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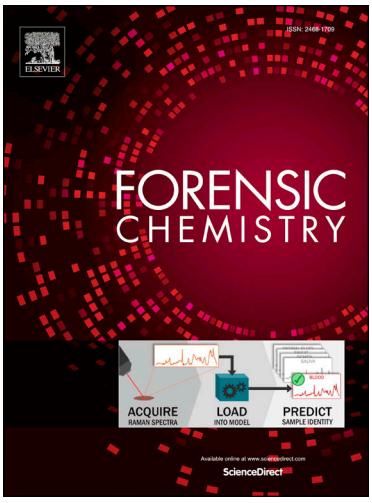

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Origami Chips: Development and validation of a paper-based Lab-on-a-Chip device for the rapid and cost-effective detection of 4-methylmethcathinone (mephedrone) and its metabolite, 4-methylephedrine in urine

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ABSTRACT: 4-methylmethcathinone (mephedrone) has emerged in drug seizures as a new psychoactive substance (NPS) causing a public health risk of global concern. Currently, there are no commercial microfluidic devices for the selective detection of mephedrone and so this study presents a simple, low cost and portable paper-based Lab-on-a-Chip (LOC) device with colorimetric detection to fill this gap. Limits of detection for mephedrone in spiked urine and dissolved powder (aqueous) samples are clinically relevant at 4.34 ng mL^{-1} and 2.51 ng mL^{-1} respectively. No cross-reactivity for commonly encountered cutting agents, interferents and adulterants were detected. Mephedrone and its main metabolite were detectable in aqueous samples within 3 minutes. Stability and reproducibility measurements showed no significant difference in signal intensity over eight weeks and no significant difference within or between devices. The proposed device has the potential to provide cost-effective, rapid, on-site testing within forensic or clinical settings and therefore has wide global applicability.

Key words: detection, mephedrone, microfluidics, NPS, urine

1. INTRODUCTION

Miniaturization of bioanalytical techniques for the creation of portable toolkits has been driven by microfluidics: the use of small systems containing micron size channels allows the integration of multiple processes to produce Lab-on-a-Chip (LOC) devices. This offers significant advantages over their conventional counterparts including; reduced sample and reagent consumption which is ideal for forensic and clinical applications; reduced waste; faster reaction times; reduced cost and increased portability [1]. In particular, paper-based microfluidic devices are very cost-effective due to the nature of the substrate and are easy to fabricate. For example, using wax printing to create the required design generates hydrophobic barriers surrounding hydrophilic channels for movement of solutions. Fluid transport is driven by capillary action and therefore no external power source is required. In addition, the high surface area to volume ratio aids detection limits when colorimetric methods are used [2]. A wide variety of fabrication methods and biological applications of paper-based microfluidic devices have previously been shown and are summarized in a number of recent reviews [2-5]. The analysis of drugs of abuse from biological matrices using microfluidic devices has mainly focused on extraction and separation of samples, as presented in a review by Al-Hetlani [6].

Microfluidic immunoassays have been reported as rapid screening tests, and while the focus has mainly been on clinical diagnostics, for example the detection of circulating tumour cells [7], there has been limited interest in drugs of abuse. This has included detection of performance enhancing drugs in sport, such as testosterone [8], as well as multiplex detection of recreational drugs of abuse using electrochemical [9], optical [10], chemiluminescent [11], immunological [12] and colorimetric techniques [13].

New Psychoactive Substances (NPSs) are substances that are new to the recreational drugs market, exhibiting a psychoactive effect when taken, thus presenting a serious risk to public health [14]. NPSs (formerly known as “legal highs”) exhibit similar biological and pharmacological activity to controlled drugs of abuse, such as cannabis and amphetamines, but with limited knowledge regarding the pharmacology and potential health risks [15]. The most recent report from the United Nations Office on

Drugs and Crime (UNODC) stated that there were 803 NPSs reported globally from 2009-2017, of which 148 were reported as synthetic cathinones, including mephedrone [16]. There has been an increase in the availability of NPSs in prisons reported across Europe. This has resulted in subsequent increases in violence, bullying, aggressive behavior and debt as a consequence of high mark up prices is also having a detrimental effect, which is still a significant issue in United Kingdom (UK) prisons even after the introduction of the Psychoactive Substances Act 2016 [17]. The most recent reports from the Office for National Statistics regarding drug-related deaths in England and Wales states that deaths involving NPSs are increasing with 125 NPS related deaths registered in 2018 [18]. Deaths directly related to NPS use within prisons can be complex and can often be under reported [19]. This is due to number of complications including the lack of reference standards for NPSs, detecting low concentrations of NPSs, and the commonly encountered issue that NPSs are taken with other recreational drugs, known as poly-drug use [19,20]. For example, 79 NPS-related deaths were reported in prisons in England and Wales between June 2013 and September 2016 even after the introduction of the NPS Act 2016 in the UK on the 26th of May 2016 made it illegal to supply, possess, export or import NPSs [19,20].

