raman spectroscopy as the best methods for point-of-care drug testing. From a cost-benefit perspective, these portable units are superior to other methods in nearly every regard. Manufacturers have made these technologies user-friendly and effective for identifying unknown substances. However, the major downsides include the need for advanced expertise for quantification and the relatively high cost of the units. Qualitative use of these devices typically requires minimal technical training. Designed for field use, these units are compact, portable, and sturdy, while offering near-laboratory-level identification capabilities. Although currently used primarily in law enforcement, their application in harm reduction settings merits exploration.

Because of their durability and low maintenance needs, IMS spectrometers are perfect for detecting drugs in airports all around the world. Training is quick and straightforward, with high sensitivity and selectivity. Long-lasting and reasonably priced consumables, along with non-destructive sampling, enable quantification without the need for specialized knowledge. IMS is the greatest choice for clinics with modest financing because of its quick and precise analysis. Online updates provided by certain gas analyzers allow for quick analysis of liquid, solid, and gas samples as well as the capacity to distinguish between several interfering compounds in complicated mixes. Point-of-care testing is kept up to date thanks to its online updating capabilities, which enables clinics to rapidly communicate procedures and information about novel molecular species.

Alternative methods for point-of-care drug testing include mass spectrometry (MS), thin-layer chromatography (TLC), and UV spectroscopy. MS is currently regarded as the gold standard in forensic drug analysis. As MS units have been in use for quite some time, it is possible to find pre-owned devices in the low-to-mid thousands range. However, acquiring a newer device optimized for drug testing or capable of detecting extremely low concentrations can cost hundreds of thousands of dollars. This price disparity poses a challenge; clinics must conduct extensive research to perform a cost-benefit analysis between new and used machines to ensure that the chosen device meets their needs. Additionally, the complexity of operating and maintaining MS machines requires clinics to consider training, operational demands, maintenance, and ongoing costs, which may outweigh the benefits.

In contrast, UV spectroscopy and TLC are more affordable options but offer less specificity. Both methods are generally simpler to operate, maintain, and interpret, yet they lack the quantification capabilities of more advanced techniques. Despite their lower cost, when used together, TLC and UV spectroscopy can effectively identify a broad range of substances, including mixtures, and provide a basic level of quantification.

A simpler option is spot/color tests, which are purely presumptive. However, they can be relatively specific for identifying compounds or mixtures when standardized procedures and a battery of tests are employed. Optimal techniques can be easily found online. These color tests are easy to conduct, quick, and reasonably priced. The color profile can be identified with the help of a smartphone app, and for precise identifications, it can then be compared with a searchable database. This technology stands out from many other presumptive approaches due to its low cost, simplicity of use, and low sample size. In Europe, this kind of exam is frequently used. To increase accuracy, however, appropriate standardization of procedures at the clinic level is crucial because they are not perfect and can be done wrong.

X-ray diffractometry, microcrystalline testing, and immunoassays are less appropriate techniques for point-of-care drug testing. Because immunoassays rely on antibody-antigen interactions and are generally developed for biological samples, they are best suited for evaluating excreted metabolites, including those found in urine. While immunoassays can indicate the presence of drugs, they are also prone to high false-positive rates. This can lead to individuals continuing to use substances or tarnishing the clinic's reputation, potentially discouraging users from returning for testing. Nonetheless, immunoassays are affordable, portable, and capable of detecting potentially lethal drugs like fentanyl [**8, 9**].

Because microcrystalline testing necessitates that the medication be mostly or entirely pure, it is a limited approach. To detect pharmaceuticals based just on their crystal structure, this method requires a high level of ability and knowledge and lacks quantitative capabilities. Despite its great degree of discrimination, X-ray diffractometry requires cooperation with specialized laboratories or organizations. In addition, X-ray diffractometers are

costly—usually tens of thousands of dollars—and challenging to maintain and use. Concerns about safety and health are also raised by the usage of radiation.

MASS SPECTROMETRY

When it comes to drug testing, mass spectrometry (MS) is the most selective method available. It is the gold standard in forensic drug investigation and determines the exact molecular mass of ions based on their mass-to-charge ratio (m/z). The three main processes of separation, ionization, and detection are typically involved in mass spectrometry. Capillary electrophoresis (CE), liquid chromatography (LC), and gas chromatography (GC) are methods for achieving separation. Several ionization techniques are available, but the most popular ones for analyzing drug substances are electron ionization (EI), atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI), atmospheric pressure photoionization (APPI), fast atom bombardment (FAB), and the more recent direct analysis in real time (DART) [**10**].

