# Lab-on-a-Chip approaches for the detection of controlled drugs, including new psychoactive substances: A systematic review

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A B S T R A C T

According to the World Drugs Report (2019) from the United Nations on Drugs and Crime there were over half a million drug related deaths, 35 million people were treated for drug use disorders across the globe, and it is estimated that more than 270 million people used drugs during 2017. Lab-on-a-Chip (LOC) technology is an increasingly popular choice of detection method for drugs of abuse. We systematically reviewed the published literature available on LOC methods for the detection of drugs of abuse including New Psychoactive Substances (NPS) from January 1999 to March 2021 and identified 45 publications. A total of 28 different drugs of abuse were investigated, with cocaine the most widely studied (58%). The LOC devices were capable of accepting a wide range of biological and non-biological samples. A total of 18 countries have been involved in LOC research into detection of drugs of abuse, with locations generally following local trends in drug use. LOC devices employed a range of detection methods with immunoassays most commonly incorporated (34%). Recommendations are made for expanding the use of real-world samples, improved validation and further analysis of practicality (in terms of providing information on cost, speed of analysis and ease of use). More than a third of all the publications included in this review were published since 2019, representing a recent increase in research using LOC devices for the detection of drugs of abuse. There is currently an extensive range of LOC approaches available offering potential for these devices as cost-effective, rapid and portable detection systems.

## Introduction

Figures published by the United Nations on Drugs and Crime (UNODC) World Drugs Report (2019) stated that in 2017, there were around 585,000 drug related deaths as a result of 271 million people abusing drugs worldwide [1]. This highlights the significant number of individuals taking drugs, but also the significant number of deaths globally as a direct consequence of drug use. Well established drugs of abuse are very prevalent, with levels of global cocaine production at their highest to date, with 1976 tons reported in 2017 (a 25% increase from 2016) [1]. There has also been an increase in polydrug use and disorders, for example over 65% of cocaine drug users required treatment for other substances including cannabis and alcohol [1]. More recently, new psychoactive substances (NPS) are finding prominence as drugs of abuse. These issues highlight a requirement to have rapid detection methods for both NPS and drugs of abuse, for which there is currently no standardised approach.

### *New psychoactive substances (NPS)*

NPS produce a psychoactive effect when taken and are relatively new to the recreational drugs market. NPS are not collectively listed under the International Drug Control Conventions, with drug legislation varying from country to country, but they do present a significant risk to public health [2]. NPS exhibit similar biological and pharmacological activity to established drugs of abuse, such as cannabis and amphetamines, but less is known about the pharmacology and potential health risks [3,4]. The most recent report from the UNODC published in October 2020 states that there were 1004 different NPS across 125 countries since NPS first emerged on the recreational drugs market [5]. This represents an increase in 54 NPSs and an additional 5 countries from the previous report [6], with NPS use exhibiting region specific trends throughout the world [1,7,8].

In the World Drugs Report (2019) [1], the largest group of NPS present in the global recreational drugs market were stimulants,

comprising of 34% of those available, followed by opioids (29%), and then synthetic cannabinoids (24%). Stimulant NPS mimic the effects of established stimulant drugs of abuse such as amphetamine, cocaine, 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine. Mephedrone (4-methylmethcathinone or 4-MMC), methylone, and 3,4-methylenedioxypyrovalerone (MDPV) are examples that have successfully established a place on the recreational drugs market [8]. In Canada and the United States (US) there is an issue with opioid use, in particular fentanyl derivatives of synthetic opioids [6], and this has led to an increase in the number of deaths reported due to overdose [7]. The third largest group of NPS is synthetic cannabinoids, also known as synthetic cannabinoid receptor agonists, which act on the cannabinoid receptor 1 ($CB_1$) and cannabinoid receptor 2 ($CB_2$) receptors and mimic the effects of cannabis [7]. There are a number of synthetic cannabinoids on the recreational drugs market, such as JWH-018, AB-FUBINACA, and 5F-APINACA, which have been monitored by the UNODC since 2009 [7]. There are high levels of use within prisons and the homeless community worldwide, but particularly within the United Kingdom (UK) [7]. With the number of available NPS constantly increasing, this poses challenges in detecting these substances in order to keep up with determining the current trends.

A previous review by Smith *et al.* discussed methods for the detection of different types of NPS [9]. Many of these methods involved the use of gas chromatography-mass spectrometry (GC–MS) [10–11] or high performance liquid chromatography-mass spectrometry (HPLC-MS) [12–14]. As a result, these detection methods are often non-portable, expensive and require specialist facilities and staff to operate.

Although legislation for NPS differs throughout the world [1], there is a current and timely global requirement for developing rapid drug detection tests that are accurate, portable and cost effective to aid identifying NPS, and other drugs of abuse. This would be especially useful within Accident and Emergency (A&E) departments, prisons, police departments and for occupational drug testing. A Lab-on-a-Chip (LOC) based testing system has the ability to meet these requirements compared to conventional laboratory techniques.

### *Lab-on-a-Chip (LOC)*

An LOC device allows for multiple laboratory-based analytical techniques to be miniaturised by incorporating microfluidic methodology. Microfluidics involves the manipulation of fluids within channels on a micrometre scale. This offers significant advantages over more traditional methodologies, but also enables new developments, which would not be possible on a larger scale. These wide-ranging advantages include: cost-effectiveness in terms of equipment needed and lack of specialist facilities/staff, reduced sample requirements, reagent consumption, waste effluent and sample requirements, faster reaction times (due to a larger surface area to volume ratio), and increased portability [15]. In recent years, the field of microfluidics has become an extremely multidisciplinary area of research.

While the development of fully integrated 'sample in-answer out' LOC devices have focussed on fields such as clinical diagnostics [15], an example of a completely integrated LOC for forensic purposes is the RapidHIT® ID System that analyzes buccal swab samples for human identification purposes and can produce a DNA profile in just 90 min [17]. The first research journal article reporting a fully integrated LOC device capable of producing a DNA profile was published by Hopwood *et al* in 2010 [18], and from this publication to the first commercially available LOC system it took approximately two years. Since the introduction of the Rapid DNA Act of 2017, such LOC technology has been used by law enforcement for the analysis of reference samples [18]. This demonstrates that LOC systems have the potential to be used effectively as part of forensic investigations. With that in mind this review identifies the current state of LOC methods for the detection for drugs of abuse and NPS.

