

**DETECHIP®: A sensor for drugs of abuse**

Journal:	<i>Journal of Forensic Sciences</i>
Manuscript ID:	JOFS-08-661.R1
Manuscript Type:	Technical Note
Date Submitted by the Author:	
Complete List of Authors:	Burks, Raychelle; Nebraska Wesleyan University, Forensic Science Program Pacquette, Shari; Doane College, Chemistry Guericke, Mike; Doane College, Chemistry Wilson, Mark; Doane College, Chemistry Symonsbergen, David; NOVEL Chemical Solutions Lucas, Kerry; Doane College, Chemistry Holmes, Andrea; Doane College, Chemistry
Keywords:	Forensic Science, criminalistics, drugs, spot test, colorimetric assay, fluorimetric assay



Review

DETECHIP[®]: A sensor for drugs of abuse*

Raychelle M. Burks,^{1†} M.F.S.; Shari E. Pacquette,² B.S.; Mike A. Guericke^{2††}; Mark V. Wilson,² Ph.D.; David J. Symonsbergen,³ M.S.; Kerry A. Lucas,² Ph.D.; and Andrea E. Holmes,² Ph.D.

Corresponding authors: Andrea E. Holmes, Doane College 1014 Boswell Ave, Crete, NE 68333; ph: 402.826.6762; fax: 402.826.8278; email: andrea.holmes@doane.edu

Kerry A. Lucas, Doane College 1014 Boswell Ave, Crete, NE 68333; ph: 402.826.8243; fax: 402.826.8278; email: kerry.lucas@doane.edu

¹Nebraska Wesleyan University, Forensic Science Program 5000 St. Paul Ave, Lincoln, NE 68504

²Doane College, Department of Chemistry, 1014 Boswell Ave., Crete, NE 68333

³NOVEL Chemical Solutions, info@NOVELCS.com, 1155 E. Hwy 33, Crete, NE 68333

[†] Will be receiving Ph.D. August 2009

^{††} Will be receiving B.S. May 2009

The work has been presented in form of oral presentations at the American Chemical Society 43rd Midwest Regional Meeting (MWRM) in October, 2008 and at the Northwest Forensic Sciences Meeting in November, 2008.

*Financial Support: The project is supported by the NIH grant number P20 RR016469 from the INBRE Program of the National Center for Research Resources and by the NSF CHE-0747949.

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

1
2
3 Modern, portable instrumental methods (1-11) for drugs of abuse have yet to replace wet
4
5 chemical colorimetric assays (12-15) for rapid lab and field screening of suspected material.
6
7 Instrumental techniques, although the most sensitive and accurate of the drug testing methods,
8
9 can be time intensive and costly, requiring technical expertise in addition to being mainly
10
11 laboratory-bound. Common methods based on immunoassays, which have high sensitivity and
12
13 are portable as testing kits, often have limited shelf-lives, prohibitive costs per unit, and can lack
14
15 specificity (16, 17). Alternatively, conventional colorimetric assays (i.e. “spot tests”) offer
16
17 speed, simplicity of operation, portability, and affordability (12-15). Where spot tests often lack
18
19 is in the occurrences of false positives, as these tests typically have poor specificity and
20
21 sensitivity compared to the methods described above (18). However, the stability and versatility
22
23 of these spot tests enable lab scientists to “triage” samples for additional drug analysis, as well as
24
25 providing quick answers to law enforcement officers or crime scene analysts in the field.
26
27
28
29
30
31
32
33

34 A number of spot tests, e.g. Marquis, Duquenois-Levine, and Scott, utilize an array of reagents
35
36 with various handling requirements (12-15). These tests often use corrosive or caustic reagents,
37
38 such as strong acids or bases (12-15). Users are typically required to carry several different test
39
40 kits in order to test a range of substances and these spot tests are often characteristic for a class of
41
42 compounds relying on the reactivity of a specific chemical functional group (18-20). Herein we
43
44 introduce DETECHIP[®] (21), a new, all-in-one spot test device for lab and potential field use.
45
46