The synthetic cathinone, mephedrone first appeared on the recreational drug market in the 2007, where it has established a place for itself on the recreational drugs market globally, but is a significant problem in the UK [21]. This is especially true in the UK where it has been identified as the most common widely abused NPS [21]. Mephedrone is a synthetic derivative of cathinone, naturally found in the *Catha edulis* plant and exhibits similar effects to amphetamine [22]. Mephedrone is most commonly found in powder or tablet forms [22]. In 2015, at the 58th Commission on Narcotic Drugs (CND) in Vienna, mephedrone was the first NPS to be listed in Schedule II of the United Nations 1971 Convention of Psychotropic Substances (decision 58/1), placing it under international control [23,24]. In relation to other drugs of abuse, such as cocaine and heroin, these numbers may be deemed as small in consequence but the use of NPSs has increased throughout the UK severely putting strain on public services, including Accident and Emergency departments and prisons.

Smith *et al.* [15] reviewed methods for the detection/quantification of mephedrone, however many of these methods are both non-portable and expensive, and as such are not suitable for in-field detection including gas chromatography-mass spectrometry (GC-MS) [25,26], high performance liquid chromatography (HPLC-MS) [27-29] as well as some more advanced tandem mass spectrometry (HPLC-HRqTOFMS) [30]. However, a recent paper by Elbardisy *et al* (2019) shows a novel electrochemical method using both cyclic voltammetry and differential pulse voltammetry for a laboratory-based detection method but has the potential for being developed as a portable detection method for mephedrone and its metabolites [31]. Though supply and production of mephedrone is now controlled in the UK, global prevalence is still cause for concern and there are no commercial microfluidic devices for the selective detection of mephedrone in either pure and/or adulterated samples.

Competitive immunoassays are readily utilised for the detection of drugs and require only one antibody, along with a labelled 'tracer' that is a known amount of labelled antigen [32,33]. Due to the lack of availability of reference standards for the continually increasing number of NPSs available within the recreational market, a competitive immunoassay is ideal for analysis of NPS. The specific antibody is immobilised to a surface, then the tracer and the target antigen within the sample compete for the active sites of the antibody [32,33]. An unknown amount on unlabelled antigen and a known amount of labelled antigen compete for the active sites of the antibody. Therefore, as there is a limited number of active sites on the antibody, the amount of labelled antigens decreases if there is an increase in the concentration of unlabelled antigens, lowering detection response. If the sample contains high concentrations of antigen, a lower response is produced by 3,3',5,5'-tetramethylbenzidine (TMB), and therefore less color, demonstrating that antigen is competing effectively for the active sites of the antibody with the HRP-labelled antigen [32,34]. An important and invaluable feature of a competitive immunoassay is that structurally similar compounds cross-react with one another to varying degrees. For example, an antibody specific for 4-MMC with cross-react to varying degrees with structurally related compounds, such as its metabolites of other synthetic cathinones [32,33]. Due to a current lack of reliable low-cost detection methods for NPS, there is a timely requirement for a robust portable LOC based solution. We address this by presenting a novel paper-microfluidic device capable of detecting mephedrone using a competitive immunoassay. This device is simple to use, low-cost and effective. Its performance was tested by conducting stability studies and cross reactivity testing, and its ability to detect the presence of mephedrone at clinically relevant levels was proven for aqueous and biological (urine) matrices.

2. EXPERIMENTAL SECTION

The controlled reference standards (cathinone hydrochloride, cocaine hydrochloride, amphetamine sulfate and ketamine hydrochloride) for this research were obtained from either Sigma-Aldrich (Gillingham, UK) or Fluorochem Ltd (Hadfield, UK) under UK Home Office license, by authorized personnel and in compliance with both the UK Misuse of Drugs Act (1971) and UK Misuse of Drugs Regulations (2001). All controlled/restricted materials were stored, transferred, used and destroyed in compliance with the UK Misuse of Drugs Act (1971) and UK Misuse of Drugs Regulations (2001).