### *Aims*

This study presents a systematic review of the use of LOC devices for the detection of controlled drugs. This has been achieved through:

- A review of the different drugs investigated by LOC methods (Section 3)
- A review of sample matrices and sample types analysed by LOC devices (Section 4)
- Global trends on research into LOC use for drug detection (Section 5)
- A comparison of LOC detection methods with regards to manufacturing materials, limits of detection and analysis time (Section 6)
- Identification of knowledge gaps and recommendations for future research (Section 7)

## Methodology

Using the key criteria for a systematic review [19], a literature search of peer-reviewed articles published from 1999 to March 2021 was conducted using the Scopus database. Due to the number of non-relevant publications exceeding 1000 for other search engines, such as Google Scholar and Web of Science, Scopus was the only one included in this systematic review. The following four search terms were used:

1. "LOC" OR "lab-on-a-*" OR "microfluidic*" AND "detection" AND "drug* of abuse" OR "new psychoactive substance*" OR "controlled drug*"
2. "lab-on-a*" OR "LOC" OR "microfluidic" AND "detection" AND "legal high*" OR "cathinone*" OR "cannabinoid*" OR "illegal drug*" OR "illicit drug*" OR "opiate*" OR "opioid*"
3. "portable" OR "handheld" OR "disposable" OR "presumptive*" AND "detection" AND "drug* of abuse" OR "controlled drug*" OR "illegal drug*" OR "illicit drug*" OR "legal high*" OR "cathinone*" OR "cannabinoid*"
4. "portable" OR "handheld" OR "disposable" OR "presumptive*" AND "detection" AND "new psychoactive substance*" OR "opiate*" OR "opioid*"

A total of 451 publications were identified. An initial suitability screen of titles and abstracts was performed with the following inclusion criteria; must be a publication containing primary research data incorporating an LOC device or a device with a microfluidic component for the detection of drugs of abuse/NPS. All other forms of literature, such as case reports, were not included, and the only literature included had to be published in a peer-reviewed journal. The publications were considered from any country, but needed to be published in English. This process resulted in the identification of a total of 87 manuscripts which were subject to a more rigorous full review. Duplicate manuscripts (from the 4 different combinations of search terms) were removed and the remaining papers screened for suitability. Each paper was reviewed blindly by two individuals and the following information gathered: LOC method detection, drug(s) investigated, LOC material, biological specimen(s) or sample, detection time, description, limits of detection (LOD) and limits of quantification (LOQ). Following all the essential steps of the method for this systematic review Supporting Informationresulted in a total of 45 publications included in this study (See Supplementary Information – Table S1).

## Drugs of abuse analysed by LOC methods

A total of 28 different drugs of abuse including NPS were reported in the 45 accepted publications. Fifty-five percent of publications reported more than one drug of abuse, reflecting the ability to perform multiplex detection. Four main drugs (cocaine, methamphetamine, morphine, amphetamine) have been detected in more than 11 of the publications,

all of which have a high level of abuse which is reflected by the latest reports on drug trends [1,7,8] (Fig. 1). These were followed by drugs of abuse that were less commonly investigated ($\Delta^9$-tetrahydrocannabinol, $\Delta^9$-THC), codeine, ketamine, MDMA, heroin, benzoylecgonine), which were reported in between 9% and 16% of the accepted publications. Then the final group includes several drugs of abuse, metabolites, precursors and NPS that were reported in between 2% and 7% of publications. Importantly, it is worth noting that the existing literature has focused on the detection of the more established drugs of abuse, with only three publications reporting the detection of NPS[20].

The most commonly investigated drugs of abuse using LOC devices reflected ongoing global trends in drug prevalence. Cocaine was the most investigated drug of abuse using LOC detection methods and was reported in 58% of the publications accepted in this review. This follows the global trends with cocaine being the main stimulant used in North and South America, as well as Central and Western Europe, Australia and New Zealand, with 19 million users globally [21]. Twenty seven million users of amphetamines were reported in the latest World Drug Report, overall globally encompassing the most popular group of stimulant drugs [21]. This correlates with methamphetamine and amphetamine being the second and fourth most reported drugs of abuse for LOC detection at 38% and 24% of publications, respectively. Methamphetamine dominates the manufacture of amphetamines [8,21] and this is reflected in the slightly higher proportion of publications detecting methamphetamine in comparison to amphetamine. Morphine was the most commonly reported opiate, the third most commonly reported drug of abuse and was targeted for LOC detection by 29% of publications. Morphine continues to be one of the most commonly abused opiates throughout the world [1,7,21,22]. However, with the increase in more potent opioids available on the recreational drug market, such as fentanyl and carfentanil, and the likely increase in resulting deaths globally [1,16,21,22], it is probable that trends in the use of LOC detection methods will change in response.

For some drugs of abuse, their widespread global prevalence is not reflected in the number of publications related to their analysis using LOC devices. $\Delta^9$-THC is the main psychoactive substance in cannabis, which was reported as the most commonly abused drug worldwide, with 192 million users estimated in 2018 [21], yet only 16% of articles reported $\Delta^9$-THC detection. Codeine and ketamine were included in 14% of the accepted publications. Ketamine is not currently under international control, but is a widely abused drug of abuse and is the main hallucinogen seized internationally accounting for 87% in the last five years, mostly from East and South Asia. Heroin is one of the most commonly used established drugs of abuse worldwide since it emerged into the recreational drugs market in the 1960's and is still one of the most abused opiates throughout the world, but there were only 4 publications detecting heroin included in this review [23–25,35]. With over 66% of all globally reported drug-related deaths were related to opioid use [1], overall there were six opiates (36%) and four opioids examined, with the most commonly detected being morphine and codeine, respectively. With a distinct gap in the detection of $\Delta^9$-THC, hallucinogens and opioids this indicates that perhaps research into detection using LOC do not necessarily follow the global drug use statistics, but more the societal and health impacts.