47 DETECHIP[®] is different from current spot tests, relying on molecular interactions between
48
49 suspect materials and non-toxic dyes rather than functional group reactivity. DETECHIP[®] is a
50
51 mix-and-measure assay providing a lasting color *and* fluorescent signal for the rapid detection of
52
53 commonly abused plant-derived and synthetic drugs. Unlike other color tests which provide a
54
55
56
57
58
59
60

1
2
3 single “yes or no” response, DETECHIP[®] gives twenty simultaneous responses, in the form of
4 color and fluorescent changes using two different buffers, allowing users to quickly characterize
5 suspect materials. DETECHIP[®] also allows users to test controls alongside suspect materials,
6 unlike other color testing kits that only describe the control. Here we describe the design and
7 preliminary characterization of DETECHIP[®] using several controlled substances and over-the-
8 counter medications.
9
10
11
12
13
14
15
16
17
18
19

20 **Standards and Reagents**

21 All standards and reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless noted.
22
23
24
25
26

27 *Drug sample preparation:* All scheduled drugs were purchased with licensing approval from the
28 U.S. Drug Enforcement Administration (DEA). In addition to the scheduled drugs, a selection of
29 common adulterants (including cutting agents) was tested. A complete list of all scheduled drugs
30 and adulterants used in this study are listed in Table 1. Stock solutions were prepared at
31 micromolar to millimolar concentrations, using less than 25 mg (most require less than 10 mg)
32 for each analyte, in the solvents according to Table S2 in the supplementary data. A wide variety
33 of drugs and adulterants are water soluble, thus water, preferably de-ionized, was used as both
34 solvent and control solution. Analytes insoluble in water, such as flunitrazepam or thebaine,
35 were solubilized in ethanol (200 proof, USP grade) or methanol (ACS grade). In all
36 experiments, the solvents, either water, ethanol, or methanol, served as the control solution.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51 Based on initial experiments, only 1.5 mL of analyte stock solution is required per DETECHIP[®]
52 testing platform.
53
54
55
56
57
58
59
60

1
2
3 *Over-the-counter (OTC) sample preparation:* All samples were purchased from the local grocery
4 store and subjected to passive extraction in water or ethanol at room temperature. For coated
5 tablets, the coating was carefully scraped off prior to dissolution or removed with the aid of the
6 solvent, when possible. This was to ensure that dyes or colored tablets did not interfere with the
7 evaluation of the analyte, as it has been previously noted that dyes used in coatings may interfere
8 with results (15). If it is not possible to remove the coating, secondary detection assays may be
9 necessary. For each OTC, a single tablet was placed in 10 mL of solvent. After approximately 2
10 hours, each tablet was crushed, mixed, and left undisturbed for up to 48 hours. Samples were
11 then centrifuged at 6000 rpm for 5 min to settle the undissolved materials. The supernatant was
12 used for analysis. The OTC samples used in this study contain the salt form of the active
13 ingredient and information found in Table S1 includes a complete list of the OTCs used and the
14 active ingredient information.

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34 *Dye preparation:* Dyes were dissolved in methanol to yield a 150 μM stock solution for ease of
35 use when preparing DETECHIP[®] assay. Information regarding the identity of the dyes used for
36 DETECHIP[®] is proprietary and will be published at a later date (21).

37
38
39
40
41
42
43
44 *Buffer:* The buffers used for DETECHIP[®] were both made at 400 mM and pH 7 (Buffer A and B,
45 other specifications regarding the buffers is proprietary (21)). Buffers at a neutral pH and 400
46 mM were selected to avoid an acid/base induced dye color change and to ensure solutions
47 remained within their buffering capacity even with the addition of secondary solvents.

48 Preliminary experiments showed that dye-analyte interactions were different between the two
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 buffers for certain analytes. In addition, using two buffers provides additional modes of analyte
4
5
6 characterization.

