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TECHNICAL NOTE
CRIMINALISTICS

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DETECHIP®: A Sensor for Drugs of Abuse*

ABSTRACT: The design and preliminary characterization of a novel sensor for drugs of abuse, DETECHIP®, is described in this proof-of-concept note. Combining both colorimetric and fluorimetric assays, DETECHIP® is suitable for lab and field use. More than a conventional spot test which provides a single "yes or no" answer, DETECHIP® provides twenty responses for a more complete characterization of suspect material. This is accomplished by visually noting colorimetric and fluorescent changes of carefully selected dyes upon the addition of test analytes, including drugs of abuse, with respect to controls. Color and fluorescence changes are recorded numerically so that a 20 digit identification code can be constructed for comparison of test analytes and known compounds. DETECHIP® is applicable to a variety of drugs, both plant-derived and synthetic, addressing the need to use several different spot tests simultaneously for a single sample.

KEYWORDS: forensic science, criminalistics, drugs, spot test, colorimetric assay, fluorimetric assay

Modern, portable instrumental methods (1–11) for drugs of abuse have yet to replace wet chemical colorimetric assays (12–15) for rapid lab and field screening of suspected material. Instrumental techniques, although the most sensitive and accurate of the drug testing methods, can be time intensive and costly, requiring technical expertise in addition to being mainly laboratory-bound. Common methods based on immunoassays, which have high sensitivity and are as portable as testing kits, often have limited shelf-lives, prohibitive costs per unit, and can lack specificity (16,17). Alternatively, conventional colorimetric assays (i.e., "spot tests") offer speed, simplicity of operation, portability, and affordability (12–15). Where spot tests often lack is in the occurrences of false positives, as these tests typically have poor specificity and sensitivity compared to the methods described above (18). However, the stability and versatility of these spot tests enable lab scientists to "triage" samples for additional drug analysis, as well as providing quick answers to law enforcement officers or crime scene analysts in the field.

A number of spot tests, e.g., Marquis, Duquenois-Levine, and Scott, utilize an array of reagents with various handling requirements (12–15). These tests often use corrosive or caustic reagents, such as strong acids or bases (12–15). Users are typically required to carry several different test kits in order to test a range of substances and these spot tests are often characteristic for a class of compounds relying on the reactivity of a specific chemical functional group (18–20). Herein we introduce DETECHIP® (21), a new, all-in-one spot test device for lab and potential field use. DETECHIP® is different from current spot tests, relying on molecular interactions between suspect materials and non-toxic dyes rather than functional group reactivity. DETECHIP® is a mix-and-measure assay providing a lasting color *and* fluorescent signal for the rapid detection of commonly abused plant-derived and synthetic drugs. Unlike other color tests which provide a single "yes or no" response, DETECHIP® gives twenty simultaneous responses, in the form of color and fluorescent changes using two different buffers, allowing users to quickly characterize suspect materials. DETECHIP® also allows users to test controls alongside suspect materials, unlike other color testing kits that only describe the control. Here we describe the design and preliminary characterization of DETECHIP® using several controlled substances and over-the-counter (OTC) medications.

Standards and Reagents

All standards and reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless noted.

Drug Sample Preparation

All scheduled drugs were purchased with licensing approval from the U.S. Drug Enforcement Administration (DEA). In addition to the scheduled drugs, a selection of common adulterants (including cutting agents) was tested. A complete list of all scheduled drugs and adulterants used in this study are listed in Table 1. Stock solutions were prepared at micromolar to millimolar concentrations, using less than 25 mg (most require <10 mg) for each analyte, in the solvents according to Table S2 in the supplementary data. A wide variety of drugs and adulterants are water soluble, thus water, preferably de-ionized, was used as both solvent and control solution. Analytes insoluble in water, such as flunitrazepam or thebaine, were solubilized in ethanol (200 proof, USP grade,

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TABLE 1—*Scheduled drugs and common names along with common adulterants used to dilute the quality of homemade illegal drugs.*