Hard techniques, such as EI, FAB, and APCI, cause molecules to fragment, producing complex mass spectra. Fragmentation is advantageous in analysis, as molecules have known fragmentation patterns that can be matched against a spectral database, allowing for quick identification of molecular species. However, hard techniques are primarily limited to detecting small molecules, which comprise most drugs, except for those consumed in their raw biological forms.

Soft ionization techniques, such as MALDI and ESI, minimize fragmentation and allow the analyzed molecules to remain intact, making them particularly useful for large biomolecules like proteins. DART is especially noteworthy because it enables non-destructive testing, operates quickly, and can rapidly quantify results when paired with an internal standard. For instance, a pill can be placed in front of the gas stream, allowing for the identification of the molecular species present within seconds. Additionally, DART does not require the separation of each molecular species before analysis, which makes it accessible for untrained personnel to collect data.

In principle, nearly any substance can be identified using mass spectrometry (MS) in conjunction with a separation technique, such as chromatography. The sensitivity of modern mass spectrometers enables the detection of analytes at concentrations in the attomolar range. MS offers enhanced sensitivity compared to some other analytical methods because the mass-charge filter effectively reduces background interference, resulting in clearer readings and analyte fingerprints. Its excellent specificity arises from characteristic fragmentation patterns, high resolution, and unique filtering capabilities, particularly in tandem or higher-order mass spectrometry [**11**].

Highly accurate identification is made possible by mass spectrometry, which offers vital information regarding molecular mass, isotopic abundance, and time resolved chemical data. In addition to being more smaller and easier to use than their predecessors, newer devices can interface with computers to improve database searches and expedite the drug detection process.

With the exception of DART, a significant disadvantage of mass spectrometry is that the analyzed material is usually destroyed during the analysis procedure. Consumable materials, some of which may be harmful, come with continuous costs, yet only a very small sample size (in milligrams) is needed. Furthermore, unless DART is used, complex mixtures must be separated using chromatographic techniques (either gas or liquid chromatography) in order to precisely identify each component [**12**].

Utilizing this technology requires intermediate to expert expertise. Users should possess some theoretical understanding of the technology and specific instruments, along with specialized training from an expert. The cost of a mass spectrometer is too high. While an older, used mass spectrometer may be less expensive initially, it may not be suitable for point-of-care drug testing. Additionally, there are significant ongoing operational costs, including chromatography reagents, gas consumables (such as nitrogen and helium), sample preparation materials, and routine maintenance and servicing.

X-RAY DIFFRACTOMETRY

In X-ray diffractometry (X-ray D), a drug sample is subjected to high-energy X-ray radiation. The crystalline atoms in the substance cause the incident X-ray beams to diffract in various directions. This diffraction enables the determination of the spatial structure of molecules by measuring how the X-ray radiation is scattered by the molecular crystal lattice. By analyzing the angles and intensities of the diffracted X-rays, a three-dimensional representation of the electron density within the crystal can be created. From this data, researchers can ascertain the positions of the atoms within the crystal, along with information about their chemical bonds and other structural details.

X-ray D can identify any crystalline or partially crystalline substance, including solids that are evidently crystalline or in powder or pill form, such as methamphetamine, ketamine, and cocaine. It can

also detect substances in mixtures or compounds with unidentified structures. This method is primarily applicable to solid substances and is used to identify specific chemical forms rather than quantify them. It is effective in detecting diluents or adulterants. The technique is sensitive to both polymorphs and contaminants, which are commonly found in drugs. X-ray diffractometry gives structural information that makes it possible to identify substances with great accuracy because each one has its own diffraction lines, or "X-ray fingerprint." It can also detect the presence of cutting agents and medication concentrations. Research has shown that even at concentrations as low as 5% of the entire pharmaceutical formulation, this technique may detect a particular medication.

One benefit of X-ray D is that it doesn't harm the material being examined and doesn't require sample preparation. Additionally, it only requires a very small sample size, usually in the milligram to microgram range. Although it can examine molecules that are currently unknown and is the most dependable method for determining structure, it is not appropriate for usage outside of a laboratory context [**13**]. Due to the highly radioactive nature of X-rays, which can be damaging to organic cells and DNA, this method requires extensive training and adherence to safety protocols, making it limited to laboratory environments. The operation of X-ray D involves a skill level ranging from advanced to expert.

