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THE M-CAT's OUT OF THE BAG: A PAPER-BASED MICROFLUIDIC IMMUNOASSAY FOR THE RAPID DETECTION OF MEPHEDRONE

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ABSTRACT

This paper reports a paper-based microfluidic competitive immunoassay for the detection of mephedrone, a new psychoactive substance (NPS). Using the proposed system, limits of detection of 4.078 µg mL^{-1} and 1.597 µg mL^{-1} for aqueous mephedrone and spiked urine samples, respectively, were obtained, with these values enabling the detection of clinically relevant concentrations of mephedrone. The proposed device has the opportunity to provide rapid, on-site testing, within either a forensic or clinical setting, for NPSs.

KEYWORDS: Immunoassay, Mephedrone, New Psychoactive Substances, Paper-Fluidic

INTRODUCTION

Mephedrone (Figure 1: **2**, R = Me) is a synthetic cathinone that has emerged in drug seizures as a replacement for controlled stimulants including amphetamines such as methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA). 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 [1]. For example, micellar electrokinetic capillary chromatography and laser-induced fluorescence has been used to separate and detect amphetamine and analogous compounds including cathinone (**3**), methcathinone (**2**, R = H), ephedrine and norephedrine [2]. However, microfluidic immunoassays have also been reported as rapid screening tests for banned substances including morphine [3], cocaine [3] and methamphetamine [4] but they have not yet been applied to NPSs.

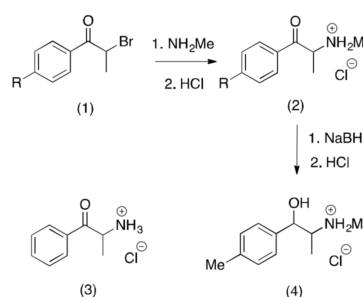

Figure 1: Synthesis of mephedone and its primary metabolite.

EXPERIMENTAL

Paper microfluidic devices were printed onto Whatman Grade 1 filter paper using a Xerox Phaser 8500 solid ink printer and then placed in an oven at 130ºC for 180 seconds to melt the wax. The design of the device was adopted from Ge *et al*. [5], and an overall reaction scheme is provided in Figure 2. The reaction wells of the microfluidic device were then activated using chitosan and glutaraldehyde prior to addition of the anti-methcathinone antibody. The wells were then blocked with 1% milk powder in phosphate buffered saline (PBS) and 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. Aqueous or biological (urine) samples were mixed 50:50 with horseradish peroxidase (HRP)-conjugated cathinone (produced using a HRP conjugation kit [Abcam, UK]). Colourimetric detection was then achieved through addition of 3,3',5,5'-tetramethylbenzidine (TMB) and intensity values examined using Image J.

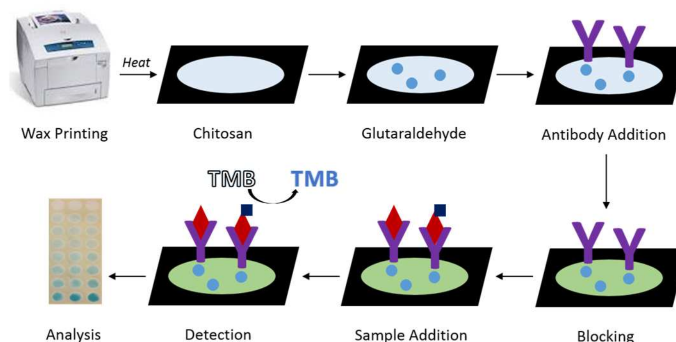

Figure 2: Overall schematic showing preparation of the paper microfluidic devices including i) wax printing, ii) antibody addition and iii) colourimetric detection and analysis using Image J. Design adapted from [5].

RESULTS AND DISCUSSION

Mephedrone hydrochloride (**2**, R = Me) and its corresponding metabolite (**4**) were synthesized from (**1**) using the method by Santali *et al.* (Figure 1). Initial experiments showed that optimum results were obtained using an antibody concentration of 0.7068 ng mL^{-1} and labelled-antigen concentration of 7.083 ng mL^{-1}. Mephedrone containing samples were prepared from both aqueous media and spiked urine to represent the drug in both its pure form and as a clinical specimen. Regression analysis showed limits of detection (LOD) of 4.078 µg mL^{-1} and 1.597 µg mL^{-1} for the aqueous mephedrone and spiked urine samples, respectively. Comparing these values with clinically relevant concentrations for mephedrone in urine (LOD = 2 µg/mL and LOQ = 4 µg/mL) shows that this method has good sensitivity [6]. A comparison was then made using mephedrone, cathinone (**3**) and the principle metabolite of mephedrone (4-methylephedrine, **3**) (Figure 3). Stability and reproducibility of devices was examined and showed no significant different in signal intensity recorded over four weeks (Between ANOVA: $F (3, 47) = 1.682$, $p = 0.185$) and no significant difference within or between devices (Figure 4).

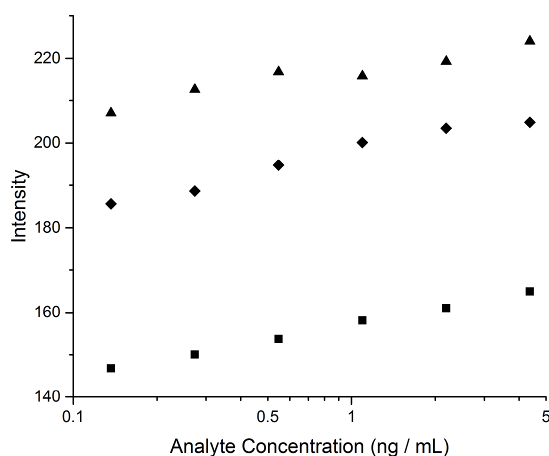

*Figure 3: Comparison of the analysis of urine samples spiked with cathinone (**3**, ◆), mephedrone (**2**, R=Me, ■) and its main metabolite, 4-methylephedrine (**4**, ▲).*

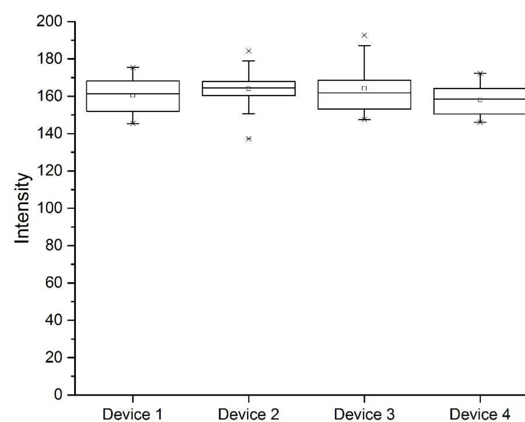

Figure 4: Inter-chip variability study showing signal intensity values recorded across four different microfluidic device. No significant different (Between ANOVA for 1:32,000: $F (3, 47) = 2.103$, $p = 0.114$). Variation within individual microfluidic devices also assessed using Levene's test, which indicated equal variances ($p = 0.638$) ($n=12$).

CONCLUSION

The paper microfluidic device presented has the opportunity to provide rapid, on-site testing, within either a forensic or clinical setting, for NPSs.

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