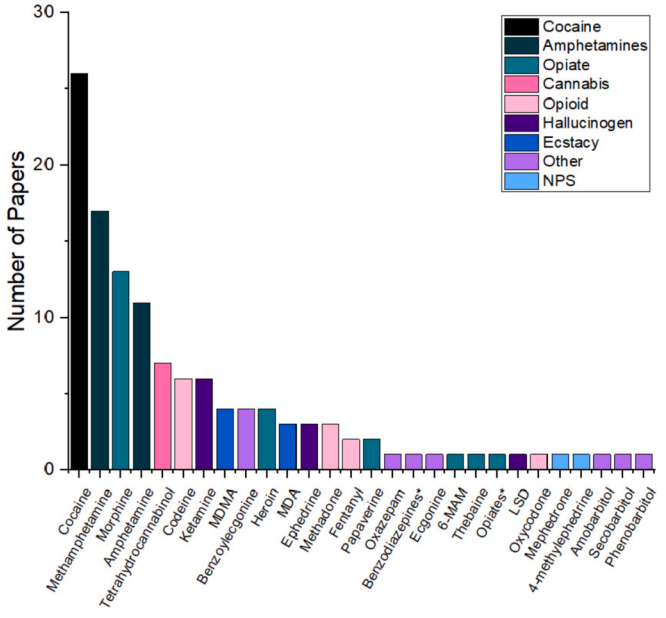


**Fig. 1.** Number of publications for each compound detected in the publications categorized according to the drug definitions used by the UNODC. *Li *et al* (2015) states opiates and benzodiazepines, however further clarification is not provided.

A review of the existing literature shows that there is also significant gap in research using LOC technology for NPS detection with only 3 out of the 45 publications investigating NPS. Two of the articles detected fentanyl using Surface-Enhanced Raman Spectroscopy (SERS)-based methods [24,27], and one used a paper-based competitive immunoassay LOC device for the detection of mephedrone and its metabolite, 4-methylephedrine [20]. As the articles were published between 2019 and 2021, this may hint at a potential increase in the number of future publications investigating the detection of NPS, as worldwide prevalence increases.

*Cutting agents, diluents, adulterants and pro-drugs*

Cross-reactivity can have a significant effect on the accuracy of any drug detection methods, however, only 29% of the publications investigated the potential cross-reactivity of cutting agents, diluents, adulterants or pro-drugs using an LOC device [20,29–37]. The most extensive research on cross-reactivity has been carried out on those devices which utilise colorimetric detection. Sixty-four compounds were investigated for their potential interference in the colorimetric detection of four different drugs of abuse (cocaine HCl, crack cocaine, heroin and methamphetamine) using a paper-based LOC device [35]. Of these 64 compounds, there were only five that reacted, including levamisole, Xanax®, and procaine. Musile *et al* (2015), investigated the effects of four cutting agents, six diluents and eight common powders using an LOC device for multiplex detection of nine drugs of abuse using presumptive testing reagents [31]. False positives for baking soda, caffeine, procaine and quinine were observed [31]. Da Silva *et al* (2018) recorded false positives for paracetamol when investigating the cross-reactivity of the adulterant, phenacetin, as well as six commonly encountered cutting agents with a colorimetric LOC device for the detection of cocaine [30], as well as a 10% colour suppression for both procaine and aminopyrine [30].

When cross-reactivity was examined in LOC devices that incorporated immunoassay-based detection systems, the results showed a lack of cross-reactivity as would be expected due to the specificity of the antibody-antigen interaction. Krauss *et al* (2016) investigated the cutting agents acetylsalicylic acid (aspirin), caffeine, dextrose and lidocaine and observed no cross-reactivity with cocaine and methamphetamine [29]. While Bell and Hanes (2007) investigated the use of five commonly encountered cutting agents (aspirin, caffeine, dextrose, lidocaine, and starch) and showed no cross-reactivity when detecting amphetamine, cocaine, methamphetamine and oxycodone [32]. A combination of 11 adulterants, interferents and cutting agents were investigated using a competitive immunoassay by McNeill *et al* (2021) with no cross-reactivity identified in detecting mephedrone and its metabolite 4-methyephedrine [20].

In terms of electrochemical systems, Yehia *et al* (2020) investigated the effects of 6 interferents commonly encountered in beverages when producing a LOC device for detection of ketamine in spiked drinks and

found that tryptamine and phenylethylamine affected potentiometric detection [37]. Ameku *et al* (2021) could successfully detect cocaine adulterated with 4-dimethy-aminoantipyrine using electrochemical detection but some cross-reactivity was observed with other cutting agents such as lidocaine and levamisole [33]. A paper-based electrochemical LOC device used for the detection of lysergic acid diethylamine (LSD) investigated interference testing with three compounds: methamphetamine showed no response, MDMA showed a well separated peak from LSD, but the structurally similar lysergic acid amide was indistinguishable due to similar voltametric peaks [34].

The publications that investigated the effects of cutting agents, diluents, and adulterants identified some degree of cross-reactivity with the LOC devices. This is predominantly because the detection mechanisms investigated here were mostly colorimetric (59%), so can be influenced by coloured impurities and reactivity with reagents. Due to the potential issues with cross-reactivity, it is important that assessments are performed when validating a new LOC device, in order to avoid false positive or false negative responses. However, from reviewing the available literature it appears that this is not always the case across a wide variety of different detection methods and is something that should be always be considered in such research to validate efficacy. These issues are not specific to the LOC devices though as standard colorimetric tests would also experience the same cross-reactivity, yet the LOC devices offer the potential to include in-built controls, analysed in parallel, that could identify these and therefore enable the LOC device to be more reliable and accurate.

## Sample matrices

LOC devices have been used to detect drugs of abuse including NPS in five different biological matrices (urine, oral fluid, plasma/serum, sweat and hair), as well as in powder form or in aqueous solution (Fig. 2). Twenty-four percent of the publications studied more than one type of sample matrix, reflecting the adaptability of the LOC device to different types of sample matrices.