10 **Experimental Procedure**

11
12 *Preliminary work:* Fourteen dyes were initially tested against each drug for noticeable color or
13
14 fluorescent changes. This selection was narrowed to five dyes based on their selectivity for
15
16 drugs of abuse and adulterants, easy-to-see color and fluorescence changes, and easy handling
17
18 and disposal. These five dyes were used in all subsequent experiments.

19
20
21
22
23
24 *DETECHIP[®] Design and Protocol:* Fabrication of DETECHIP[®] is a simple process. First, 150
25
26 μL of each dye stock solution is placed into the appropriate wells of a 96 well optical bottom
27
28 plate (Thermo Fisher Scientific, Rochester, NY). A single dye occupies all 12 wells of its row
29
30 with sets of 4 wells per row comprising the analysis sequence for a single analyte. Thus, for
31
32 each DETECHIP[®], three testing platforms are generated per 96 well plate, as illustrated in FIG.
33
34 1A. Each DETECHIP[®] platform is 5 rows by 4 columns/wells, resulting in a 20 digit “code” for
35
36 each analyte. The final step in preparing DETECHIP[®] is passive evaporation (less than 16
37
38 hours) of the dye solvent, leaving a deposit of solid dye within each well. Prior to analysis, 150
39
40 μL aliquots of Buffer A is added to dye occupied wells in columns 1, 2, 5, 6, 9, 10 with the
41
42 remaining columns (3, 4, 7, 8, 11, 12) similarly wet with Buffer B. To the control columns
43
44 (every odd number), 150 μL aliquots of control solution is added (as described earlier). Once
45
46 dyes are in solution, 150 μL aliquots of analyte solution are added to the sample columns (every
47
48 even number). Mixing of solutions in wells is unnecessary but can be easily accomplished
49
50 during pipetting.
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6 *Analysis:* Visual color and fluorescent changes, as a result of dye-analyte interactions, were
7
8 noted and confirmed by spectrophotometry and tested in triplicate. Results are described in the
9
10 following section. Dye-analyte interactions were analyzed using a Varian Cary 50 UV-Vis
11
12 Spectrophotometer (Palo Alto, CA) equipped with a microplate reader. A wavelength scan from
13
14 400 nm to 800 nm was used to determine λ_{\max} values and to confirm color changes for each dye-
15
16 analyte pair in the visible range. Fluorescence changes noted visually using a low UV
17
18 wavelength lamp (254 nm) were confirmed using a Shimadzu RF-5301 Spectrofluorophotometer
19
20 (Columbia, MD).
21
22
23
24
25
26

27 *Results Table:* A typical DETECHIP[®] ready for analysis is shown in FIG. 1A. A simple 12
28
29 column x 5 row blank table using common spreadsheet software (hereafter “results table”) is
30
31 shown. Color (CC) and fluorescence (FC) changes in the sample well relative to the control well
32
33 are also noted (FIG. 1B). A “0” indicates no change while “1” denotes a change in the sample
34
35 versus the control. The corresponding results table for DETECHIP[®] in FIG. 1A is shown in FIG.
36
37 1B.
38
39
40
41
42
43

44 *Construction of the Codes:* Once the twenty simultaneous, visual responses are converted to
45
46 either a “0” or “1” as in FIG. 1B, a twenty digit binary code is generated for each analyte.
47
48 Beginning with row DC1, the “0” or “1” for the color change in Buffer A starts the binary code,
49
50 followed by the color change for Buffer B. The third digit of the binary code is the fluorescence
51
52 value (“0” or “1”) for Buffer A, row DC1, with the fourth digit the fluorescence value for Buffer
53
54 B. The binary code’s next four digits are sequenced in the same fashion using values for row
55
56
57
58
59
60

1
2
3 DC2, followed by rows DC3, DC4, and DC5. A twenty digit binary code will result (FIG. 1C),
4
5 which can be compared to codes available from the manufactures of DETECHIP[®] (NOVEL
6
7 Chemical Solutions) or generated in-house using standards. Table S2 shows the codes for all of
8
9 the illicit drugs, while Table S3 shows the codes for the OTCs and cutting agents tested with this
10
11 assay.
12
13