These chemical tests lead to the formation of unique microcrystals of a specific analyte when a designated reagent is applied. The resulting microcrystal formations are then compared to a reference standard or control using a standard light microscope. The microcrystals are evaluated based on their shape, size, color, and spatial arrangement.

Several commonly abused substances include cocaine, heroin, methadone, GHB (gamma hydroxybutyrate), ketamine, phencyclidine, amphetamines, and methamphetamine. In microcrystalline assays, the analyte and a reference or control standard are combined with particular reagents to create distinct microcrystals. Since the crystals that form rely on the reagent and analyte selection, these tests can be quite specific, provided that no other chemicals interfere with the reagent in a comparable way. Additionally, the development of the distinctive microcrystals for the substance under test must not be impeded or obscured by impurities, diluents, or adulterants. Microcrystalline tests work best with pure or separated samples because, under the right circumstances, they yield a highly distinctive but insufficiently specific result for confirmation. High sensitivity means that just micrograms of the material are needed for testing [**14**].

The advantages of microcrystalline tests include their relatively low cost and minimal reagent requirements. The instrumentation is straightforward; however, this method does not quantify the amount of substance present. A drawback is that the sample tested is destroyed in the process, which may be inconvenient for individuals seeking identification. The method requires intermediate to advanced expertise and skilled interpretation of results.

INFRARED SPECTROMETRY

Infrared (IR) spectroscopy is a highly discriminatory technique that measures the amount of IR radiation absorbed or emitted by a sample as a function of wavelength. A spectrum is generated by passing infrared radiation through a sample and quantifying the incident radiation absorbed at each IR frequency. Analyzing the spectra enables the identification of molecular functional groups. The IR spectrum of a pure molecular compound provides a unique fingerprint that distinguishes it from the IR absorption patterns of other compounds, including isomers—compounds with the same chemical formula but different atomic arrangements [**15**].

Since almost all substances have IR-active vibrational modes, IR techniques have the benefit of enabling both qualitative and quantitative examination. However, using unknown samples and mixes for quantitative analysis can be difficult. In harm reduction clinics, the specific expertise needed for forensic investigation and quantification could be lacking. The majority of studies outlining simple quantification techniques are carried out in pharmaceutical research using regulated standards and procedures. Although it is technically possible to quantify unknown compounds, this is usually done on an individual basis and frequently calls for highly skilled technicians and chemists in forensic labs. As such, quantification using this technology is unlikely to be practical in such settings. Recent advancements in IR technology have led to the development of portable IR devices.

When reference spectra are available, most compounds can be clearly identified based on their infrared (IR) spectra. Drugs can be identified using a searchable database, such as http://webbook.nist.gov/. However, IR cannot distinguish between enantiomers, similar to mass spectrometry (MS). IR can generate structural information that provides sufficient selectivity, yielding high discriminating capability. IR can differentiate between diastereomers, such as pseudoephedrine and ephedrine, as well as between free base/acid and salt forms of substances. The

terms free base/acid and salt forms refer to variations in physical properties that can influence how the substance is used. Free base substances are typically more volatile and have lower boiling points, making them suitable for smoking. In contrast, salt forms are generally more stable, often crystalline, and soluble in water, allowing for methods of ingestion, insufflation (inhaling through the nose), or injection. A common example is crack cocaine (free base) and cocaine (salt); although both are the same drug, the differences in absorption and dosage methods can lead to varying physiological responses. One significant advantage of IR spectroscopy is that it does not destroy the sample—an important consideration when working with drugs and their users. Additionally, it requires only a very small sample size, usually in the milligram range or less, and can analyze samples in virtually any physical state, primarily solids or liquids. However, interference is common and can complicate identification [**16**].

Depending on the gadget, different levels of knowledge are needed to operate this technology. The market's portable infrared devices are designed for outreach workers and other users with rudimentary to intermediate understanding. Depending on the device's settings, these instruments can detect chemicals in a combination within defined concentration limits by analyzing the acquired spectrum and searching internal databases. Since it may only offer a precise breakdown of the constituents in a material or mixture and occasionally provide a semi-quantitative analysis (i.e., ranking constituents from most to least abundant), this procedure is referred to as presumptive or qualitative testing. For accurate quantification (as a percentage of mass relative to total mixture weight), It has demonstrated that some portable IR devices can be used for low-error quantitative analysis. However, interpreting the obtained spectrum in a quantitative manner requires advanced to expert-level knowledge, as these devices do not perform this task automatically. Many other IR devices also necessitate at least an intermediate understanding of the procedures, with some requiring advanced expert knowledge for proper analysis and quantification, including equipment operation and database searching.