### *Non-biological matrices*

Aqueous solutions were the most commonly used matrices in more than half of the publications (51%) and demonstrated using all the different types of detection methods discussed in this review. Aqueous samples offer advantages as these solutions can be representative of bulk or seized drug samples, and can be easily prepared by dissolving the analyte in solution. A variety of non-biological matrices were reported including water [20,34,36,38–42], acetonitrile [25,29,34], phosphate buffered saline (PBS) [44–47], methanol [27,34], combination of acetone and deionised water [31], 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer [48] well as beverages including energy drinks [37] and fruit juices [37] to represent 'spiked drink' samples. Only 7% of the publications using LOC devices tested powder drug samples. This is likely because the design of the sample interface with the LOC device is more complex for powdered samples compared to liquids that can be added to the device more easily. One disadvantage of these types of matrix is that they are not representative of biological samples, which may also include drug metabolites and additional interferants.

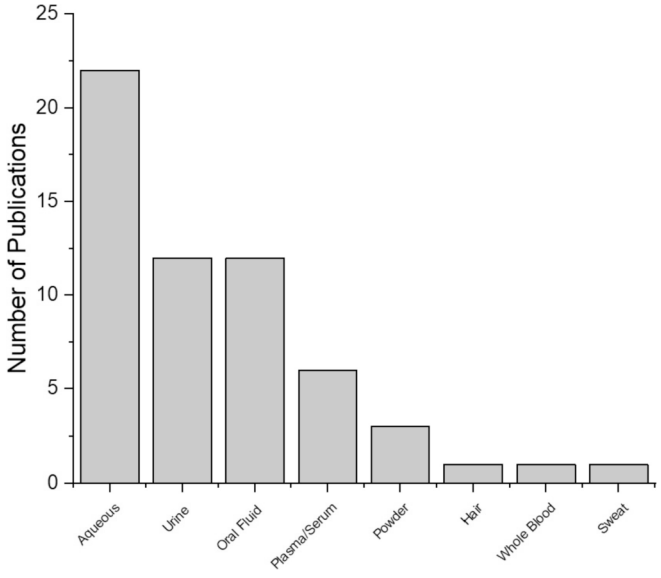


**Fig. 2.** The number of publications and the sample matrixes investigated.

### *Biological matrices*

While aqueous solutions were the most commonly used sample matrix, collectively biological matrices were included in the vast majority (58%) of publications testing drug of abuse including NPS using LOC devices (Fig. 2). Non-invasively collected samples (urine [20,23,38,47,54,57,64,65,67,73,74], oral fluid [28,38,45,51,66,70,71,74,85–87], sweat [69] or hair [68]) were predominantly analysed in over half of the publications (23 out of 45) as they are easier to collect, with urine and oral fluid in particular being compatible with current law enforcement practices [49]. Urine and oral fluid were the second most commonly used matrices included in 27% of the publications and were the most commonly encountered sample matrix in 50% of the immunoassay-based publications [28,38,45,50,51]. Only two publications focussed solely on an invasively collected biological matrix of plasma [52,72]. Three more publications demonstrated adaptability of their LOC devices in accepting multiple sample matrices including urine and plasma [54], oral fluid and plasma [50], and whole blood, plasma and urine [64]. The use of different matrices showed similar results for all these studies in terms of usability of the LOC device [50,54,62], for example Far *et al.* (2005), showed clinically similar levels of amphetamine in plasma (6 ng $mL^{-1}$) and urine (20 ng $mL^{-1}$) [54].

The analysis of biological samples matrices provides the opportunity to examine both the parent drug and any metabolites which can provide additional information on how much of the drug has been administered in order to aid both clinical and forensic analysis [49]. In addition the use of different biological samples allows for flexibility in testing with varying detection windows, with oral fluid (from hours), urine (days), sweat (weeks) to hair (months) [49]. For the drugs of abuse including NPS investigated, a total of eight metabolites were examined: two cocaine metabolites (benzoylecgonine, ecgonine methyl ester), benzodiazepine metabolite (oxazepam), three heroin metabolites (codeine, 6-monoacetylmorphine [6-MAM], and morphine), MDMA metabolite (MDA) and one of the metabolites of mephedrone (4-methylephedrine). These eight metabolites were reported in over a third (40%) of the overall publications, with morphine being the most widely detected. Aqueous solution was the most commonly encountered sample matrix in 39% of the publications. However, 22% of the publications that detected a metabolite included the use of urine as the biological matrix. Qiang *et al* (2009) detected eight drugs of abuse, including the parent drug heroin and two of its metabolites morphine and codeine in urine using capillary electrophoresis [23].

It is essential that when developing an LOC device for drug detection, that the time detection window is considered and appropriately matched to the sample matrices to be used. Across all drugs of abuse, the detection window for oral fluid is the shortest, while the detection window for hair is the longest (up to 90 days) [49]. For example, in this review cocaine was the most widely included drug of abuse and the detection windows are; urine (2–4 days), oral fluid (1–36 days), and hair (up to 90 days) [49]. Whereas, the metabolite of heroin, morphine was the third most widely used drugs of abuse and the detection windows are; urine (2–5 days), oral fluid (1–36 days), and hair (up to 90 days). With the detection windows only differing for urine from the parent drug heroin

(2–3 days), the metabolite offers the advantage of a slightly longer detection window. With all of the publications within this review detecting the drugs of abuse in 30 min or less, this highlights the advantageous speed of analysis of LOC detection for drugs of abuse including NPS.

LOC devices are available for a range of different sample matrices, but it is not possible to directly analyse all sample types with one universal device, as well as offering the opportunity for rapid analysis with short detection windows for some drugs that have a short half-life, such as 6-MAM. LOC devices are advantageous compared to traditional detection methods as they are portable which means they may encounter a range of sample matrices. Currently only 13% of publications that investigated the use of both biological and non-biological samples and it would be beneficial to LOC devices using both to capture the potential of the LOC devices that are being researched.

## Global prevalence

There were 18 countries affiliated to publications on the detection of drugs of abuse including NPS using LOC devices (Fig. 3), with the largest number of papers were published in North America (41%). It is perhaps not unexpected that the majority of publications are from more wealthy countries in terms of research capacity but here we explore links to specific drug prevalence's globally. Comparing the percentage of drugs of abuse in the six categories (amphetamines, cannabis, cocaine, ecstasy, opiates, and opioids) show a focus on amphetamines in North America (30%) whereas a focus on opiates in East and South-East Asia (29%).