Common Name	Trade Name	Street Name*
Fentanyl citrate	Fentora®	Apache, China girl, China white, Dance Fever
Hydrocodone bitartrate	Vicodin®	
Hydromorphone HCl	Dilaudid®	Dust, Juice, Smack, D, Footballs
Thebaine		Paramorphine
Levo alphacetylmethadol HCl	ORLMM®	LAAM
Ketamine HCl	Ketalar®, Ketaset®	Special K, K, Kit Kat
d-Methamphetamine HCl		Crystal Meth, Meth, Speed, Ice, Crank
l-Methamphetamine HCl		Deoxyephedrine
Amphetamine sulfate	Dexedrine®, Adderall®	
Methylphenidate HCl	Ritalin®	Ritalin®
Cocaine HCl		Coke, Snow, Crack, Rock
Flunitrazepam	Rohypnol®	Roofie
Tetrahydrocannabinol (THC)		
1-(1-phenylcyclohexyl) Piperidine HCl	Sernylan®	PCP, Angel Dust
Codeine		Pain killers, Pain Pills
Common Adulterants		
Caffeine		
Nicotine		
Quinine		

*Information was obtained from the U.S. Drug Enforcement Administration.

Aaper) or methanol (ACS grade). In all experiments, the solvents, either water, ethanol, or methanol, served as the control solution. Based on initial experiments, only 1.5 mL of analyte stock solution is required per DETECHIP® testing platform.

OTC Sample Preparation

All samples were purchased from the local grocery store and subjected to passive extraction in water or ethanol at room temperature. For coated tablets, the coating was carefully scraped off prior to dissolution or removed with the aid of the solvent, when possible. This was to ensure that dyes or colored tablets did not interfere with the evaluation of the analyte, as it has been previously noted that dyes used in coatings may interfere with results (15). If it is not possible to remove the coating, secondary detection assays may be necessary. For each OTC, a single tablet was placed in 10 mL of solvent. After *c*. 2 h, each tablet was crushed, mixed, and left undisturbed for up to 48 h. Samples were then centrifuged at 3783×*g* for 5 min to settle the undissolved materials. The supernatant was used for analysis. The OTC samples used in this study contain the salt form of the active ingredient and information found in Table S1 includes a complete list of the OTCs used and the active ingredient information.

Dye Preparation

Dyes were dissolved in methanol to yield a 150 μM stock solution for ease of use when preparing DETECHIP® assay. Information regarding the identity of the dyes used for DETECHIP® is proprietary and will be published at a later date (21).

Buffer

The buffers used for DETECHIP® were both made at 400 mM and pH 7 (Buffer A and B, other specifications regarding the buffers is proprietary [21]). Buffers at a neutral pH and 400 mM were selected to avoid an acid/base-induced dye color change and to ensure solutions remained within their buffering capacity even with the addition of secondary solvents. Preliminary experiments showed that dye-analyte interactions were different between the two buffers for certain analytes. In addition, using two buffers provides additional modes of analyte characterization.

Experimental Procedure

Preliminary Work

Fourteen dyes were initially tested against each drug for noticeable color or fluorescent changes. This selection was narrowed to five dyes based on their selectivity for drugs of abuse and adulterants, easy-to-see color and fluorescence changes, and easy handling and disposal. These five dyes were used in all subsequent experiments.

DETECHIP® Design and Protocol

Fabrication of DETECHIP® is a simple process. First, 150 μL of each dye stock solution is placed into the appropriate wells of a 96-well optical bottom plate (Thermo Fisher Scientific, Rochester, NY). A single dye occupies all 12 wells of its row with sets of 4 wells per row comprising the analysis sequence for a single analyte. Thus, for each DETECHIP®, three testing platforms are generated per 96-well plate, as illustrated in Fig. 1*A*. Each DETECHIP® platform is 5 rows by 4 columns/wells, resulting in a 20 digit "code" for each analyte. The final step in preparing DETECHIP® is passive evaporation (less than 16 h) of the dye solvent, leaving a deposit of solid dye within each well. Prior to analysis, 150 μL aliquots of Buffer A is added to dye-occupied wells in columns 1, 2, 5, 6, 9, 10 with the remaining columns (3,4,7,8,11,12) similarly wet with Buffer B. To the control columns (every odd number), 150 μL aliquots of control solution is added (as described earlier). Once dyes are in solution, 150 μL aliquots of analyte solution are added to the sample columns (every even number). Mixing of solutions in wells is unnecessary but can be easily accomplished during pipetting.