14 15 16 17 **Results and Discussion**

18
19 DETECHIP[®] analysis is simple; a visual check for a color change in sample versus control wells,
20
21 followed by monitoring fluorescence changes using a hand-held, low wavelength UV lamp.
22
23

24
25
26
27 *Confirmation by spectrophotometric analysis:* After the addition of analytes, UV-Vis and
28
29 fluorescence data were collected. FIG. 2A shows an example of a UV-Vis spectrum of fentanyl
30
31 in Buffer A with the dye DC1. Samples were scanned from 400 nm to 800 nm for confirmation
32
33 of either a wavelength shift or absorbance change in the presence of the test analyte. In all cases,
34
35 when a color change was noted, the UV-Vis data showed λ_{max} shifts or decreases in absorbance
36
37 similar to the visual color change shown in FIG. 2A (the addition of fentanyl caused a 23 nm
38
39 shift). Additionally, when fentanyl was added to DC2, a color change occurred from neon green
40
41 to very faint green accompanied by a shift from 456 nm to 433 nm and a significant decrease in
42
43 absorbance. With DC3, a 17 nm shift and a color change from light pink to bright pink was
44
45 observed, while for DC4 a 20 nm shift and a color change to bright pink. A significant decrease
46
47 in absorbance was noted for DC5, with a shift from 498 nm to 502 nm and a color change from
48
49 red to nearly colorless (data not shown).
50
51
52
53
54
55
56
57
58
59
60

1
2
3 For each dye sample, the λ_{\max} was determined and used to measure fluorescent changes on the
4 spectrofluorophotometer (FIG. 2B). In many cases, the addition of the analyte would quench the
5 fluorescence signal as seen with the UV lamp and confirmed by fluorescence measurements. As
6 shown in FIG. 2B, quenching was measured by fluorescence and confirmed visually (data not
7 shown). The other fluorescent sensors (DC2 and DC4) showed similar fluorescent quenching
8 profiles. Of all the drugs, adulterants, and OTCs tested, only aspirin was found to be fluorescent
9 under experimental conditions. This is due to the conversion of acetylsalicylic acid (aspirin) to
10 salicylate ion in aqueous solutions with pH > 5 (22). Salicylate ion is easily excited using a low
11 UV wavelength lamp, as its excitation wavelength is approximately 310 nm with emission
12 around 400 nm (23). The uniqueness of aspirin's fluorescence makes this common street drug
13 diluent and adulterant easy to spot. Simply dissolving aspirin in water and examining with a UV
14 lamp is enough of a presumptive test. It should be noted that certain concentrations of aspirin,
15 mixed with drugs of abuse, will likely result in a unique code.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

36 *OTC and adulterant analysis:* In addition to studying controlled substances, several OTC drugs
37 and supplements, as well as common cutting agents (i.e. quinine and caffeine) were subjected to
38 DETECHIP[®] analysis (results shown in Table S3). The reasons for this were fourfold: (1) select
39 OTC active ingredients are precursors for scheduled drugs, (2) a variety of OTCs are used as
40 cutting or bulking agents, (3) suspect tablets may simply be OTCs for personal use or used to
41 “dupe” a buyer, and (4) the specificity of DETECHIP[®] for OTCs and cutting agents versus drugs
42 of abuse can be studied.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 eleven OTCs or cutting agents. DETECHIP[®] design modification is currently underway to boost specificity, with the aim of providing no occurrence of false positive 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.

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.