### *North America*

North America accounted for the largest number at 41% of the total of publications in this review, with the second largest number of publications affiliated solely to the USA (37%) (Fig. 3). The highest global annual prevalence of cocaine use was observed in North America [21]. Between 2013 and 2017 cocaine seizures were reported in over 140 countries worldwide, however this review shows LOC technologies were only developed to detect cocaine in 11 different countries. In 2017, there were 238 tons of seizures in North America a dramatic increase from 94 tons in 2013 [1]. Half of the publications reporting the detection of cocaine affiliated USA and also 100% of the publications reporting the detection of its metabolite, benzoylecgonine. Highlighting that developing LOC devices that detect both the parent drug cocaine, and its main metabolite could be invaluable to addressing the high levels of prevalence in North America.

North America has the second highest prevalence for amphetamines, ecstasy and opioids [21]. The trends for publications are well linked to geographical use, as 70% of the publications reporting the detection of amphetamine were published in the USA. An increase in the stimulant methamphetamine use in North America [1,8], is supported with a large number (73%) of the publications detecting methamphetamine affiliated to North America. The trends in the scientific literature appear to follow this increased drug use as 89% of the methamphetamine studies from the US were published between 2015 and 2019. North America had the highest number of the publications reporting the detection of MDMA (75%) and MDA (67%). Seventy-five percent of the publications reported the detection of an opioid in North America which correlates with the significant number of overdose deaths (~70,000) linked to opioid use in 2018 [21]. The highest annual global prevalence of cannabis use was observed in North America [21], which is reflected with 71% of the publications reporting the detection of $\Delta^9$-THC published in the USA. However, it is worth noting that the overall number of publications in this review reporting the detection of $\Delta^9$-THC is relatively low in comparison to extremely global prevalence levels, which could be due to fewer health risks in comparison to other drugs of abuse.

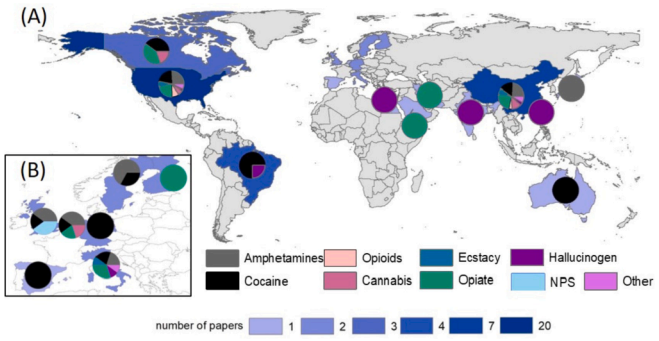


**Fig. 3.** Geological distribution of the accepted publications (all affiliations included) reporting the use of LOC for the detection of drugs of abuse including NPS, with pie charts illustrating the classifications of the drugs analysed.

### *Asia*

Asia accounted for the joint second largest number of publications (at 23%) in this review, with the second largest number of publications affiliated to solely to China (16%) (Fig. 3). Publications were associated with the major drug trends/usage in these countries. For example, the main drug of abuse requiring treatment is methamphetamine [1] and 57% of the publications in this review detecting methamphetamine were affiliated with Asia. The highest prevalence for amphetamines was reported in Eastern and South-East Asia [21], reflected in the percentage of publications in China reporting the detection of amphetamines (57%). The second highest number of publications (14%) reporting the detection of $\Delta^9$-THC were published in China (Fig. 3), with prevalence levels in East and South East Asia being the third highest globally. However, 97% of global morphine seizures were located to three countries (Iran, Afghanistan and India) [56] and there was only one publication affiliated to each of India [47] (reporting the detection of ketamine) and Iran [57] (reporting the detection of morphine, codeine and papaverine). The most recent of the two publications, Farahani *et al* (2020) addressed the requirement for the detection of morphine in relation to seizure trends [57].

### *Europe*

Europe accounted for 23% of the total number of publications in this review, the joint second largest number of publications, with 7 countries represented. In the World Drug Report (2020), Western and Central Europe reported the second highest annual prevalence of cocaine [21,56]. There has also been an increase in the number of people requiring treatment for the first time as a result of cocaine use in Europe, with the large majority (75%) of these drug users requiring treatment in the UK, Spain and Italy [1]. Within Europe the combined contributions was 27% of the total of publications reporting the detection of cocaine. These trends show that LOC devices are predominantly being developed in westernised countries where cocaine is used recreationally.

Western and Central Europe have the third highest prevalence for amphetamines, and ecstasy [21], with amphetamines reported as the main stimulant in Europe [8]. The trends for publications are well linked to geographical use, reflected in the results as 42% of the publications reporting the detection of amphetamine were published in Europe. There has been no increase in research in European countries (25% of methamphetamine studies) where the levels of methamphetamine use have declined or remain at a stable level [8] as the articles were published in prior to 2016. The second highest at 25% of the publications reporting the detection of MDMA were published in Europe, reflective of the prevalence levels. There was only one publication reporting the detection of $\Delta^9$-THC using LOC devices, published in the Netherlands in 2009 with no further research in Europe, which is not reflective of current figures of cannabis use within Europe [21].

*Rest of the world*

South America accounted for 9% of the overall publications reviewed. Seventy-five percent of the accepted publications published in South America investigated cocaine detection using seized samples and were published recently (between 2018 and 2021), supporting their potential for use in examining seized drug samples with South America being one of the main trafficking routes of cocaine to North America [8,21]. Both the Africa and Oceania were affiliated with single publications, based around detection of ketamine and cocaine, respectively.