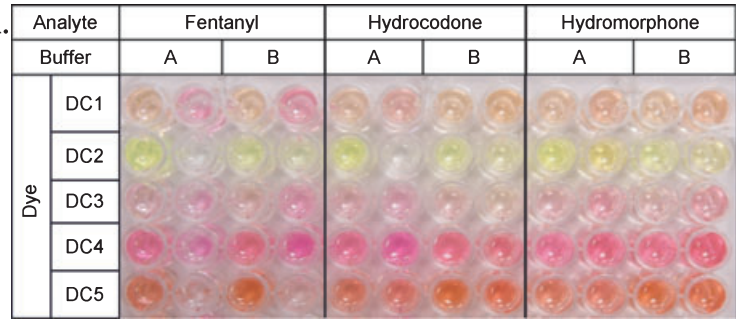
Analysis

Visual color and fluorescent changes, as a result of dye-analyte interactions, were noted and confirmed by spectrophotometry and tested in triplicate. Results are described in the following section. Dye-analyte interactions were analyzed using a Varian Cary 50 UV-Vis Spectrophotometer (Palo Alto, CA) equipped with a microplate reader. A wavelength scan from 400 nm to 800 nm was used to determine λ_{max} values and to confirm color changes for each dye-analyte pair in the visible range. Fluorescence changes noted visually using a low UV wavelength lamp (254 nm) were confirmed using a Shimadzu RF-5301 Spectrofluorophotometer (Columbia, MD).

Results Table

A typical DETECHIP® ready for analysis is shown in Fig. 1*A*. A simple 12 column × 5 row blank table using common spreadsheet software (hereafter "results table") is shown. Color (CC) and fluorescence (FC) changes in the sample well relative to the control well are also noted (Fig. 1*B*). A "0" indicates no change while "1" denotes a change in the sample versus the control. The corresponding results table for DETECHIP® in Fig. 1*A* is shown in Fig. 1*B*.

A.



B.

		CC	FC	CC	FC	CC	FC	CC	FC	CC	FC	CC	FC
Dye	DC1	1^{1}	1^{3}	1^{2}	1^{4}	1	1	1	1	1	1	1	1
	DC2	1^{5}	1^{7}	1^{6}	1^{8}	1	1	1	1	0	1	0	1
	DC3	1^{9}	0^{11}	1^{10}	0^{12}	1	0	1	0	0	0	0	0
	DC4	1^{13}	1^{15}	1^{14}	1^{16}	1	1	0	1	0	1	0	1
	DC5	1^{17}	0^{19}	1^{18}	0^{20}	0	0	0	0	0	0	0	0

C.

11111111110011111100	11111111110010110000	11110011000000110000

FIG. 1—*(A) Actual DETECHIP® assay using both Buffers A and B and the five dyes (DC1-DC5). Shown are the results with fentanyl, hydrocodone, and hydromorphone. Control samples are in even-numbered wells and test analytes are in odd-numbered wells. (B) A representative of how the code for each analyte is constructed based on color (CC) and fluorescence (FC) changes seen in A. The small numbers in the upper-right corner of each block represent the order in which the code is read. (C) The actual DETECHIP® codes for fentanyl, hydrocodone, and hydromorophone.*

Construction of the Codes

Once the 20 simultaneous, visual responses are converted to either a "0" or "1" as in Fig. 1*B*, a 20 digit binary code is generated for each analyte. Beginning with row DC1, the "0" or "1" for the CC in Buffer A starts the binary code, followed by the CC for Buffer B. The third digit of the binary code is the FC value ("0" or "1") for Buffer A, row DC1, with the fourth digit the FC value for Buffer B. The binary code's next four digits are sequenced in the same fashion using values for row DC2, followed by rows DC3, DC4, and DC5. A 20 digit binary code will result (Fig. 1*C*), which can be compared to codes available from the manufacturers of DETECHIP® (NOVEL Chemical Solutions, Crete, NE) or generated in-house using standards. Table S2 shows the codes for all of the illicit drugs, while Table S3 shows the codes for the OTCs and cutting agents tested with this assay.

Results and Discussion

DETECHIP® analysis is simple: a visual check for a color change in sample versus control wells, followed by monitoring fluorescent changes using a hand-held, short wavelength UV lamp.