The synthesis of the racemic target compounds was achieved using the previously reported method by Mayer *et al* [35] in 15% (mephedrone and 4-methylephedrine) – 37% (4-methylcathinone) overall yield. The hydrochloride salts were obtained as stable, off-white powders and determined to be soluble (10.0 mg mL^{-1}) in deionized water, methanol and dimethylsulfoxide (DMSO). To ensure the authenticity of the materials utilized in this study the synthesized samples were fully structurally characterized by nuclear magnetic resonance (^{1}H and ^{13}C NMR), GC-MS and Fourier-transform infrared spectroscopy with attenuated total reflection (ATR-FTIR) and the purity of all samples confirmed by elemental analysis (>99.6% in all cases) [35].

2.1. Preparation of Microfluidic Devices

Paper- based microfluidic devices were designed and printed onto Whatman Grade 1 filter paper using a Xerox Phaser 8500 solid ink printer to produce the design shown in Figure 1. The devices were then placed in an oven at 130 ºC for 180 seconds to melt the wax and complete the creation of the hydrophobic barriers. The reaction wells (black center tab, Figure 1) were then

activated by adding 5 µL of 0.250 mg mL^{-1} chitosan [Sigma-Aldrich, UK] followed by 5 µL of 2.50 % glutaraldehyde [Sigma-Aldrich, UK] and then washed twice with 10 µL of analytical grade water [33]. The relevant antibody (4 µL) was then added to

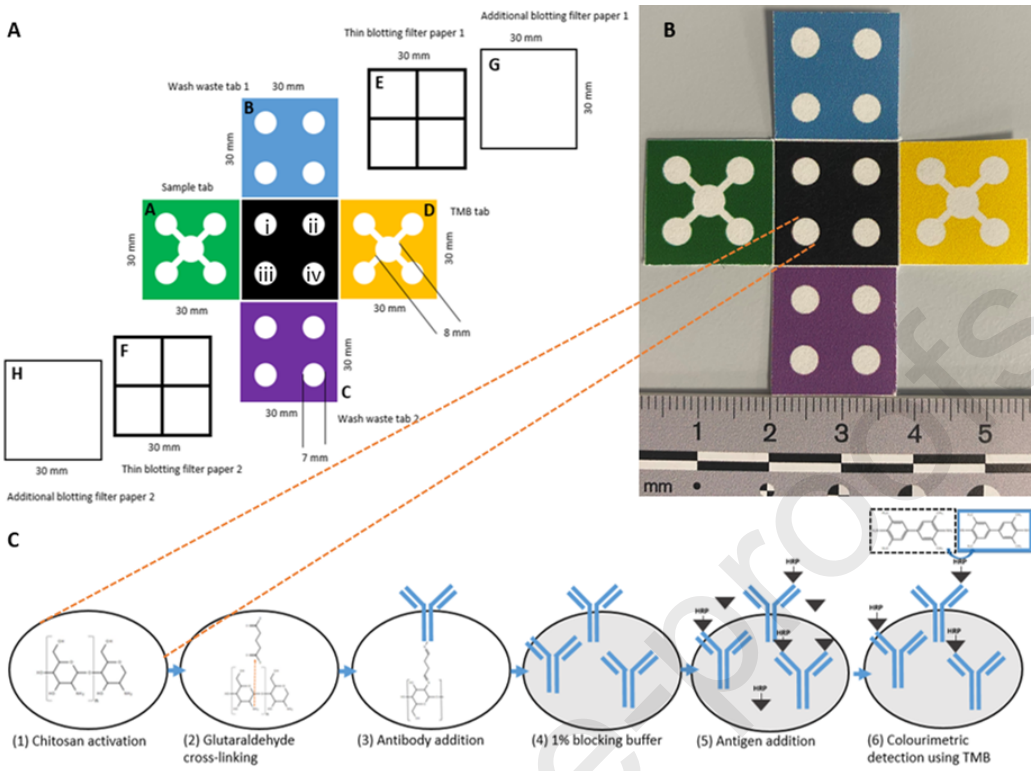

Figure 1 (A). Schematic of the LOC design with central detection zone (black tab); sample tab (green (A)); wash tabs (blue (B) and purple (C); and detection reagents tab (yellow (D)). The location of the antibodies is shown using (i) anti-methcathinone; ii) additional testing well (for proof of concept anti-amphetamine was added); iii) anti-horseradish peroxidase (HRP) and iv) negative control (analytical grade water)) on the central detection zone (black tab). (E) Thin blotting filter paper to be attached under (B), (F) Thin blotting filter paper to be attached under (C). (G) and (H) additional filter paper to be attached under (E) and (F) for excess water waste; **(B)** Image of the printed LOC device with its 'origami design'. To allow the sequential steps of the immunoassay to take place the tabs are folded over the central detection zone (black tab) in order (from A to D). **(C)** Schematic of the preparation of the LOC device; (1) chitosan addition, (2) glutaraldehyde cross-linking. After 2 hours, this was washed twice with analytical grade water. (3) antibody addition (i-iv). After 30 minutes, this was then washed twice with analytical grade water. (4) 1% milk powder (blocking buffer) was then added. After 15 minutes, this was washed twice with 0.05% PBS-Tween. (5) Labelled and non-labelled antigen addition. This was then washed twice with analytical grade water and followed by (6) colorimetric detection using TMB.