## Detection methods

The detection methods utilised in the publications included in this review were grouped into 6 different categories; aptamer, capillary electrophoresis (CE), colorimetric, electrochemical, immunoassay, and spectrometry. However, it is worth noting that there were four publications that combined one or more detection method, including colorimetric and electrochemical [33], colorimetric and immunoassay [87], colorimetric, electrochemical and fluorimetric [37], and electrochemical and immunoassay [38]. Immunoassay-based detection techniques were the most common detection method used and accounted for 34% of the studies in this review (Fig. 4). This could be due to ease of manufacturing, simplicity of immunoassays as well as being relatively inexpensive. However, another important consideration is that immunoassays are the preferred initial screening tests in laboratories throughout the world for drugs of abuse, highlighting a beneficial advantage of this method for miniaturisation using microfluidics [89,60–63]. A substantial number of studies also employed detection methods using spectrometric (27%) or colorimetric tests (19%), whereas electrochemical (12%), CE (4%) and aptamer (2%) methods were used less (Fig. 4).

*Multiplex detection*

Multiplex detection was reported in more than half (51%) of the accepted publications. The most commonly encountered number of drugs for multiplex detection was 2 at 39% and the largest number of drugs detected using a multiplex LOC device was 12 using immunoassay-based programmable bio-nano-chips (p-BNC) [28]. One-hundred percent of the publications using CE methods reported multiplex detection [23,48], followed by 79% of colorimetric detection and 65% of the immunoassay-based publications [28,44,51]. For the spectrometry techniques, 67% of the publications used multiplex detection [24,39,67]. It is worth noting that there was no multiplex detection reported for the publications utilising either electrochemical or aptamer detection techniques. The World Drug report (2020) states an increasing trend in polydrug use, for example greater than 65% of cocaine drug users requiring treatment for using with other substances [1], therefore multiplex detection would be invaluable, especially with a LOC device.

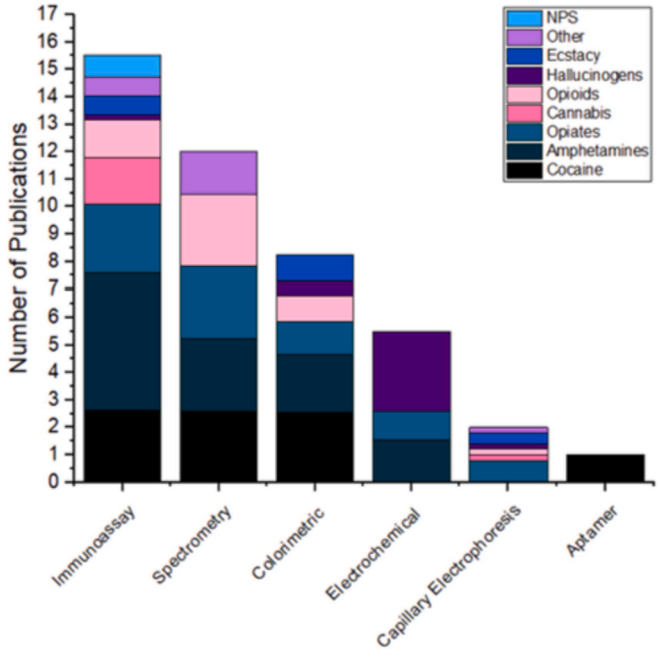


**Fig. 4.** The number of publications as analysed by different detection methods.

*Limits of detection (LODs)*

An integral aspect of developing a detection device is to ensure that it is fit for purpose and able to detect to a both clinically and forensically relevant levels. It is worth noting the limits of detection (LODs) were not clearly stated in 22% of the publications reviewed, but this is because the devices were designed to be quantitative. For some studies, the LOC device was designed to qualitatively identify a pure substance, however by including an indicative LOD it makes it much easier to identify the benefits of the device and establish if it may be applied to a wider range of applications. Where accepted publications clearly stated LODs, overall the majority of these were to clinically relevant levels (low ng level) highlighting the potential for LOC devices to be used to be used alongside (or replace) traditional laboratory-based methods.

Immunoassay-based publications reported clinically relevant low LODs between 1 and 1000 ng $mL^{-1}$ [20,28,38,45,50–52,54]. The LODs for spectrometry techniques ranged from 0.0178 to 51 ng $mL^{-1}$ and offered similar level of sensitivity based on the LODs for drugs of abuse reported in the publications when compared to other LOC detection methods [27,57,39,40,66,67,71–74]. All electrochemical techniques reported LODs, however, these were the least sensitive method ranging from 760.72 – 1.24 × $10^{16}$ ng $mL^{-1}$ [34,37,41,47,75]. LOC devices utilizing CE also reported slightly higher LODs than the other LOC detection methods, with Qiang *et al* (2009) reporting LODs between 1150 and 2090 ng $mL^{-1}$ for a range of drugs of abuse with the authors acknowledging further research needs to be undertaken to ensure that these are more clinically relevant [48]. Several colorimetric detection methods reported high LODs, ranging from 1200 ng $mL^{-1}$ –10 mg $mL^{-1}$ [29–31,35–37]. With Musile *et al* (2015) reported a minimum quantity detectable (MQD) for both visual and instrumental analysis of 2500–1100 ng $mL^{-1}$ and 1200–8700 ng $mL^{-1}$, respectively [31]. Whereas, Bell and Hanes (2007) reported positive results that were significantly lower than clinically relevant levels between 0.05 and 0.125 ng $mL^{-1}$ [32]. The LOD was 0.659 × $10^{3}$ ng $mL^{-1}$ for the aptamer detection method, which was higher than previously reported levels [76–80]. Due to the limited number within this detection method, it is difficult to provide further clarity using this aptamer detection. A paper by Yehia *et al* (2020) combined three different detections methods, electrochemical, colorimetric and fluorescence for the detection of ketamine with high varying LODs of 760.72 ng, 10 mg $mL^{-1}$, and 0.0475 mg $mL^{-1}$ respectively [37].

*Manufacturing materials*

The LOC devices reported were manufactured from a range of different materials, categorized into polymers, glass, paper, and combined materials (Fig. 5). The most commonly utilized material category for the publications was polymers at 33%. Sixteen percent of publications included a combination of more than one manufacturing material categories.