Confirmation by Spectrophotometric Analysis

After the addition of analytes, UV-Vis and fluorescence data were collected. Figure 2*A* shows an example of a UV-Vis spectrum of fentanyl in Buffer A with the dye DC1. Samples were scanned from 400 nm to 800 nm for confirmation of either a wavelength shift or absorbance change in the presence of the test analyte. In all cases, when a color change was noted, the UV-Vis data showed λ_{max} shifts or decreases in absorbance similar to the visual color change shown in Fig. 2*A* (the addition of fentanyl caused a 23 nm shift). Additionally, when fentanyl was added to DC2, a color change occurred from neon green to very faint green accompanied by a shift from 456 nm to 433 nm and a significant decrease in absorbance. With DC3, a 17 nm shift and a color change from light pink to bright pink was observed, while for DC4 a 20 nm shift and a color change to bright pink. A significant decrease in absorbance was noted for DC5, with a shift from 498 nm to 502 nm and a color change from red to nearly colorless (data not shown).

For each dye sample, the λ_{max} was determined and used to measure fluorescent changes on the spectrofluorophotometer (Fig. 2*B*). In many cases, the addition of the analyte would quench the FC as seen with the UV lamp and confirmed by fluorescence measurements. As shown in Fig. 2*B*, quenching was measured by fluorescence and confirmed visually (data not shown). The other fluorescent sensors (DC2 and DC4) showed similar fluorescent quenching profiles. Of all the drugs, adulterants, and OTCs tested, only aspirin was found to be fluorescent under experimental conditions. This is due to the conversion of acetylsalicylic acid (aspirin) to salicylate ion in aqueous solutions with pH >5 (22). Salicylate ion is easily excited using a short wavelength UV lamp, as its excitation wavelength is *c*. 310 nm with emission around 400 nm (23). The uniqueness of aspirin's fluorescence makes this common street drug diluent and adulterant easy to spot. Simply dissolving aspirin in water and examining with a UV lamp is enough of a presumptive test. It should be noted that certain concentrations of aspirin, mixed with drugs of abuse, will likely result in a unique code.

OTC and Adulterant Analysis

In addition to studying controlled substances, several OTC drugs and supplements, as well as common cutting agents (i.e., quinine and caffeine) were subjected to DETECHIP® analysis (results shown in Table S3). The reasons for this were fourfold: (i) select OTC active ingredients are precursors for scheduled drugs, (ii) a variety of OTCs are used as cutting or bulking agents, (iii) suspect tablets may simply be OTCs for personal use or used to "dupe" a buyer, and (iv) the specificity of DETECHIP® for OTCs and cutting agents versus drugs of abuse can be studied.