the appropriate well (black center tab, Figure 1): i) anti-methcathinone; ii) additional testing well to enable this device to be adapted in the future (for proof of concept anti-amphetamine was added); and iii) anti-horseradish peroxidase (HRP). Four microlitres of analytical grade water was added to well iv) as a negative control. The antibodies were permanently bound to the microfluidic device through the glutaraldehyde cross-linker. Following a 30-minute incubation, the wells were washed twice with 10 µL of analytical grade water in order to remove any unbound antibodies. Blocking was then achieved by adding 10 µL of 1 % milk powder (in phosphate buffered saline) solution and incubated at room temperature for 15 minutes before washing twice with 10 µL of 0.05% PBS-Tween. The devices were either used immediately or stored at 4 °C for a period of up to 4 weeks prior to use in a stability study.

2.2. Conjugation

The competitive immunoassay required a horseradish peroxidase (HRP) conjugated analyte to compete with the target analyte, mephedrone in solution. HRP-conjugation is achieved through interaction of the HRP with a primary amine within a target molecule to be conjugated (Figure 2). Mephedrone does not have this functional group, therefore cathinone was chosen as the target molecule as it has significant (2200 %) cross-reactivity with the anti-methcathinone antibody [36].

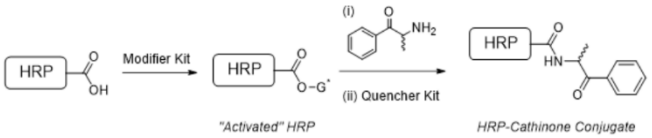

Figure 2. Proposed conjugation of cathinone (free base) with horseradish peroxidase (HRP)

In order to conjugate the HRP to the target molecule, the cathinone firstly needed to be converted into its freebase form. In order to expose the amine and inducing a nucleophilic attack in the presence of HRP. A 10.2 mg sample of cathinone hydrochloride [Fluorochem Ltd, UK] was dissolved in 500 µL of analytical grade water before being added to a 10.0 mg mL^{-1} solution of sodium hydrogen carbonate (10.0 mg in 1000 µL, Fisher Scientific, UK). Once mixed the solution began to effervesce. After the reaction had ceased, the pH of the mixture was taken. A litmus paper test indicated a pH of 8 and confirmed that the cathinone was in its freebase form. The aqueous layers of the cathinone were washed with diethyl ether (6 x 500 µL, Fisher Scientific) and the organic fractions were combined before the sample was evaporated to incipient dryness under nitrogen leaving a pale residue. The residue was reconstituted in 100 µL of dimethyl sulphoxide (Sigma-Aldrich, UK) then 10 µL was removed and diluted using 990 µL of PBS (100x dilution, Oxoid Ltd. UK) to give a 1 % DMSO/PBS solution. A HRP-conjugation kit [Abcam, UK] was then used as per the manufacturer's standard protocol. 10 µL solution of modifier reagent was added to 100 µL of the cathinone freebase solution and mixed. The cathinone freebase-modifier mixture was added to the LYNX lyophilized mix and left to incubate overnight at room temperature. After incubation, 10 µL of quencher reagent was added to the mixture and incubated at room temperature for 30 minutes prior to use and then stored in aliquots at -20°C until required.