ION MOBILITY SPECTROMETRY

Ion mobility spectrometry (IMS) separates and identifies ions based on their speed as they travel through a carrier gas. The mobility of an ion depends on three molecular characteristics: charge, reduced mass, and collision cross-section. Before samples can enter the instrument, they must be ionized. This can be achieved through techniques such as electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), atmospheric pressure photon ionization (APPI), coronal discharge, or by using radioactive sources like nickel-63. There are various designs of ion mobility spectrometers, including drift tube, ion trap, traveling wave, high-field asymmetric waveform, and differential mobility types. Drift tube IMS determines ion mobility by measuring the time it takes for ions to reach the detector, and many modern instruments utilize this method for analysis [**17**].

Interestingly, field asymmetric ion mobility spectrometry (FAIMS), a subtype of high-field asymmetric waveform IMS, uses a strong electric field to regulate ion flow through a physical filter. Then, ions with particular mobilities are subjected to a pulsed electric field. Ions that do not have the selected mobility will clash with the walls and not make it to the detector; only those that do can keep a steady path through the filter. IMS can quickly and accurately detect any small molecule of drug substance. FAIMS sensitivity relies on various characteristics of both the ion of interest and the surrounding physical environment. IMS can detect concentrations as low as one molecule in a billion (ppb) and is highly selective. This selectivity is further enhanced when using FAIMS, which can function effectively in environments with high levels of interference with minimal adjustments to operating conditions. IMS is non-destructive and requires only a minuscule sample, even when quantitative methods necessitate destructive testing. The determination process is rapid, often completing within seconds, even for complex samples [**18**].

IMS instruments do not require a trained operator, allowing for rapid sample analysis. However, identification of the sample necessitates a database of known molecules for comparison. Creating this database demands the expertise of a trained chemist using alternative techniques or established standards. Once established, the database can be accessed by any instrument without further technical assistance. Qualification is achievable through the use of internal standards or prebuilt methods. IMS is frequently utilized by law enforcement agencies at airports to detect narcotics and explosives. Its minimal maintenance, ease of use for non-technical personnel, low cost, fast and accurate results, low consumable costs, and robust methodologies make IMS a preferred choice for drug identification.

RAMAN SPECTROSCOPY

Raman spectroscopy is an optical technique that relies on the inelastic scattering of radiation when it interacts with matter. This interaction provides spectral vibrational information about the substance

being analyzed. The process involves shining a laser onto a sample and detecting the scattered light. Because of electromagnetic and chemical interactions in the sample, a tiny percentage of the dispersed light undergoes an energy shift from the laser frequency. A Raman spectrum of the sample is produced by charting the intensity of the shifted light against frequency [**19**].

An important development in this area is the development of portable, handheld Raman spectrometers. Thermo Fisher Scientific's devices, which have a straightforward "point and shoot" mechanism, are especially designed for identifying substances of abuse. In addition to giving visual readouts of compounds detected, these devices have real-time database access capabilities.

Raman spectroscopy can identify virtually any drug, including active pharmaceutical ingredients (APIs) as well as molecules with the same chemical formula but different arrangements, such as isomers, derivatives, and analogues of classical drugs of abuse. This capability is particularly important due to the emergence of novel psychoactive substances. The ability to distinguish between minor variations in physical or chemical structures greatly enhances unambiguous identification. Portable Raman spectroscopy has even been reported to detect the date-rape drug (flunitrazepam) in spiked beverages. However, Raman spectroscopy may struggle with substances that exhibit strong fluorescence, often found in plant-based narcotics like heroin. Yet, proper sample preparation can enable the analysis of these substances. The device has demonstrated a high correlation with laboratory results (MS) for cocaine, heroin, and methamphetamine; inconclusive results typically arise from the presence of drug substances at very low concentrations in the mixture. Some studies have shown that cocaine can be detected at concentrations as low as 5% when cut with sorbitol. Additionally, residues of amphetamine (in milligrams to micrograms) have been detected on paper currency using Raman spectroscopy.