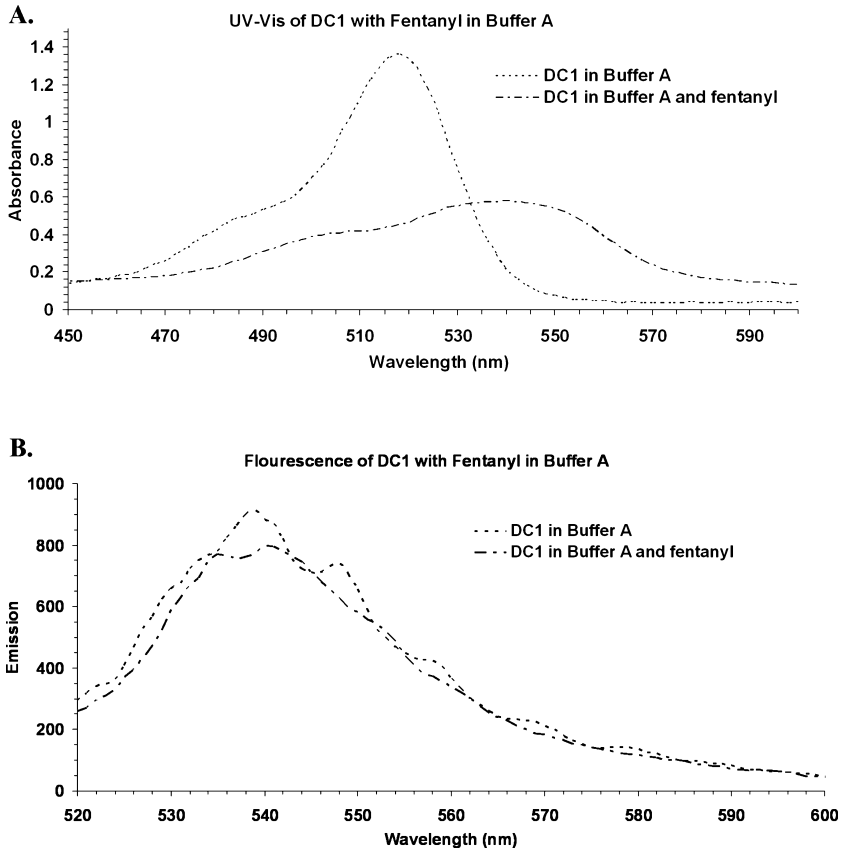


FIG. 2—*(A) An example of the spectrophotometric changes that are accompanied by a color change when fentanyl was added to DC1 in Buffer A, which led to a visible color change from peach to bright pink accompanied by a bathochromatic shift from* λ_{max} *of 517.9 nm to 539.9 nm. (B) A representative example of fluorescence quenching* (c. *20%) at 538 nm when fentanyl was added to the DC1 in Buffer A.*

Specificity

DETECHIP® produces high selectivity for a color test compared to other color tests (12–15,18–20). Only two scheduled drugs had identical codes: *l*-methamphetamine and *d*-methamphetamine (Table 2). This was not surprising because even the more sophisticated techniques rely on the detection of metabolites produced from these enantiomers in order to differentiate *l*-methamphetamine from *d*-methamphetamine (24). In all the other cases, identical codes were matched between scheduled drugs and OTC samples or adulterants and not between scheduled drugs, which can also be a common occurrence for immunoassay-based tests (16,17). Results for DETECHIP® therefore suggest changes in color and fluorescence are most likely based on intermolecular interactions between dyes and drugs, rather than chemical reactions which are functional group specific. Overall, flunitrazepam had the most matches with OTCs and cutting agents in comparison to the other drugs tested. Such identical codes suggest it may be necessary to increase the number of dyes to aid in specificity. Despite the analytes that did produce similar codes, DETECHIP® was able to uniquely identify nine illicit drugs from 11 OTCs or cutting agents. DETECHIP® design modification is currently underway to boost specificity, with the aim of providing no occurrence of false positives or false negatives for drugs of abuse, adulterants, and OTCs through such methods as using alternative or additional dyes or buffers, or customizing DETECHIP® to only test for certain classes of analytes. This preliminary work does illustrate that through the proper selection of dyes and test conditions, a reliable assay for drugs of abuse using easy-to-handle reagents can be fabricated.

TABLE 2—*List of identical code matches between analytes.*

	Ethanol Extracts	Water Extracts
Fentanyl	Multivitamin	Multivitamin
	Jet-Alert	
Hydromorphone	Tylenol® cold day	
Cocaine		Caffeine
Flunitrazepam	Quinine	Nicotine
	Allergy Relief D	Codeine
		Aspirin
THC	L-glutamine	
l-Methamphetamine		*d*-Methamphetamine

Portability

DETECHIP® has excellent potential for use in the field. The dyes are immobilized, being "inactive" until use (as described in DETECHIP® Design and Protocol). All reagents are fairly innocuous and readily available in convenient storage bottles with droppers for easy use. Solutions of suspect material can be made using sterile, rugged, and disposable supplies available from a number of chemical supply companies.

Conclusion

DETECHIP® is an "all-in-one" spot test, yielding 20 simultaneous responses to generate an identification code for each analyte while allowing users to test controls alongside suspect material.

Practical benefits of DETECHIP® include ease-of-use, low sample volume requirements, and the use of safe and non-toxic reagents. Preliminary data reveal reasonably high specificity among scheduled drugs, OTCs, and common cutting agents. DETECHIP® has the potential to be designed in such a way that false positives and negatives are minimal. Overall, DETECHIP® is a portable, simple, and selective spot test that can be used with a variety of test analytes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of over the counter samples used and the active ingredients according to the manufacturer.

Table S2. List of illicit drugs, cutting agents, and their respective binary codes.

Table S3. Binary codes for over the counter samples in water and ethanol.

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