2.3. Immunoassay Protocol

The LOC device incorporates a competitive immunoassay for the selective detection of mephedrone and 4-methylcathinone in both urine and aqueous samples. Aqueous samples were prepared by dissolving the target analytes of varying concentrations (70.0 ng mL^{-1} – 0.137 ng mL^{-1}) in molecular grade water. Drug and alcohol-free urine samples were donated by healthy volunteers and spiked with target analytes and metabolites at clinically relevant concentrations [30]. Ethical approval for this study was obtained through the Research Ethics and Governance Committee at Manchester Metropolitan University (Ethics approval number: SE151633A1). Samples were mixed in a 50:50 ratio with the HRP-conjugated cathinone and 5 µL was added to each reaction chamber on the microfluidic device (sample tab (A), Figure 1). Samples were incubated for 210 seconds before washing twice with molecular grade water (wash waste tabs (B) and (C), Figure 1). Finally, 5 µL of 3,3',5,5'-tetramethylbenzidine (TMB) [Thermo Scientific, UK] was added (TMB tab (D), Figure 1) and an image of the color change in the central detection zone (black tab) by taken on an iPhone 7 and measured using ImageJ analysis (version 1.52a). The color change was proportional to the amount of HRP present. Optimisation of the immunoassay was achieved by testing a range of antibody (anti-methcathinone) and labelled antigen (cathinone-HRP) concentrations, as well as testing a range of antibody (anti-HRP) and labelled antigen (cathinone-HRP) concentrations. The inclusion of the anti-HRP antibody provides the microfluidic device a positive control. A negative control was also included on the device by replacing the antibody with analytical grade water. The additional testing well was included as part of the design to offer future flexibility of the device. As proof of concept, a range of concentrations between 0.200 ng mL^{-1} to 0.050 ng mL^{-1} of an anti-amphetamine antibody, and labelled-antigen concentrations, ranging from 0.200 ng mL^{-1} to 0.050 ng mL^{-1} were tested.

2.4. Stability Study

The signal intensity was recorded over an 8-week period to investigate the stability of the microfluidic device, which was subjected to four different storage conditions: fridge (2-8 ºC), freezer (-20 ºC), in the dark at room temperature and in the light at room temperature.

2.5. Cross reactivity using the paper-based LOC device

The cross-reactivity of the anti-methcathinone antibody with investigated using the immunoassay protocol with adulterants (amphetamine, cocaine and ketamine), cutting agents (benzocaine, caffeine, lidocaine, paracetamol, procaine and taurine), and interferents (cornflour and flour). A blank sample (no adulterant, cutting agent, or interferent present) and a concentration range (7 samples from concentrations 0.156 to 10.0 mg mL^{-1}) of commonly encountered adulterants, cutting agents, and interferents was spiked to an aqueous sample and the signal intensity was recorded.

2.6. Data Analysis

Photographic images were taken of the microfluidic devices using an iPhone 7 once the immunoassay had been carried out. Image J software version 1.52a (http://imagej.nih.gov/ij/) was then used to analyze the average intensity of each of the wells. The RGB values (blue component) were then transferred into Origin (version 2019) for the graphical representations, and SPSS (version 22) and R Studio (version 3.6.1) to allow statistical analysis to be performed. Limits of detection (LOD) were determined for the aqueous and biological samples. ANOVA was used to determine if there was any difference in signal intensity over an eight-week period. The variation between devices was determined using Levene's test. Linear regression was used to determine if there was any cross-reactivity with the anti-methcathinone antibody and any of the adulterants, cutting agents and interferents investigated.

3. RESULTS AND DISCUSSION

3.1. Method development of Immunoassay Parameters

Anti-methcathinone antibody concentrations, ranging from 0.020 µg mL^{-1} to 2.04 mg mL^{-1}, and labelled-antigen concentrations, ranging from 0.200 ng mL^{-1} to 0.025 ng mL^{-1}, were evaluated. It was found that from these parameters an antibody concentration of 0.511 mg mL^{-1} and a labelled-antigen concentration of 0.2 ng mL^{-1} obtained the best results. Anti-HRP antibody concentrations, ranging from 0.200 ng mL^{-1} to 0.050 ng mL^{-1}, and labelled-antigen concentrations, ranging from 0.200 ng mL^{-1} to 0.025 ng mL^{-1}, were also evaluated in order to determine the most effective concentration for this positive control The most effective concentrations were 0.050 ng mL^{-1} for the anti-HRP antibody and 0.200 ng mL^{-1} for the labelled-antigen concentration.

Therefore, for all future experiments these conditions were used. The additional testing well demonstrates the flexibility of this microfluidic device, offering an additional antibody to be included for the detection of an NPS or drugs of abuse.
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When mephedrone is present, the TMB will subsequently produce a lower response as the unlabeled target antigen (mephedrone) outcompetes the labelled cathinone-HRP for the active sites of the anti-methcathinone antibody. Therefore, the higher the average light intensity values, the lower the response of the labelled antigen and the whiter the color. If no mephedrone is present, the TMB will subsequently produce a higher response as the unlabeled target antigen (mephedrone) is outcompeted the labelled cathinone-HRP for the active sites of the anti-methcathinone antibody. Therefore, the lower the average light intensity values, the more intense the reaction color indicating a greater presence of HRP (labelled antigen) and therefore less of the unlabeled antigen. The positive control (anti-HRP) and the negative control (analytical grade water) were both included in order to increase the reliability of each result when testing using this microfluidic device. A positive control was indicated by a blue color change as the HRP (labelled antigen) is successfully competing for the active sites on the anti-HRP antibody. Whereas, the negative control was indicated by a white color as there is no antibody present in the immunoassay. The positive (anti-HRP antibody) and negative (analytical grade water) controls were effective within the LOC device with signal intensity ranges between 122-139 and 178-198, respectively.