1
2
3 Solutions of suspect material can be made using sterile, rugged, and disposable supplies
4
5 available from a number of chemical supply companies.
6
7
8
9

10 **Conclusion**

11
12 DETECHIP[®] is an “all-in-one” spot test, yielding twenty simultaneous responses to generate an
13
14 identification code for each analyte while allowing users to test controls alongside suspect
15
16 material. Practical benefits of DETECHIP[®] include ease-of-use, low sample volume
17
18 requirements, and the use of safe and non-toxic reagents. Preliminary data reveal reasonably
19
20 high specificity among scheduled drugs, OTCs, and common cutting agents. DETECHIP[®] has
21
22 the potential to be designed in such a way that false positives and negatives are minimal.
23
24
25

26
27 Overall, DETECHIP[®] is a portable, simple, and selective spot test that can be used with a variety
28
29 of test analytes.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Golovko AI, Golovko SI. The influence of ethanol on the functional status of GABA receptors. *Biochemistry* 2002;67:719-29.
2. Karle J, Witt MR, Nielsen M. The use of in vivo antisense oligonucleotide technology for the investigation of brain GABA receptors. *Neurochem Int* 1997;31:437-46.
3. ElSohly MA, Salamone SJ. Prevalence of drugs used in cases of alleged sexual assault. *J Anal Toxicol* 1999;23(3):141-6.
4. Kollroser M, Schober C. Simultaneous analysis of flunitrazepam and its major metabolites in human plasma by high performance liquid chromatography tandem mass spectrometry. *J Pharm Biomed Anal* 2002;28:1173-82.
5. Armstrong D, Rundlett KL, Nair UB. Enantioresolution of amphetamine, methamphetamine and deprenyl (Selegiline) by LC, GC, and CE. *Curr Sep* 1996;15:57-61.
6. Huang Q, He X, Ma C, Liu R, Yu S, Dayer CA, et al. Pharmacore/receptor models for GABA/BzR Subtypes via comprehensive ligand mapping approach. *J Med Chem* 2000;43:71-95.
7. Negrusz A, Moore C, Deiterman D, Lewis D, Kaleciak K, Kronstarnd R, et al. Highly sensitive micro-plate enzyme immunoassay screening and NCI-GC-MS confirmation of flunitrazepam and its major metabolite 7-aminoflunitrazepam in hair. *J Anal Toxicol* 1999;23:429-35.
8. Negruz A, Moore C, Hinkel K, Stockham TL, Verma M, Strong MJ, et al. Deposition of 7-aminoflunitrazepam in hair after a single dose of Rohypnol. *J Forensic Sci* 2001;46:1143-51.

- 1
2
3 9. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J. *Molecular Biology of the Cell*.
4
5 3rd ed. New York: Garland Publishing Inc., 1994.
6
- 7
8 10. Dams R, Benijts T, Lambert WE, De Leenheer AP. Simultaneous determination of in
9
10 total 17 opium alkaloids and opioids in blood and urine by fast liquid chromatography-
11
12 diode-array detection-fluorescence detection, after solid phase extraction. *J Chromatogr*
13
14 *B Analt Technol Biomed Life Sci* 2002;773:53-61.
15
- 16
17 11. Hanna GM. NMR regulatory analysis: enantiomeric purity determination for (R)-(-)-
18
19 desoxyephedrine and antipode methamphetamine. *Pharmazie* 2006;61(3)7144:188-93.
20
21
- 22 12. O'Neal CL, Crouch DJ, Fatah AA. Validation of twelve chemical spot tests for the
23
24 detection of drugs of abuse. *Forensic Sci Int* 2000;109:189-201.
25
26
- 27 13. Morris JA. Modified Cobalt thiocyanate presumptive color test for ketamine
28
29 hydrochloride. *J Forensic Sci* 2007;52(1):84-7.
30
31
- 32 14. National Institute of Justice, Office of Science and Technology. Color test reagents/kits
33
34 for preliminary identification of drugs of abuse. Washington, DC: U.S. Government
35
36 Printing Office; 2000 July. Report No.: NIJ Standard-0604.01.
37
38
- 39 15. United Nations International Drug Control Programme. Rapid testing methods of drugs
40
41 of abuse. Vienna: United Nations; 1994. Report No.: ST/NAR/13/REV.1.
42
43
- 44 16. Marielle G, Pirjo L. A comparison between on-site immunoassay drug-testing devices
45
46 and laboratory results. *Forensic Sci Int* 2001;121(1):37-46.
47
- 48 17. Mule SJ, Bastos ML, Jukofsky D. Evaluation of immunoassay methods for detection, in
49
50 urine, of drugs subject to abuse. *Clin Chem* 1974 Feb;20(2):243-8.
51
52
- 53 18. Department of Forensic Science. Controlled substances procedures manual. Virginia: The
54
55 Department; 2009. Report No.: DFS Document 221-D100.
56
57
58
59
60