It is crucial to remember that while the technology offers very precise and intuitive qualitative assessment, it does not supply numeric data during its "point and shoot" identification procedure. All things considered, even when pollutants and adulterants are present, Raman spectroscopy can detect and measure a large variety of medications. Considering the different compounds used to "cut" drugs, this characteristic is essential. Chemical reagents are not needed for the quick, non-destructive method known as Raman Spectroscopy (RS). Importantly, it can assess products through transparent packaging like glass containers and plastic bags. It can also identify distinct chemicals in mixes and is not impacted by moisture or water. While little to no sample preparation is generally needed, some substances with high fluorescence, including certain cutting agents, may require preparation. RS is suitable for both organic and inorganic compounds and can be employed for qualitative and quantitative analysis. However, like Infrared (IR) spectroscopy, RS faces challenges with quantitative analysis. Although quantitative analysis is feasible with Raman spectroscopy, it can be complex and may not be practical in harm reduction settings. Given the difficulties of quickly performing quantitative analysis on numerous unknown samples, it is important to note that portable handheld devices specifically designed for detecting drugs of abuse are available. Qualitative results can be obtained in mere seconds to a few minutes [**20**].

Like IR, the level of skill needed to use this technology varies per device. Certain Raman spectrometers are made for "point and shoot" use, which requires only rudimentary to intermediate knowledge for presumptive analysis and yields obvious interpretations of the compounds tested. IR and quantitative analysis using portable "point and shoot" Raman spectrometers have similar needs. According to research some portable Raman spectrometers are capable of providing highly accurate quantitative analysis. It's crucial to remember that this has the same limitations as portable infrared devices. In contrast, benchtop or laboratory-specific devices tend to be more complex and may necessitate database searching and result interpretation, which can elevate the required level of expertise to intermediate, advanced, or expert, depending on the chosen device.

COLORIMETRIC TESTS

Spot/color tests provide presumptive testing through chemical reactions between analytes and indicators. Numerous indicator tests exist, including cobalt thiocyanate, nitric acid, para-dimethylaminobenzaldehyde, and ferric chloride. The indicator reacts chemically with the analyte, producing a color change specific to the substance tested. These color spots are then visually compared to reference charts, with the Munsell color charts being the current standard. An innovative method utilizes a smartphone app to identify colors with high precision, supported by software that matches results in a searchable database. This approach enhances accuracy by providing more precise color quantification [**21**].

Cocaine, other prescription opioids, amphetamines, LSD (lysergic acid diethylamide), cathinones (bath salts), heroin, and fentanyl are among the majority of drugs of abuse that can be detected by

colorimetric assays. It's possible that some new psychoactive compounds may not yet have colorimetric tests linked to them. The analytes that each particular test can identify are listed, but it's crucial to remember that the sample is destroyed during the testing process. Thankfully, color tests only need a tiny portion of the sample; anything that can be seen can be examined.

Depending on the particular spot test and analyte utilized, these tests can have detection limits in the microgram range and be quite sensitive. With appropriate standards, these tests can be quite specific, though achieving high specificity may require multiple analyses. Knowledge of the expected substance and its general appearance can further enhance specificity. Colorimetric tests are considered presumptive because they can only indicate the presence or absence of a specific substance based on the administered test. Each test or reagent is designed to detect either the presence or absence of a particular drug or class of drugs. A single test may not be adequate for analyzing a suspected or unknown mixture, especially if there are any concerns about the substance in question. Below is an example of a protocol for testing suspected mixtures. Actual color results may vary depending on factors such as concentration, whether the drug is in salt or free base form, and the presence of additional diluents or contaminants. While a positive result may suggest a specific drug or class of drugs, it does not always confirm a single substance. Colorimetric tests utilize straightforward chemical reactions to produce visible results that can be interpreted visually.

The reagents and laboratory materials required for these tests are inexpensive and readily available, making them accessible to users with minimal training. However, since color perception is subjective and lighting conditions in non-laboratory settings can vary, the accuracy of the results can be significantly improved by using smartphone apps that provide quantitative assessments of color test results. The skill level required ranges from basic to intermediate. A basic user can perform a simple test and obtain results, while an intermediate user would follow a standard protocol. For example, an intermediate protocol might involve running a battery of tests based on the amount of sample collected without objection from the user. These tests should be guided by educated guesses, narrowing down possibilities through analysis and questioning [**22**].

Tests should be interpreted within a maximum of 10 minutes. They can be analyzed using a smartphone or, if that is not available, under good lighting conditions to ensure accurate color determination. The results can then be compared against a database if a computer or internet access is available. With such a system, a presumptive test can become a significantly more powerful tool.

