GUILTY BY DISSOCIATION: PAPER-FLUIDIC PRESUMPTIVE TESTING OF THE NEW PSYCHOACTIVE SUBSTANCE, DIPHENIDINE

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ABSTRACT

This paper reports a paper microfluidic device which can presumptively test for the new psychoactive substance (NPS), diphenidine. A simple 'dip-stick' test has been developed in which Scott's and Marquis reagents are stored on the paper-fluidic device and a colour change is observed upon sample addition if the drug is present. The limit of detection for diphenidine was determined to be 2.5 mg mL^{-1} and 5 mg mL^{-1} for the Scott's and Marquis reagents, respectively, which is within the range normally found within bulk samples encountered by law enforcement agencies. A range of street samples were tested and the results showed strong correlation with conventional laboratory methods.

KEYWORDS: Diphenidine, New Psychoactive Substances, Paper-Fluidic, Presumptive Testing

INTRODUCTION

Diphenidine (Figure 1), a new *N*-methyl-*D*-aspartate (NMDA) receptor inhibitor, has recently emerged in drug seizures as a legal replacement for the controlled dissociative anesthetic ketamine and a number of fatalities associated with its use have been reported [1, 2]. Microfluidic devices have previously been used to detect drugs of abuse [3, 4], however, currently there are no commercial presumptive tests and/or microfluidic devices for the selective detection of diphenidine in either pure and/or adulterated samples.

EXPERIMENTAL

The paper-fluidic device was produced by wax printing onto Whatman filter paper to produce the design shown in Figure 2. Devices were incubated at 130ºC for 180 seconds to melt the wax and then laminated using a layer of cellotape and backed with white paper to provide stability upon addition of the acidic Marquis reagent. To complete the device, 1 µL of Scott's reagent and 1 µL of Marquis reagent were added to the respective test zones and allowed to dry. Aqueous samples were then added to the device via capillary action by placing the wick into the test solution.

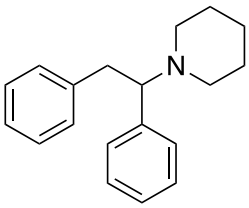

Figure 1: Structure of diphenidine

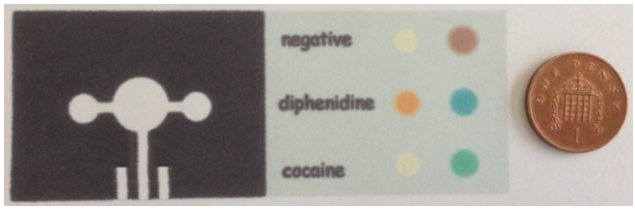

Figure 2: Microfluidic device design incorporating test regions for Scott's and Marquis reagents, wick for aqueous sample addition and reference list for interpretation of results.

RESULTS AND DISCUSSION

Optimization: Serial dilutions of diphenidine from 10 mg mL^{-1} were produced to evaluate the sensitivity of the system. Visual inspection showed clear colour changes upon addition of the analyte. Diphenidine, gives a strong positive blue colour with Scott's reagent and a positive orange colour with Marquis reagent. The limit of detection for diphenidine was determined to be 2.5 mg mL^{-1} (Scott's) (Figure 3) and 5 mg mL^{-1} (Marquis) respectively, which indicates that the device would be able to detect levels normally found within bulk samples encountered by law enforcement agencies.

Forensic application: Twenty-two diphenidine street samples were analysed using the paper-fluidic device and compared against a number of standard tests including: gas chromatography-mass spectrometry (GC-MS), infra-red (IR) spectroscopy and presumptive testing using a ceramic white tile. The results (Table 1) showed excellent agreement across the techniques, in terms of both qualitative detection of diphenidine *vs.* other drugs of abuse, and quantitative analysis – where samples (No. 16 – 22) containing significantly lower levels of diphenidine gave a marked reduction in intensity of the colour observed with Marquis reagent. Though diphenidine is principally encountered as a pure substance, the selectivity of our method was determined by screening against other common recreational drugs (mephedrone, cocaine and ketamine), related diphenidine derived NPSs (2-, 3- and 4-methoxphenidine (MXP)) and common adulterants (paracetamol, caffeine and sucrose). In the case of recreational drugs and adulterants, the device could easily discriminate between the six analytes and diphenidine, however, in the case of structurally-related substances (MXPs) the selectivity was less clear cut implying discrimination between these three positional isomers may not be possible. However, the observation that structurally related substances exhibit the same behavior could indicate the devices suitability as a selective field test for this class of dissociative recreational substances.

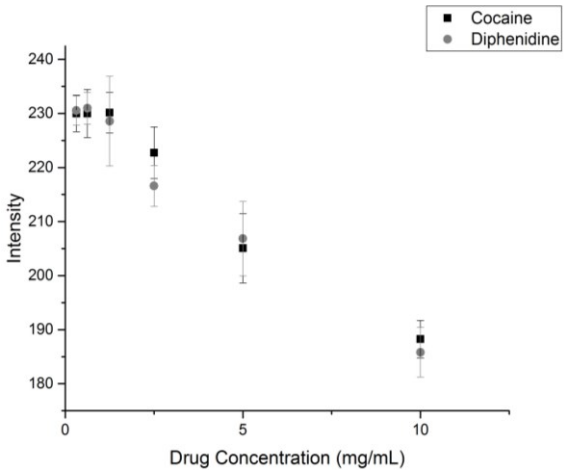

Figure 3: Standard curve based on RGB values for varying concentrations of cocaine and diphenidine for Scott's reagent (n = 6).

Table 1: Comparison of results obtained from conventional white tile Scott's (S) and Marquis (M) tests, paper-fluidic devices, IR spectroscopy and GC-MS.

Sample	Method of Analysis					
	White Tile		LOC		IR (% match)	GC-MS (% w/w)
	S	M	S	M		
Street samples 1 - 15	+	++	+	++	Diphenidine (96.29 - 98.12)	Diphenidine (97.5 - 99.8)
Street samples 16 - 22	+	+	+	+	Diphenidine (63.28 - 66.96)	Diphenidine (34.9 - 59.4)
Mephedrone[a]	-	-	-	-	Mephedrone (100)	Mephedrone (100)
Cocaine[a]	+	-	+	-	Cocaine (100)	Cocaine (100)
Ketamine[a]	-	-	-	-	Ketamine (100)	Ketamine (100)
Caffeine[a]	-	-	-	-	Caffeine (100)	Caffeine (100)
Paracetamol[a]	-	-	-	-	Paracetamol (100)	Paracetamol (100)
Sucrose[a]	-	-	-	-	Sucrose (100)	Sucrose (100)
Diphenidine[a]	+	++	+	++	Diphenidine (100)	Diphenidine (100)
2-MXP[a]	+	++	+	++	2-MXP (100)	2-MXP (100)
3-MXP[a]	+	++	+	++	3-MXP (100	3-MXP (100
4-MXP[a]	+	++	+	++	4-MXP (100)	4-MXP (100)

[a]*Reference standards purchased from Caymen Chemicals.*

CONCLUSION

Our paper-fluidic device offers the ability to perform rapid presumptive testing of suspected diphenidine-containing drug samples in the field. The test is an easy-to-use dip-test which produces a simple colour change reaction, allowing visual distinction of diphenidine and a range of common controlled drugs and/or adulterants. The device is able to act as a discriminatory test between diphenidine and other classes of drugs, however, at present it is unable to distinguish between substituted derivatives of diphenidine (e.g. methoxphenidine, 2-MXP) and efforts to develop a more selective system for these compounds is currently underway.

ACKNOWLEDGEMENTS

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