19. U.S. Department of Justice, Bureau of Narcotics and Dangerous Drugs. Basic training program for forensic drug chemists. Washington, DC: U.S. Government Printing Office, 1972.
20. Feigel F. Spot tests in organic analysis. 7th ed. New York: Elsevier Publishing Co., 1966.
21. Holmes A, inventor DETECHIP[®] is a registered trademark and information disclosed in this paper is patent pending. U.S. Patent NO. 61/080,711.
22. Carey F, editor. Organic Chemistry 7th ed. New York: McGraw-Hill, 2008.
23. Miles CI, Schenk GH. Fluorescence of acetylsalicylic acid in solution and its measurement in presence of salicylic acid. *Anal Chem* 1970;42(6):656-9.
24. Rasmussen LB, Olsen KH, Johansen SS. Chiral separation and quantification of *R/S*-amphetamine, *R/S*-methamphetamine, *R/S*-MDA, *R/S*-MDMA, and *R/S*-MDEA in whole blood by GC-EI-MS. *J Chromatogr B Analt Technol Biomed Life Sci* 2006;842(2):136-41.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Additional Information - Reprints Not Available from Author

For Peer Review

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

<i>Common Name</i>	<i>Trade Name</i>	<i>Street Name*</i>
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
<i>Common Adulterants</i>		
Caffeine		
Nicotine		
Quinine		

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

Table 2. List of identical code matches between analytes.

	<i>Ethanol extracts</i>	<i>Water extracts</i>
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

For Peer Review

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

<i>OTC</i>	<i>active ingredient</i>	<i>Reported amount per tablet (mg)</i>
Equate® Nighttime sleep-aid	diphenhydramine	25
Equate® 24hr Allergy relief D	pseudoephedrine	240
	loratadine	10
Equate® Allergy medication	diphenhydramine	25
Equate® Suphedrine sinus headache	acetaminophen	325
	phenylephrine HCl	5
Equate® Ibuprofen	ibuprofen	200
Equate® Naproxen	naproxen	220
Equate® Complete Multivitamin	various supplements	various
Tylenol® cold day	acetaminophen	325
	dextromethorphan HBr	10
	phenylephrine HCl	5
Tylenol® cold night	acetaminophen	325
	dextromethorphan HBr	10
	phenylephrine HCl	5
	chlorpheniramine	2
Rexall™ Natural L-glutamine	L-glutamine	500
Spring Valley glucosamine & chondroitin	glucosamine	1500
	chondroitin	1200
Schiff® DHEA	dehydroepiandrosterone (DHEA)	25
Jet-Alert™	caffeine	200
Valu-Rite® Enteric Coated Aspirin	aspirin	325
Dollar General® Antacid (calcium rich)	calcium carbonate	500

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

<i>Analyte</i>	<i>Solvent</i>	<i>Binary Code</i>
Fentanyl citrate	Water	11111111110011111100
Hydrocodone bitartrate	Water	11111111110010110000
Hydromorphone HCl	Water	11110011000000110000
Thebaine	Ethanol	00111111000000110000
Levo alphacetylmethadol HCl	Water	11000111010011010100
Ketamine HCl	Water	00110011000001110000
d-Methamphetamine HCl	Water	01000000111111110000
l-Methamphetamine HCl	Water	01000000111111110000
Amphetamine sulfate	Water	11111011000001111100
Methylphenidate HCl	Water	00000000010001110000
Cocaine HCl	Water	11111111110000111100
Flunitrazepam	Ethanol	00110011000000110000
Tetrahydrocannabinol	Methanol	00000011000000000000
1-(1-Phenylcyclohexyl) piperidine HCl	Water	00111111010000110000
Codeine	Methanol	00110011000000110000
Caffeine	Water	11111111110000111100
Nicotine	Water	00110011000000110000
Quinine	Ethanol	00110011000000110000