IMMUNOASSAY

Immunoassay is a technique that involves the binding of an antibody, which is selective for a specific drug or drug group (the antigen), along with a detectable label that becomes part of the antibody-antigen complex (such as a fluorescent marker). The immune system's normal reaction, in which antibodies in biological tissues bind to antigens to neutralize or eradicate them, is mimicked by this binding. Since immunoassays were first created to evaluate biological materials, mainly metabolites in urine, they are rarely utilized for drug analysis. As a result, although immunoassays give doctors useful information about their patients, they cannot identify the kind or quantity of a drug before it is consumed or administered. To find an analyte in liquid samples, ELISA can be used for various biochemical tests. There isn't much academic research on the particular medications that ELISA can identify outside of biological samples (post-ingestion/metabolization).

Immunoassay technology can rapidly and effectively detect various opioids and cocaine. However, issues with specificity can lead to false positives due to structural similarities between drugs or their metabolites. The sensitivity of these assays is quite high, allowing for detection in the microgram range as antibody-antigen interactions occur at the molecular level [**23**]. Immunoassay is a fast and relatively inexpensive method that typically requires only basic scientific knowledge for execution and interpretation. Running these tests may demand an intermediate skill level. Nonetheless, there is a scarcity of scientifically published information on the use of immunoassays for whole drug analysis. These assays are most commonly employed to detect drug use post-incident, such as in urine drug screens.

URINE DIPSTICK TEST

This method has recently gained attention as a cost-effective, easy-to-use presumptive test for fentanyl. In this approach, a sample of the drug is dissolved in water, and if fentanyl is present above a specified cutoff concentration, a visual indicator on the strip will change. The method operates via chromatographic immunoassay, and the strip changes color in the presence of the appropriate analyte. Currently, fentanyl is the only drug reported to be tested using this method, and there is limited published data regarding its efficacy. While the strips are designed to detect fentanyl in urine and are specific to this substance and its metabolites, there

is no scientific data available on their sensitivity.

The provided sample is destroyed during testing. Urine dipsticks are user-friendly, quick to interpret, specific for fentanyl, and have recently been shown to be effective in identifying unknown drug mixtures containing fentanyl. However, since dipsticks were designed for urine detection, there is potential for false positives in other mediums due to low specificity. Additionally, many retailers restrict sales to health professionals, making it challenging for harm reduction agencies to obtain these tests unless affiliated with a health clinic. Some medical device companies may also object to using such tests in harm reduction settings due to liability concerns, even with qualified health professionals present [**24**].

THIN-LAYER CHROMATOGRAPHY (TLC)

A sample is placed on a planar stationary phase in thin-layer chromatography (TLC), which is followed by a liquid mobile phase that promotes capillary action. The retention time of the analyte depends on how long it is in each phase, whether it is adsorbed to the stationary phase or remains in the mobile phase. Depending on their size and affinity for the mobile phase, the sample's constituent parts move at different speeds. A plate with dots of the separated components that have traveled varying distances on the stationary phase is the result.

Benzodiazepines, barbiturates, GHB, heroin, morphine, opium, oxycodone, other opiates, amphetamines, cocaine, methamphetamine, ecstasy, ketamine, LSD, marijuana, mescaline, synthetic cannabinoids, and cathinones are among the compounds that TLC can detect. However, separating and identifying novel psychoactive substances can be challenging. TLC may perform poorly with complex mixtures, with sensitivity in the micro-nanogram range. Specificity can vary from intermediate to high, depending on the mixture, and retention factors can provide preliminary substance identification but are not unique to a single compound. To enhance specificity in cases with similar retention factors, TLC should be combined with techniques such as Raman spectroscopy, colorimetric testing, or UV analysis for UV-active species [**25**].

TLC is a cost-effective method for testing substances, offering good sensitivity and rapid separation. It can serve as a presumptive test with a relatively high degree of accuracy, depending on the purity of the sample. While TLC can identify certain known substances in the samples provided, it does not quantify the amount present. For optimal results, TLC should be combined with more precise techniques, such as Raman spectroscopy, mass spectrometry (MS), or infrared spectroscopy (IR).

TLC is straightforward to use and interpret, making it suitable for users with basic to advanced skill levels. A novice may follow instructions to perform a test but may struggle with result interpretation. In contrast, someone with intermediate to advanced skills will be better equipped to interpret the results and supervise less experienced users.