3.2. Aqueous and Biological Matrices

Mephedrone containing samples were prepared from both dissolved powder (aqueous) and spiked urine to represent the drug in both its pure form and as a clinical specimen. Analysis of the data sets showed a linear range of 0.078 to 10.0 mg mL^{-1} for the aqueous and urine samples (Figure 3). The LOD were calculated using regression analysis of the aqueous mephedrone and urine sample. The LOD was reported at 3 times the standard deviation (SD) of the intercept (3x SD). A LOD of 2.51 ng mL^{-1} and 4.34 ng mL^{-1} was calculated for the aqueous mephedrone and urine sample, respectively. This is comparable to clinically relevant levels with traditional laboratory methods for mephedrone in urine (LOD = 2.00 ng mL^{-1}) achieved by analysis with HPLC-HRqToFMS [30] It is worth noting that the standard deviation between replicates of the same mephedrone concentration for both the urine and aqueous samples did show some variation within the data.

Whilst the linear response indicates the potential for the device to be used for full quantitative analysis, there is an appreciable degree of variation in measurements, which would hamper the accurate quantitative determination of cathinones. It is therefore recommended that this device is used only to confirm the presence of a cathinones in concentrations greater than 2.51 ng mL^{-1} in aqueous samples and 4.34 ng mL^{-1} in urine.

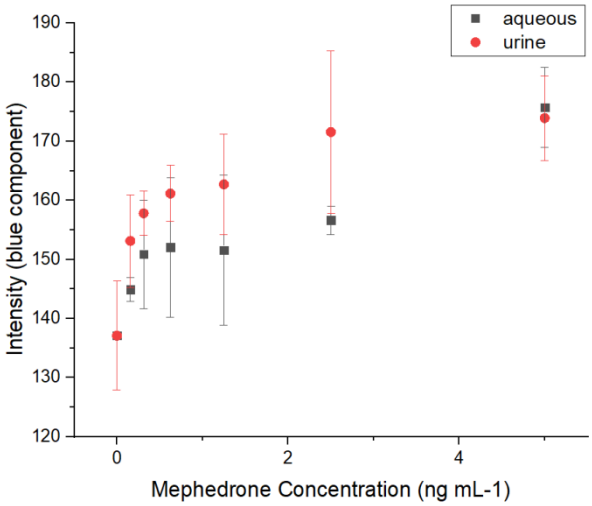

Figure 3. Detection of mephedrone in both aqueous media and spiked urine samples (n = 6)

Mephedrone is metabolised in the body via two different Phase 1 processes (Figure 4), the main route of metabolism is via a primary route of demethylation in order to form a cathinone product. Due to the high degree of cross-reactivity associated with the anti-methcathinone antibody and the positive results obtained with both mephedrone and cathinone-HRP conjugate, the same concentration range was applied to samples of methcathinone, cathinone, 4-methylcathinone and 4-methylephedrine. Analysis of the data collected confirmed that the anti-methcathinone antibody did in fact cross-react with all members of the cathinone family tested at clinically relevant levels (0.137 to 70 ng mL^{-1}). With the metabolite and parent compound cathinone demonstrating the greatest affinity with the greatest response, followed by methcathinone and its metabolite, 4-methylephedrine.

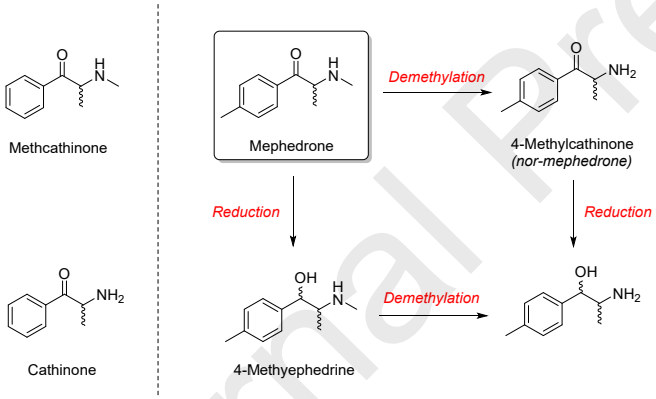

Figure 4. Structures of cathinone, methcathinone and the proposed route for the Phase I metabolism of mephedrone (Adapted from Khreit *et al.* [37]).