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

<i>OTC</i>	<i>Water</i>	<i>Ethanol</i>
Equate® Nighttime sleep-aid	11010000110011110000	00000000000000000000
Equate® Allergy relief D	00000000000000000000	00110011000000110000
Equate® Allergy medication	11010000110011110000	00000000000000000000
Equate® Suphedrine sinus headache	11111111010011111100	11110000000000110000
Equate® Ibuprofen	00000000000000000000	00111111000000000000
Equate® Naproxen	11111111000000100000	11111111000000110000
Valu-Rite® Enteric Coated Aspirin *	00110011001100110011	11111111111111111111
Equate® Complete Multivitamin	11111111110011111100	11111111110011111100
Tylenol® cold day	11110011110011110000	11110011000000110000
Tylenol® cold night	11110011110011110000	11110011110000110000
Rexall™ Natural L-glutamine	11111111110011110000	00000011000000000000
Spring Valley glucosamine & chondroitin	11111111000000110000	00000000000000000000
Schiff® DHEA	00000000000000000000	00000000000000000000
Jet-Alert™	11110011110011111100	11111111110011111100
Dollar General® Antacid (calcium rich)	00000000000000000000	00000001000000000000

*Aspirin under experimental conditions is fluorescent. The addition of aspirin automatically produces a fluorescent change. No other analyte tested had similar properties.

Figure Legends

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 represents the order in which the code is read. *C*, The actual DETECHIP[®] codes for fentanyl, hydrocodone, and hydromorphone.

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 bathochromic shift from λ_{\max} of 517.9 nm to 539.9 nm. *B*, A representative example of fluorescence quenching (approximately 20%) at 538 nm when fentanyl was added to the DC1 in Buffer A.

Figure 1.