ULTRAVIOLET SPECTROSCOPY

This method relies on the absorption of light energy in the ultraviolet (UV) wavelength range. Light in this range can elevate the energy levels of electrons within a molecule from their ground state to higher energy levels. Each transition to a higher energy level necessitates a specific amount of energy, which is provided by light at particular wavelengths. By using a specific wavelength, a characteristic UV absorption spectrum can be obtained, reflecting the electronic structure of the molecule. This structure determines which wavelengths are absorbed and which pass through the sample. UV-visible (UV-vis) spectrophotometers measure the intensity of light passing through a sample and compare it to the intensity of light before it enters the sample, capturing this data to create a characteristic spectrum.

The UV spectra of drugs may be same if their structures are comparable. MDMA, ketamine hydrochloride, cocaine hydrochloride, diazepam, phenobarbital, and barbital concentrations in the microgram range have all been effectively detected using UV-vis spectroscopy. Additionally, it has successfully distinguished six distinct chemicals and, for the first time, some mixes. Although other chemicals might be identified, there isn't much research to support the technique's wide application for different illegal narcotics. Although UV spectrometry is non-destructive and can be used on solid materials, some samples may need to be prepared in a way that makes them unfit for use in other processes. UV gives rough structural information with low selectivity for some discriminating capabilities, and it can be utilized both qualitatively (for identification) and quantitatively (for measuring amounts).

Selectivity and specificity can be improved by combining chromatographic methods with UV spectroscopy. However, because samples must be diluted to prevent saturated spectra, it is not the best method for identifying numerous medicines in a mixture. Without further sample preparation, compounds lacking appropriate chromophores do not generate a signal, making UV-vis analysis more difficult. However, because of the existence of aromatic ring structures in their chemical composition, the majority of drugs of abuse contain appropriate chromophores. Furthermore, the pH of

the sample solution might affect the UV spectrum, and during analysis, the chemical composition may alter [**26**]. The expertise required for UV analysis ranges from basic to advanced. While the technique can be taught easily to individuals with minimal theoretical knowledge, interpreting the results necessitates intermediate to advanced understanding.

CONCLUSION

There are a number of factors to take into account when choosing technology for drug checks in harm reduction. In addition to assessing the tests' quantitative capabilities, harm reduction organizations that want to offer drug testing services must also determine if they have the financial and human resources to support the technology. As a result, the suggestions stress a thorough cost-benefit analysis and bring up the crucial issue of whether some less accurate interventions are better than none at all. The guidelines that follow will describe point-of-care drug testing procedures while keeping these considerations in mind. The strongest candidates for drug testing techniques, considering all factors, are ion mobility spectrometry (IMS), infrared (IR) spectroscopy, Raman spectroscopy, and spot/color tests, each with its own limitations. Spot/color tests are purely presumptive, and quantification often depends on expert interpretation. In some cases, the therapeutic index is so narrow, and the amounts involved so small, that only the most precise techniques can definitively establish whether the quantity falls within the therapeutic range (i.e., sufficient to produce a high but not fatal effect, barring other circumstances).

For drug identification and quantification, a variety of proven methods are available, each with unique benefits and drawbacks. This review is intended to serve as a useful manual for the application of these techniques and suggestions for point-of-care harm reduction, not to offer a comprehensive scientific examination of them. This paper is intended to assist in reducing the number of possibilities available for each methodology, enabling clinics to select a more manageable subset of approaches for possible use. Since many of these technologies are now exclusively utilized in research and law enforcement, clinics with funding resources might think about contacting the manufacturers to propose their use in harm reduction contexts.

***Author(s) Contribution**:* resources and review; E. Ayesha.

Funding: No funding involved.

***Ethical* Approval:** Not applicable

Conflict of Interest: Nil.

Consent for Publication: All authors approved the manuscript for publication.