The majority (43%) of the publications including a polymer as the manufacturing material incorporated immunological detection. The inclusion of polymers can be an advantageous in comparison to other LOC manufacturing materials as they offer rapid prototyping and are

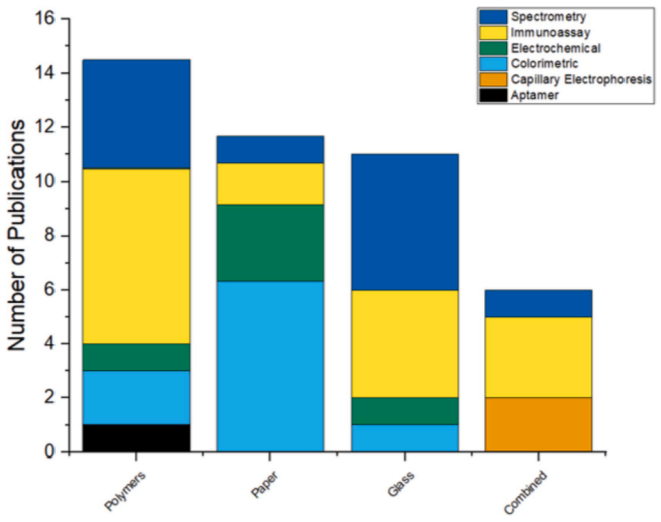


**Fig. 5.** Number of publications and manufacturing materials included categorised by the detection method incorporated within the LOC device. The polymers category includes polydimethylsiloxane (PDMS) [46,51,71], polyethylene glycol (PEG) [52], polyvinyl chloride/ polytetrafluoroethylene (PVC/PFTE) [66], polyurethane/polyaniline (PU/PANI) [57], PDMS/PFTE, polyester [25,29] and plastic* [50,54]. *There were two publications that stated the inclusion of plastic as the LOC material, but did not clarify further. Silica-based LOC devices were included as part of the glass category.

cost-effective when produced in large numbers. This combined with being extremely biocompatible therefore offering flexibility in potential detection methods is an ideal feature for a LOC detection device designed to test potential drugs of abuse in biological samples [75].

The second most commonly utilised manufacturing material was glass (24%), and was frequently combined with immunological detection methods (36%). Glass is not as biocompatible as other manufacturing materials but does have excellent optical properties, reflected by the inclusion of glass using spectrometry detection methods (33%). A significant advantage of using glass is that there are certain reagents, such as Marquis and Mandelin's reagent used in a number of presumptive test reagents for colorimetric detection that use concentrated sulphuric acid, which are only compatible with glass [32].

Twenty-two percent of the publications reported the use of paper, including 60% of the total publications published since 2020 [47,31], with chromatography paper and office paper as example substrates. Ninety-one percent of the publications included samples in a liquid form (aqueous solutions or biological fluids). However, the publications that utilised paper as the manufacturing material only included two different biological matrixes (oral fluid and urine) in this review, even though paper is compatible with biological fluids. However, paper does have limitations, as hair and powder samples require an aqueous matrix for capillary action to take place. The use of paper-based LOC devices offer numerous advantages to their traditionally conventional counterparts: they are extremely cost-effective, environmentally friendly, and simple to manufacture for example using just a wax printer [82–84].

Seventeen percent of the publications reported the use of a combination of different materials which allows the LOC device to utilise the benefits the different materials types, however due to the complexity of combining materials this has only been reported in 20% of the publications in the last five years. The combined materials category includes publications that reported a combination of different manufacturing materials [19,23,28,40,44,48], such as glass and PDMS [40], or a programmable bio-nano-chip for the detection of 12 drugs of abuse in oral fluid using agarose bead sensors [28]. For further information on fabrication methods for creation of LOC devices there are a number of detailed reviews, such as that by Scott and Ali (2021) [59].

*Analysis time*

One major advantage of LOC devices is their ability to provide a rapid analysis, however for a third (33%) of the publications the total analysis time was not clearly stated. The total analysis time for the publications that did report this ranged from seconds up to 30 min (Fig. 6). With more than half (51%) of publications reporting a total analysis times under 10 min [25,31,54,64,66,71,29,40,41,50,51]. It is also worth noting that for the total analysis times that were stated, they were not always easy to obtain from the publications either due to timings given for individual processes rather than full analysis, lack of clarity or simply no discussion of the topic.

All of the publications incorporating electrochemical techniques stated the total analysis times of 2.5 min and under [41,47], offering the fastest total analysis time of the detection methods in this review. One-hundred percent of the colorimetric-based publications reported detection of the drugs of abuse in 6 min and under [25,29,31,32]. Immunoassay-based techniques showed the largest variations in total analysis times from 1 to 30 min, with 62% of these publications 10 min and under. Less than half (46%) of the spectrometry detection methods clearly stated a total analysis time, however, those that did showed a range from 1 to 15 min. Statistical analysis was performed to investigate if there were associations between analysis time and either detection methods, manufacturing material or sample matrices. There was no significant difference observed across these three statistical tests using Kruskal-Wallis ($p > 0.05$). With low numbers in each category, in the future with more publications on LOC devices for the detection of drugs of abuse including NPS this could be investigated further.

## Knowledge gaps and recommendations

*Sample types*

This review has identified a number of knowledge gaps which could provide a direction for further research into the use of LOC devices for detection of drugs of abuse including NPS. Current statistics published by the UNODC [1,8,21] show high levels of prevalence of particular drugs of abuse including $\Delta^9$-THC and also NPS (including synthetic cannabinoids), which are both under investigated globally. For example, 22 countries in Europe reporting a significant problem of synthetic

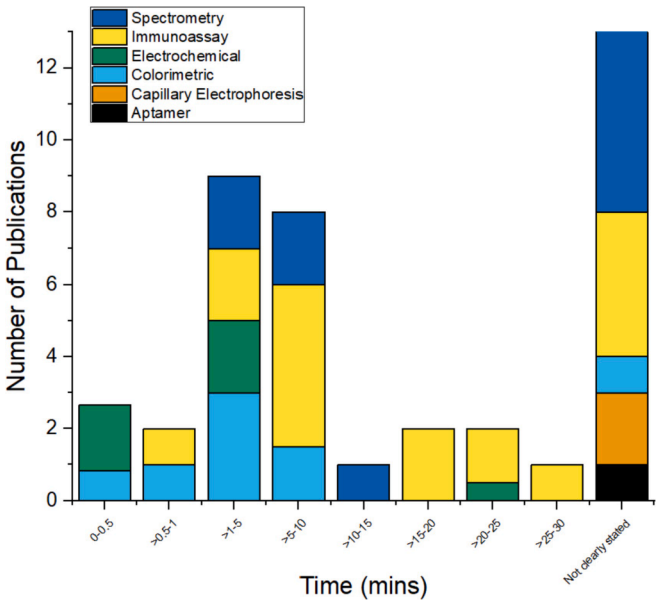


**Fig. 6.** The number of publications for the total analysis time included in the publications in this review.

cannabinoid use, observed in both prisons and homeless communities [21], however, there were no LOC devices reported for detection of synthetic cannabinoids in any of the publications to our knowledge. It is therefore recommended that existing LOC devices could be adapted to look at additional drugs of abuse. The rapid prototyping nature and relatively easy manufacture of LOC devices means that investigating emerging drug trends is feasible.