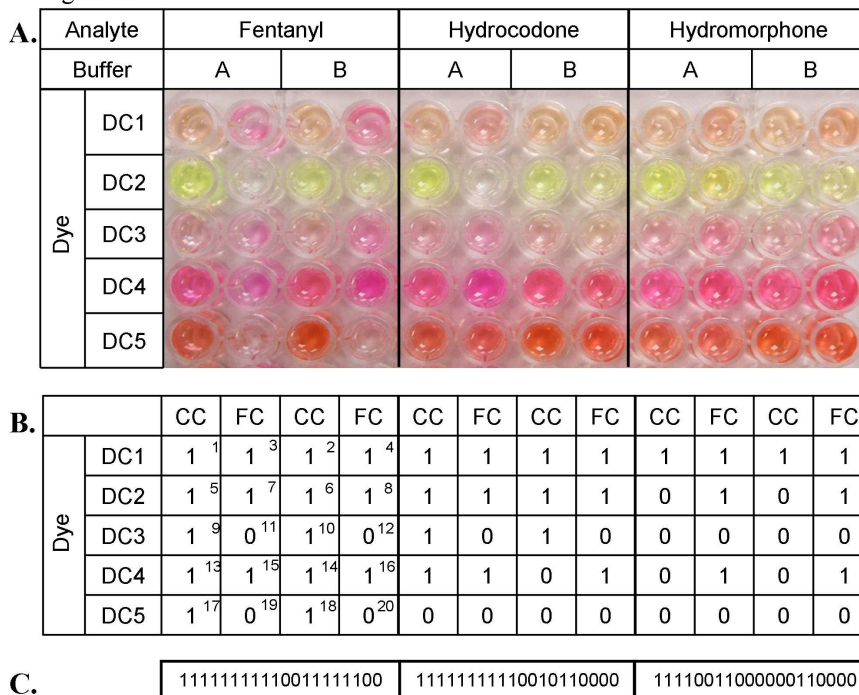


FIG. 1. A, Actual DETECHIP® assay using both Buffers A and B and the five dyes (DC1-DC5). Shown are the results with fentanyl, hydrocodone, and hydromorphone. Control samples are in even numbered wells and test analytes are in odd numbered wells. B, A representative of how the code for each analyte is constructed based on color (CC) and fluorescence (FC) changes seen in A. The small numbers in the upper-right corner of each block represents the order in which the code is read. C, The actual DETECHIP® codes for fentanyl, hydrocodone, and hydromorphone. 190x154mm (307 x 307 DPI)

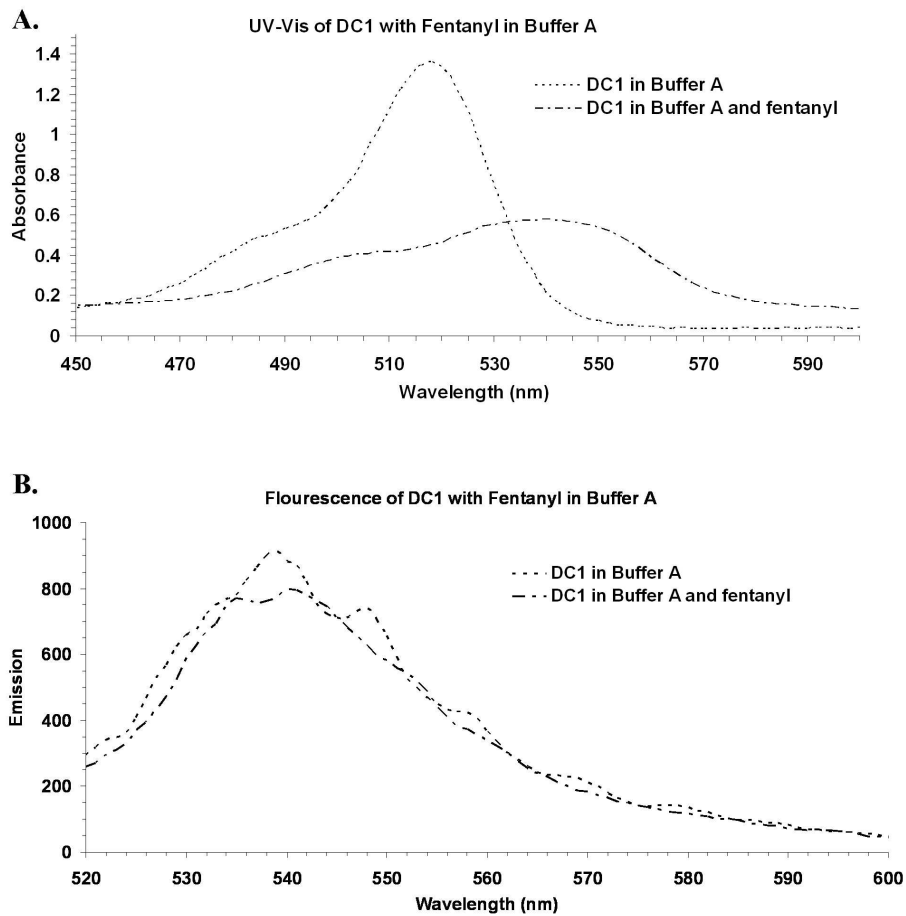


FIG. 2. A, An example of the spectrophotometric changes that are accompanied by a color change when fentanyl was added to DC1 in Buffer A, which led to a visible color change from peach to bright pink accompanied by a bathochromatic shift from λ_{max} of 517.9 nm to 539.9 nm. B, A representative example of fluorescence quenching (approximately 20%) at 538 nm when fentanyl was added to the DC1 in Buffer A.
174x171mm (307 x 307 DPI)