Both cathinone and 4-methylcathinone had linear ranges between 0.20-5.00 ng mL^{-1}. Thus, demonstrating that both the 4-methylcathinone and the cathinone are successfully competing with the cathinone-HRP for the active sites on the antibodies and that even small changes in the drug concentration affect the amount of cathinone-HRP present. Positive results were also observed for another common cathinone derivative, methcathinone and its primary metabolite, 4-methylephedrine. However, both the light intensity values and the calibration curves were weaker than those previously seen indicating that even though they both successfully compete for the active sites on the antibodies their binding affinity is not as strong as the mephedrone metabolites, making them harder to detect.Therefore, this LOC device can identify the presence, but it is for non-quantitative analysis and cannot distinguish between structurally similar synthetic cathinones at varying concentrations.

3.3. Stability Study

A control immunoassay experiment to investigate the stability of the microfluidic device was performed by subjecting the device to four different storage conditions; fridge (2-8 ºC), freezer (-20 ºC), in the dark at room temperature and in the light at room temperature. The signal intensity was recorded over the 8 weeks and showed that there was no significant difference between the values recorded over the time period (ANOVA: $F = 2.134$, $df = 1$, $p = 0.146$) (Figure 5). However, there was a significant difference between the four conditions (ANOVA: $F = 12.741$, $df = 3$, $p < 0.001$). Three of the four conditions (fridge, freezer, and light at room temperature) were not significant indicating that these three conditions were stable for the eight week period and could all therefore effectively used as suitable storage

conditions. The significant difference in the LOC devices stored in the dark at room temperature ($p < 0.001$) indicates that the LOC devices were not stable in this condition, and therefore storage in this was is not recommended.

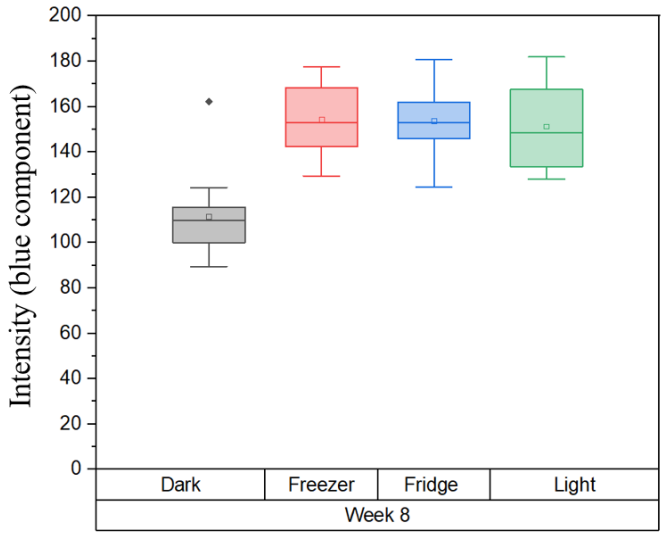

Figure 5. Signal intensity recorded over a 8 week period to investigate the stability of the microfluidic devices (n = 18).

Variation within four individual microfluidic devices was assessed using Levene's test, which indicated equal variances ($p = 0.638$) indicating that intra-device variability was observed. Inter-device variation was then examined to examine the reliability of the manufacturing (Figure 5). This showed that there was no significant difference between devices (ANOVA: $F(3, 47) = 2.10$, $p = 0.114$).

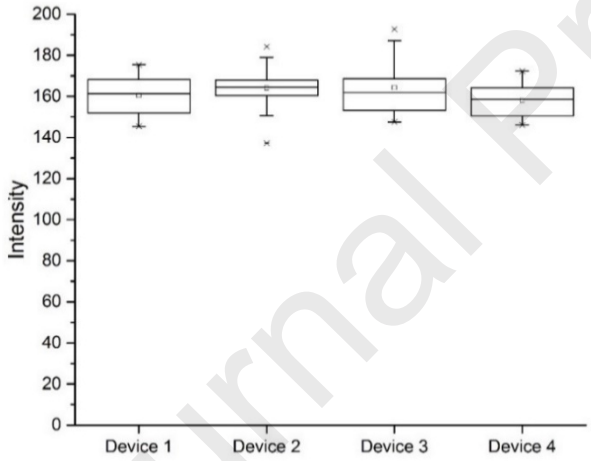

Figure 5. Signal intensity recorded across four different microfluidic devices (n = 12).