There were a limited number of publications that investigated real-world samples with whole blood and urine to investigate parent drugs and metabolites, 'spiked drinks', as well as seized drug samples. The inclusion of real-world samples offers an insight into the potential of the LOC device for the detection of drugs of abuse including NPS in the field and if they are fit for purpose as future commercial devices.

*Quality assurance*

Quality assurance and validation is an important element for any newly emerging techniques, particularly to enable comparison with conventional methodologies. The most commonly encountered sample matrix was aqueous solution using spiked drugs of abuse, which is beneficial in assessing whether the LOC device can test bulk or seized samples. In addition to this, the inclusion of cutting agents or adulterants should be considered for investigation to check for any potential cross-reactivity. However, another important aspect would be to analyse biological samples (rather than sample solutions) with the addition of investigating metabolites as well as parent drugs, and the effect of interferents to establish whether the device is fit for purpose in a wider range of scenarios. LODs need to be investigated if the LOC device offers quantitative analysis to ensure that the developed LOC device is fit for purpose and that they are applicable to real-world samples and can be easily used in the field to detect drugs of abuse and NPS to clinical and forensically relevant levels. In addition, there are those LOC devices which offer semi-quantitative detection, such as work by our group which uses a paper-LOC device employing an immunoassay for the detection of cathinones [20]. These semi-quantitative methods can provide additional information compared to a simple presence/absence result but without the increased cost usually associated with quantitative methods. The use of positive and negative controls within the LOC devices should be considered to increase the reliability and integrity of each resulting test. While these controls are routinely applied in conventional laboratory settings, they are not always integrated onto LOC devices. This quality assurance is important for future validation of the LOC devices and acceptance within local criminal justice systems.

*Practicality*

Practicality of LOC devices is another important element as they need to be easy to use and so that potential viable portable detection methods can be used by non-scientifically trained personnel. This would include taking the devices out of the laboratory and conducting field tests to investigate the ruggedness and portability of the devices. The majority of studies (84%) claim that their LOC devices are "easy to use" but there was little/no quantitative data to help explain how easy these techniques are, which makes any comparison between detection methods difficult. A small number of these included end user testing in order to assess not only the accuracy of the devices but also if they were user friendly which is a positive indication. This review has highlighted a lack of field testing and future publications should consider the inclusion of rigorous in-field test of LOC devices.

Storage is also an important element that is often overlooked, as this is vital requirement to produce a commercial device for the detection of drugs of abuse including NPS. Investigating a range of storage conditions over a period of time in order to determine if the device has the same level of sensitivity and selectivity in 6 months' time, for example. An ideal LOC device will be able to be stored at room temperature to avoid additional storage requirements (e.g. access to a freezer) to simplify field deployment.

A major advantage to using LOC devices is that they are highly cost-effective in comparison to other traditional laboratory-based detection methods. They are relatively cheap to produce, require less reagents, and subsequently produce less waste, as well as lack a requirement for expensive, specialist laboratory space and highly-trained scientists. Whilst many studies stated the financial benefits of their device (86%) there was a lack of quantitative information that makes a comparison of cost of detection methods difficult. Most studies used terms such as "low-cost", "less expensive" or "inexpensive" to describe the costs of detection methods with only two stating the overall production cost of each LOC device, at 10 cents [36] and less than $2 to produce [35]. Therefore, it is recommended that this is included in future publications to enable comparison with traditional laboratory methods and between different detection methods and materials for the LOC devices.

Total analysis times, where reported, were all under 30 min (Fig. 6) and this highlights another potential advantage for portable testing compared to traditional laboratory-based methods. With a third of publications not reporting a total analysis time, it is recommended that future publications include this rather than giving more subjective statements. Evaluating these elements, cost, time and ease of use, will all aid in determining whether the LOC devices could be applicable for commercialisation.

## Conclusion

Drugs of abuse including NPS are continuing to be a worldwide challenge, therefore the development of new portable methods for their rapid detection is pertinent. This review has highlighted the wide range of detection methods, manufacturing materials, drugs of abuse that have been targeted, and the diverse range of sample matrices that can be incorporated into an LOC device.

Global trends in drug abuse were reflected in the number of publications which were aimed at detection of particularly drugs of abuse, for example, the majority of studies (58%) reported using LOC detection methods for cocaine detection, which reflects cocaine being the main stimulant used worldwide, with 18 million users. However, there were some exceptions to this such as cannabis as, although reported as the most commonly abused drug worldwide, only 16% of the publications reported the detection of $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC). Multiplex detection was common (55% of cases) which again is aligned with trends in poly drug use.

LOC devices are capable of accepting a wide range of sample types, both biological and non-biological, enabling all commonly encountered sample types to be analysed. This was linked to a range of detection methods, with immunoassays being the most commonly incorporated (34% of publications) due to their high sensitivity and specificity.

LOC devices for detection of drugs of abuse is still a rapidly evolving field, with 42% of articles published since 2019. Related technologies, without a microfluidic component, such as Lab-on-a-Glove for the electrochemical detection of fentanyl [86] and Lab-on-a-screen-printed electrochemical cell for the detection of the "rohypnol" drug flunitrazepam [88] represent alternative portable detection methods that are of interest. Going forward this emerging scientific field could offer commercially viable detection, either qualitatively or quantitatively, for the portable and rapid detection of real-world drug samples.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.forc.2021.100370.

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