REFERENCES

1. Canadian Community Epidemiology Network on Drug Use. Deaths involving fentanyl in Canada, 2009–2014. Ottawa: Canadian Centre on Substance Abuse; 2015.
2. Canadian Institute for Health Information. Hospitalizations and emergency department visits due to opioid poisoning in Canada. Ottawa: Author; 2016.
3. Mounteney J, Griffiths P, Sedefov R, Noor A, Vicente J, Simon R. The drug situation in Europe: an overview of data available on illicit drugs and new psychoactive substances from European monitoring in 2015. Addiction. 2016;111(1):34–48.
4. European Monitoring Centre for Drugs and Drug Addiction. An inventory of on-site pill-testing interventions in the EU: fact files. Lisbon: European Monitoring Centre for Drugs and Drug Addiction; 2001.
5. Butterfield RJ, Barratt MJ, Ezard N, Day RO. Drug checking to improve monitoring of new psychoactive substances in Australia. Med J Aust. 2016; 204(4):144–5.
6. Brunt TM, Nagy C, Bücheli A, Martins D, Ugarte M, Beduwe C, Ventura Vilamala M. Drug testing in Europe: monitoring results of the Trans European Drug Information (TEDI) project. Drug Test Anal. 2017;9(2):188–98.
7. Tsai JSC, Lin GL. Drug-testing technologies and applications. In: Wong RC, Tse HY, editors. Drug-testing technologies and applications. Totowa: Humana Press; 2005. p. 29–69.
8. Cody RB, Laramee JA, Nilles JM, Durst HD. Direct analysis in real time (DART) mass spectrometry. JEOL News. 2005;40(1):8–12.
9. Forsgard N, Salehpour M, Possnert G. Accelerator mass spectrometry in the attomolar concentration range for 14C-labeled biologically active compounds in complex matrixes. J Anal At Spectrom. 2010;25(1):74–8.
10. Manimala YS, Gautam S, Reddy BG. Mass spectrometry: an analytical method. J Pharm Anal. 2016;5(2):118–25.
11. Verkouteren JR, Staymates JL. Reliability of ion mobility spectrometry for qualitative analysis of complex, multicomponent illicit drug samples. Forensic Sci Int. 2011;206(1–3):190–6.
12. Sisco E, Verkouteren J, Staymates J, Lawrence J. 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. 2017;4:108–15.
13. Bunaciu AA, Aboul-Enein HY, Fleschin S. Application of Fourier transform infrared spectrophotometry in pharmaceutical drugs analysis. Appl Spectrosc Rev. 2010;45(3):206–19.
14. Sorak D, Herberholz L, Iwascek S, Altinpinar S, Pfeifer F, Siesler HW. New developments and applications of handheld Raman, mid-infrared, and near- infrared spectrometers. Appl Spectrosc Rev. 2012;47(2):83–115.
15. de Oliveira Penido CAF, Pacheco MTT, Lednev IK, Silveira L. Raman spectroscopy in forensic analysis: identification of cocaine and other illegal drugs of abuse. J Raman Spectrosc. 2016;47(1):28–38.
16. Ali EMA, Edwards HGM. The detection of flunitrazepam in beverages using portable Raman spectroscopy. Drug Test Anal. 2017;9(2):256–9.
17. Frederick KA, Pertaub R, Kam NWS. Identification of individual drug crystals on paper currency using Raman microspectroscopy. Spectrosc Lett. 2004; 37(3):301–10.
18. Engh RA. X-ray crystallography: basic principles. In: Encyclopedic reference of genomics and proteomics in molecular medicine. Berlin, Heidelberg: Springer Berlin Heidelberg; 2006. p. 2026–9.
19. Rendle DF. X-ray diffraction in forensic science. Rigaku J. 2003;19(2):11–22.
20. Trzybiński D, Niedziałkowski P, Ossowski T, Trynda A, Sikorski A. Single-crystal X-ray diffraction analysis of designer drugs: hydrochlorides of metaphedrone and pentedrone. Forensic Sci Int. 2013;232(1):e28–32.
21. Elie MP, Elie LE. Microcrystalline tests in forensic drug analysis. In: Meyers RA, editor. Encyclopedia of analytical chemistry, vol. 1. Hoboken: Wiley; 2009. p. 471–81.
22. Kanai K, Takekawa K, Kumamoto T, Ishikawa T, Ohmori T. Simultaneous analysis of six phenethylamine-type designer drugs by TLC, LC-MS, and GC- MS. Forensic Toxicol. 2008;26(1):6–12.
23. O'Neal CL, Crouch DJ, Fatah AA. Validation of twelve chemical spot tests for the detection of drugs of abuse. Forensic Sci Int. 2000;109(3):189–201.
24. Elkins KM, Weghorst AC, Quinn AA, Acharya S. Colour quantitation for chemical spot tests for a controlled substances presumptive test database. Drug Test Anal. 2017;9(2):306–10.
25. Dasgupta A. Clinical chemistry: analysis of drugs of abuse. In: Meyers RA, editor. Encyclopedia of analytical chemistry, vol. 2. Hoboken: Wiley; 2009. p. 1242.
26. Li Q, Qiu T, Hao H, Zhou H, Wang T, Zhang Y, Li X, Huang G, Cheng J. Rapid and on-site analysis of illegal drugs on the nano-microscale using a deep ultraviolet–visible reflected optical fiber sensor. Analyst. 2012;137(7):1596–603.