3.4. Cross-reactivity using the paper-based LOC device

The cross-reactivity of the anti-methcathinone antibody with the adulterants, cutting agents and interferents at varying concentrations was investigated. Linear regression analysis (Table 1) showed that there was no detrimental cross-reactivity for any adulterants, cutting agents, or interferents investigated. There was no statistically significant increase between the response, and the concentration of the adulterants, cutting agents, or interferents in the spiked samples. This indicates that the level of cathinone-HRP binding to the antibody is consistent across the dilution ranges of the adulterants, as well as the cutting agents and interferents.

Table 1: Summary table for linear regression analysis cross-reactivity of cutting agents, adulterants and interferents

Adulterants/ cutting agent	**Compound**	***P*-Value**	**Adjusted R-squared**
Adulterants	amphetamine	0.121	0.0651

	cocaine	0.837	0.0902
	ketamine	0.610	0.0329
Cutting agents	benzocaine	0.203	0.0308
	caffeine	0.998	0.0217
	lidocaine	0.327	0.000166
	paracetamol	0.0883	0.0866
	procaine	0.304	0.00471
	taurine	0.0685	0.109
Interferents	corn flour	0.0789	0.00321
	flour	0.0983	0.00289

3.5. Additional adaptable functionality using the paper-based LOC device

This microfluidic device offers future flexibility for additional testing of other NPS or drugs of abuse. This was demonstrated by using well ii (Figure 1) for the simultaneous determination of amphetamine. For this proof of concept, a range of anti-amphetamine antibody concentrations (0.200 ng mL^{-1} to 0.050 ng mL^{-1}), and labelled-antigen concentrations, (0.200 ng mL^{-1} to 0.050 ng mL^{-1}) were trialed. By using an optimum concentration of anti-amphetamine antibody at 1.75 ng mL^{-1} and a labelled-antigen concentration of 0.200 ng mL^{-1}, this enabled the microfluidic device to detect amphetamine in at a level of 10 mg mL^{-1} in dissolved powder (aqueous). These results demonstrate how this microfluidic device can be easily adapted to suit future and specific research trends in different geographical locations around the globe.

4. Conclusions

This study has successfully demonstrated that this paper-based LOC device can detect clinically significant levels of mephedrone and its metabolites in dissolved powder (aqueous) and spiked urine samples. The limits of detection of the device are 2.51 ng mL^{-1} (aqueous) and 4.34 ng mL^{-1} (urine), which is comparable with traditional laboratory methods of 2 ng mL^{-1} achieved by HPLC-HRqToFMS [30]. The devised method has successfully encompassed many of the advantages associated with microfluidic devices; it is both low cost and easy to produce, the incubation and overall reaction time has been reduced from days on a standard 96 well plate to minutes on the paper-based device and both the reagent and sample volumes required have been reduced to microlitre quantities.

Thus, supporting the reliability of this microfluidic device for future testing of mephedrone and its metabolites. This microfluidic device also has the advantage for future flexibility as the additional testing well was easily adaptable by effectively optimizing a range of anti-amphetamine antibody concentrations and labelled-antigen concentrations for the specific detection of amphetamine. Therefore, providing proof of concept that this microfluidic device has multiplex potential, showing flexibility to suit future research projects for the detection of other NPSs or drugs of abuse.

The application of an intra-inter stability study showed that there was no significant difference between different LOC devices, successfully signifying that tests carried out across a range of different plates were reproducible. Stability studies indicated that the LOC devices stored in the four different conditions were stable for up to eight weeks. The stability demonstrated by this LOC device would allow for more widespread use of the point-of-care (POC) test and enable optimisation and pre-coating with the primary antibody to occur prior to distribution.

This method has proved successful at differentiating cathinones from other adulterants, cutting agents, and interferents. Spiking studies identified no cross-reactivity in the presence of cocaine, ketamine, paracetamol, benzocaine, caffeine, lidocaine, procaine, taurine, sucrose, cornflour and flour. The results show that this proposed device has the potential to provide rapid, on-site testing within either a forensic or clinical setting in biological samples (urine), but also in aqueous samples that may have been ‘cut’ with diluents or administered with other drugs of abuse. The low-cost of the device is highly beneficial as it can be used in low income countries, giving it a wide global applicability, to support efforts to reduce NPS use, with clear health, social and economic benefits

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Notes
The authors declare no competing financial interest.

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Highlights

- Mephedrone, a synthetic cathinone, is a widely used new psychoactive substance
- We present a Lab-on-a-Chip to detect mephedrone at clinically relevant levels
- Cross-reactivity with common adulterants, stability and reproducibility were examined
- Using a Lab-on-a-Chip enables rapid (< 3 minutes), low-cost, on-site detection